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Neuropil distribution in the cerebral cortex differs between humans and chimpanzees

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Abstract

Increased connectivity of higher-order association regions in the neocortex has been proposed as a defining feature of human brain evolution. At present, however, there are limited comparative data to examine this claim fully. We tested the hypothesis that the distribution of neuropil across areas of the neocortex of humans differs from that of one of our closest living relatives, the common chimpanzee. The neuropil provides a proxy measure of total connectivity within a local region because it is comprised mostly of dendrites, axons, and synapses. Using image analysis techniques, we quantified the neuropil fraction from both hemispheres in six cytoarchitecturally defined regions including frontopolar cortex (area 10), Broca's area (area 45), frontoinsular cortex (area FI), primary motor cortex (area 4), primary auditory cortex (area 41/42), and the planum temporale (area 22). Our results demonstrate that humans exhibit a unique distribution of neuropil in the neocortex compared to chimpanzees. In particular, the human frontopolar cortex and the frontoinsular cortex had a significantly higher neuropil fraction than the other areas. In chimpanzees these prefrontal regions did not display significantly more neuropil, but the primary auditory cortex had a lower neuropil fraction than other areas. Our results support the conclusion that enhanced connectivity in the prefrontal cortex accompanied the evolution of the human brain. These species differences in neuropil distribution may offer insight into the neural basis of human cognition, reflecting enhancement of the integrative capacity of the prefrontal cortex.

Keywords

cytoarchitecture; evolution; brain; asymmetry

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Introduction

Humans possess cognitive capacities that are clearly distinct from those of our closest relatives, the great apes (Herrmann et al., 2007; Premack, 2007; Warneken and Tomasello, 2009). However, because only a few studies have examined how human brain organization differs from that of the great apes (e.g., Aldridge, 2011; Passingham, 2009; Preuss, 2004; Rilling, 2008; Semendeferi et al., 2001; Semendeferi and Damasio, 2000; Semendeferi et al., 2002; Semendeferi et al., 2011; Sherwood et al., 2008), a satisfactory description of what makes the human neural phenotype unique is still lacking. Investigations of brain evolution that have explored species-specific differences in neocortical organization between humans and other apes have focused on variation in the size of particular regions (de Sousa et al., 2010a; Rilling and Seligman, 2002; Schenker et al., 2010; Semendeferi et al., 1998; 2001; Spocter et al., 2010; Zilles and Rehkämper, 1988), the histological architecture of cortical areas (Buxhoeveden et al., 2001b; de Sousa et al., 2010b; Hackett et al., 2001; Preuss and Coleman, 2002; Raghanti et al., 2008a; b; c; Schenker et al., 2008; Semendeferi et al., 2011; Sherwood et al., 2007a; Sherwood et al., 2010a; Sherwood et al., 2006; Stimpson et al., 2011), and gene expression (Cáceres et al., 2003; Enard et al., 2002; Fu et al., 2011; Khaitovich et al., 2004; Preuss et al., 2004; Somel et al., 2009; Uddin et al., 2004).

One quantitative approach that has been used in previous research to characterize phylogenetic differences in neocortical cytoarchitecture involves analysis of the proportion of neuropil space in the gray matter (Buxhoeveden et al., 2001a; Buxhoeveden et al., 2001b; de Sousa et al., 2010b; Schenker et al., 2008; Semendeferi et al., 1998; 2001; Semendeferi et al., 2011; Sherwood et al., 2004; Sherwood et al., 2007b; Zilles and Rehkämper, 1988). The neuropil is defined as the space between neuronal and glial cell bodies that is comprised of dendrites, axons, synapses, glial cell processes, and microvasculature. Accordingly, the proportion of cortical gray matter that is composed of neuropil may serve as a proxy for the total interconnectedness among neurons from local intrinsic circuits and extrinsic projections within a region (Wree et al., 1982). Notably, it has been reported that there is a significantly greater fraction of neuropil space in layer III of the frontopolar cortex (area 10) and Broca's area (areas 44 and 45) of humans relative to apes, while there are no phylogenetic differences in the neuropil fraction among primary sensory and motor cortical areas (areas 3, 4, and 17) (Schenker et al., 2008; Semendeferi et al., 2011). Humans, moreover, have been reported to differ from chimpanzees and macaque monkeys in displaying a leftward asymmetry of neuropil space in layer III of the cortex of the planum temporale (the posterior superior part of area 22, which is also known as area Tpt) (Buxhoeveden et al., 2001a). Interhemispheric asymmetry in neuropil distribution has also been observed in other regions of humans, with leftward dominance in the primary motor cortex (Amunts et al., 1996), parts of the visual cortex (Amunts et al., 2007), and Broca's area (Amunts et al., 1999; but see Schenker et al., 2008 for more varied results for neuropil asymmetry in Broca's area of humans). Studies of the neuroanatomical correlates of schizophrenia, furthermore, have revealed that left hemisphere abnormalities in the neuropil of the planum temporale characterize affected individuals (Chance et al., 2008), highlighting the potential role for this variable in cognitive function.

Because these studies of neocortical neuropil fraction have been based on relatively small sample sizes (Buxhoeveden et al., 2001a), focused on a narrow range of cortical areas (Schenker et al., 2008; Semendeferi et al., 1998; 2001; Semendeferi et al., 2011), or were concerned only with humans (Amunts et al., 2007; Amunts et al., 1996; Amunts et al., 1999), important issues remain unresolved. For instance, it is not known whether the significant increase in neuropil space observed in the human prefrontal cortex is specific to this region or is a more general trend that is common to other higher-order association areas. In addition, hemispheric lateralization of neuropil has never been studied across multiple

areas of the cerebral cortex of human or chimpanzee brains within the same analysis, making it difficult to reach conclusions about the evolution of neuroanatomical asymmetry patterns as they relate to lateralized functions such as communication, language, and the control of manual actions (Corballis, 2007; Hopkins and Cantalupo, 2004; Hopkins et al., 2007; Hopkins et al., 2008; Hopkins et al., 2010).

The current study tests the hypothesis that the distribution of neuropil in the neocortex of humans differs from chimpanzees. We used microscope-based image analysis techniques to quantify the fraction of neuropil space from Nissl-stained histological sections of both hemispheres in six cytoarchitectonically defined regions of the chimpanzee and human neocortex, including frontopolar cortex (area 10), Broca's area in the inferior frontal cortex (area 45), the hand area of primary motor cortex (area 4), agranular frontoinsular cortex (area FI), primary auditory cortex (area 41/42) and the cortex of the planum temporale (area 22).

Materials and Methods

Subjects

The study sample consisted of the brains of twelve chimpanzee subjects, including six females (mean age at death = 37.8 years; s.d. = 12.9; range = 13–48) and six males (mean age at death = 29.3 years; s.d. = 10.8; range = 17–41). These same twelve chimpanzee brains have been used previously in stereologic studies of language area homologues in the neocortex (Schenker et al., 2010; Spocter et al., 2010). Five of the chimpanzee subjects were wild-caught but had lived in captivity since 1973, while the remaining seven chimpanzee subjects were born in captivity. All of the chimpanzee subjects lived in social groups of two to 13 individuals, while housed at the Yerkes National Primate Research Center (Atlanta, Georgia) and all died of natural causes without neurological complications. The Yerkes Center is fully accredited by the American Association for the Accreditation of Laboratory Animal Care. American Psychological Association guidelines for the ethical treatment of animals were adhered to during all aspects of this study and institutional animal care and use approval was obtained before conducting this work. The brains of chimpanzees were removed at necropsy and immersed in 10% formalin within 14 hours of each subject's death.

In addition to the chimpanzee sample, we analyzed archival Nissl-stained sections from twelve normal adult human subjects, consisting of six females (mean age at death = 53.7 years; s.d. = 31.6; range = 11–97) and six males (mean age at death = 53.7 years; s.d. = 25.6; range = 19–86). These Nissl-stained histological sections of human brains are part of the Yakovlev-Haleem Collection, housed at the National Museum of Health and Medicine, Washington, DC. The human brains were fixed by immersion in 10% formalin. Details about the age and sex distribution of the chimpanzee and human samples can be found in Table 1.

Behavioral measurements

Handedness data were only available for the chimpanzees and have been previously reported (Hopkins, 1995; Hopkins and Cantalupo, 2004; Tagliabata et al., 2006). In brief, two different tasks were used to evaluate handedness in this sample, the 'tube task' and a manual gesturing task. The tube task required the subject to use a finger from one hand to remove peanut butter from the inside of a polyvinylchloride tube. This task remains stable across the lifespan of an individual (Hopkins et al., 2007). The manual gesturing task involved recording hand use when subjects presented with food by the experimenter performed an open hand begging gesture. Handedness data on the gesture task were available for 10 of the 12 subjects, whereas handedness data on the tube task were available for all subjects.

Tissue preparation and staining

Chimpanzee brains were prepared for histology as previously described (Schenker et al., 2010; Spocter et al., 2010). Each hemisphere was blocked with a coronal cut at the level of the precentral gyrus and another cut at the level of the angular gyrus. These slabs were cryoprotected by immersion in buffered sucrose solutions up to 30%, embedded in Tissue-Tek medium, frozen in a slurry of dry ice and isopentane, and sectioned at 40 μm with a sliding microtome in the coronal plane. Every 10th section (400 μm apart) was stained for Nissl substance with a solution of 0.5% cresyl violet to visualize cytoarchitecture.

The human brain sections from the Yakovlev-Haleem Collection were embedded in celloidin, sectioned at 35 μm in either the coronal or horizontal plane, and stained to visualize myelin using the Loyez stain and reveal cell bodies using cresyl violet in alternating series.

Area identification and image analysis

Area identification was based on published descriptions of cytoarchitecture for these regions from both humans and chimpanzees (Allman et al., 2011; Augustine, 1985; Bailey et al., 1950; Fullerton and Pandya, 2007; Galaburda and Sanides, 1980; Galaburda and Pandya, 1982; Schenker et al., 2010; Semendeferi et al., 2001; Sherwood et al., 2003; Sherwood et al., 2004; Spocter et al., 2010). Figure 1 shows examples of cytoarchitecture from the regions of interest.

We quantified the neuropil fraction (NF) from the cortical regions of interest, spanning layers I to VI, using Nissl-stained histological sections according to a method that we have previously employed (Sherwood et al., 2007b). This method measures the fraction of the projected profile of the section that is darkly stained, comprising cell bodies of neurons, glia and endothelial cells, versus the space that is unstained, which is composed of dendrites, axons, synapses, and microvasculature. Images with luminal space of vessels or tears in the tissue were removed from the analyses. This technique is similar to methods used by other laboratories to quantify the same aspect of cytoarchitecture, such as the gray level index (GLI) (Wree et al., 1982; Zilles, 1978) and the gray level ratio (GLR) (Buxhoeveden et al., 2001b; Casanova et al., 2003; Chance et al., 2008; Schenker et al., 2008; Semendeferi et al., 2011). Because of differences in the specific tissue processing techniques and algorithms used to create binary images, however, the NF values in the present analysis are not directly comparable to other studies.

Image collection for NF analysis was performed using a Zeiss Axioplan 2 microscope (Zeiss, Thornwood, NY) equipped with a Ludl XY motorized stage (Ludl Electronics, Hawthorne, NY), Heidenhain z-axis encoder, and an Optronics MicroFire color videocamera (Optronics, Goleta, CA) coupled to a Dell PC workstation running StereoInvestigator software (MBF Bioscience, Williston, VT). To measure the NF, first contours were drawn around the regions of interest under low power magnification (4 $\times$), then fractionator sampling was used (grid spacing of 1000 $\times$ 1000 μm) as implemented by the StereoInvestigator system to collect a series of 8 bit grayscale image frames obtained in a systematic-random fashion with a 20 $\times$ (0.5 N.A.) Plan-Neofluar objective lens (Fig. 2). The exposure of the digital camera was standardized to an average target intensity of 71%. Images covered 440 $\times$ 587 μm and were 1600 $\times$ 1200 pixels in size, yielding a resolution of 0.37 pixels per μm . Digital images from three Nissl-stained sections were analyzed for each region of interest. Throughout the process, image frame acquisition was monitored during fractionator sampling and all frames that fell outside of the boundaries of the region of interest were omitted from further processing. On average, 15.9 (s.d. = 5.9) image frames per section were collected for analysis of NF from each region of interest in each individual.

Once images were captured, the NF was measured by importing each image series into ImageJ (v.1.32j). Each series was converted to binary by an automated threshold routine based on the method of Rider and Calvard (1978). Dilation and erosion functions were applied to fill small holes representing light staining of cellular nuclei. After converting each image series to binary, the percentage of the measuring frame occupied by pixels representing unstained elements (i.e., neuropil) was calculated. The NF for each region of interest was calculated as the section-weighted mean of NF across all the image frames. Using this sampling approach, the mean coefficients of error of the data were 0.056 for the human sample and 0.039 for the chimpanzee sample. Photomicrographs used in figures were montaged using StereoInvestigator software and were edited for brightness and contrast using Adobe Photoshop CS3.

Data analysis

All data analyses were performed using SPSS software (version 11.0). We examined the association between NF and age for each region in each species separately using non-parametric Spearman rank order correlations. No relationship between NF and age was found, so this variable was not included in further analyses.

Because the human and chimpanzee samples used in this study were prepared with different histological techniques, all analyses of NF were performed within each species separately. Using mixed-model factorial analysis of variance for each species, we examined whether the NF differed significantly among cortical areas, and between hemispheres and sexes. Cortical area and hemisphere were included in the analysis as within-subjects factors and sex was entered as a between-subjects factor. Tukey's HSD post-hoc multiple comparisons were performed when significant overall effects were observed. We used a repeated-measures design to model the effects of cortical area and hemisphere within subjects because these dependent variables represent multiple observations of the same individual. The estimation of NF from a histological section is related to fixation conditions, plane of sectioning, tissue section thickness, method of embedding, Nissl staining protocol, and the particular microscope optics used to collect images. Interindividual variation in the manner in which brain tissue interacts with these confounding factors is impossible to control completely and variation in shrinkage can be considerable even when all technical variables are held constant (de Sousa et al., 2010). Therefore, one major advantage of using the repeated-measures design is that it reduces the error variance when there are large mean differences in a series of dependent measures among sample members. In the context of the current study, this permits analysis of patterns of within-subject variation in NF across cortical areas and hemispheres, assuming that sources of error in the measurement of NF due to technical tissue processing variability are consistent within a brain specimen.

To examine lateralization further, an asymmetry quotient (AQ) was calculated using the equation $(\text{Right}-\text{Left})/[(\text{Right}+\text{Left})/2]$. Positive values indicate a right greater than left asymmetry and negative values indicate a left greater than right asymmetry. Because the AQ is calculated as an index of the NF values from the left and right of the same individual, any artifacts related to fixation or histological preparation are expected to be consistent in both hemispheres and yield a reliable relative measure of lateralization. Such relative measures of asymmetry may then be compared across samples. Population-level asymmetry for each cortical area in each species was examined using a one-sample *t*-test to determine whether the means were significantly different from zero (i.e., symmetry).

Non-parametric Spearman rank order correlations adjusted for multiple comparisons using Bonferroni correction were used to evaluate the associations between the NF AQ for each cortical area with indices of handedness in the chimpanzee sample. Statistical significance was considered at $\alpha = 0.05$.

Results

Variation in neuropil fraction within each species

Analysis of NF data from chimpanzees revealed significant variation among cortical areas ($F_{5, 120} = 5.02$; $P < 0.001$), but no other main effects of hemisphere or sex, and no interactions (Table 2; Fig. 3). Follow-up post-hoc comparisons indicated that in chimpanzees the primary auditory cortex had a significantly lower NF than the majority of other cortical areas (i.e., areas 10, 45, and 4).

The human sample also displayed significant variation among cortical areas ($F_{5, 120} = 10.77$; $P < 0.001$), and no other main effects or interactions (Table 3; Fig. 3). Follow-up post hoc comparisons revealed that, in humans, area 10 had significantly greater NF than areas 45, 4, 41/42, and 22. The frontoinsula cortex also had significantly greater NF than areas 41/42 and 22.

Because the NF is a ratio value calculated from two variables, we sought to determine more precisely the basis of the cortical region differences between species. We examined the particle size, corresponding to the area occupied by the typical cell profile, from binarized images that were used for NF measurement. We analyzed a subset of the sample, comprising areas 10, 41/42, and FI from the left hemisphere in six individuals (3 male, 3 female) from each species. After binarization, ImageJ software was used to analyze the median size of black particles (which correspond to profiles of single cells or overlapping clusters of cells) that exceeded a minimum of 20 pixels. In the chimpanzees, particle size was significantly larger in area 10 than in both area 41/42 and area FI (Kruskal Wallis, $\chi^2 = 8.00$, $P = 0.018$, $df = 2$). In the humans, particle size did not differ significantly among areas (Kruskal Wallis, $\chi^2 = 1.49$, $P = 0.476$, $df = 2$). Median particle size across regions and individuals was not correlated with NF in humans ($r_s = -0.286$, $P = 0.302$, $n = 18$) or in chimpanzees ($r_s = -0.182$, $P = 0.470$, $n = 18$). These results indicate that the observed species-specific patterns of regional variation in NF are independent of the size of segmented cell particles in the images analyzed. This supports the interpretation that within-specimen variation in NF is based mostly on differences in the proportion of neuropil space between cells, rather than differences in the size of cell somata.

Asymmetry

We examined NF data further for evidence of population level asymmetry by using one-sample *t*-tests to determine if the distribution of AQs differed significantly from zero (i.e., symmetry). Although asymmetry was observed in individuals from both species (e.g., 5 of the 12 chimpanzees had greater than 7% asymmetry of NF in the frontoinsula cortex), we found no evidence of consistent directional lateralization at the population level for any of the cortical regions in either species (Table 4; Fig. 4).

Using a repeated measures analysis of variance, we tested whether humans and chimpanzees differed from each other in the pattern of NF AQ across cortical areas. Results revealed a significant species effect ($F_{1, 120} = 4.61$; $P = 0.03$), but no significant main effect of cortical area or sex, and no significant interactions (Table 5). The mean NF AQ across all cortical area sampled in chimpanzees was -0.02 (s.d. = 0.07), compared to -0.00027 (s.d. = 0.04) for humans. These results indicate that for the regions of interest in this study, the cerebral cortex of chimpanzees shows a tendency to have a slightly greater proportion of neuropil in the left hemisphere, whereas the distribution of neuropil is more symmetrical between hemispheres in humans. Variation in AQ scores between regions indicates that the leftward asymmetry in AQ values observed in chimpanzees was largely driven by area FI (see Fig. 4). However, despite the statistical significance of these species differences in hemispheric

lateralization, it is notable that neither species displayed population level asymmetry for NF in any cortical area after adjusting α for multiple comparisons.

We also tested for correlations between asymmetry in NF and measures of handedness from the chimpanzee sample based on tasks of bimanual coordination and manual gesturing. No significant correlations were observed between asymmetry in neuropil for any cortical region and handedness on these tasks.

Discussion

We examined the distribution of neuropil space in humans and chimpanzees across six cytoarchitectonically defined neocortical regions. Frontopolar cortex and the frontoinsular cortex of humans had significantly more neuropil than other areas. In contrast to humans, the only region showing a significant difference in neuropil in chimpanzees was the primary auditory cortex, which had relatively less neuropil than the other cortical areas. Our examination of neuropil asymmetry indicated no significant interhemispheric differences in either humans or chimpanzees.

Before discussing our results in more detail, it should be noted that our measurement of neuropil fraction (NF) is not a direct index of local connectivity, but rather is based on a two-dimensional image of a projection through the thickness of tissue sections, serving as an estimate of a complex three-dimensional intercellular compartment comprised of multiple structural elements. Furthermore, the systematic-random sampling approach used to collect image frames for NF analysis covered the entire thickness of the cortical mantle with equal probability, providing an estimate of the average NF for the regions of interest. Previous studies have employed GLI analysis of variation in profiles of cell volume density (i.e., the reciprocal of NF) along series of radial sampling lines to define cytoarchitectonic transitions between cortical areas and examine regional specializations (e.g., Amunts et al., 1999; Amunts et al., 2007). The current analysis did not investigate such laminar variation in NF, but rather compared regional variability in neuropil across the whole cortex in humans and chimpanzees. In this way, our analysis also differs from other comparative studies of human and ape neocortex that restricted their analysis of the proportion of neuropil space (GLR) and minicolumn widths only to layer III (Buxhoeveden et al., 2001a; Buxhoeveden et al., 2001b; Schenker et al., 2008; Semendeferi et al., 2011). Finally, it should be noted that the actual NF values obtained in our study are dependent on the particular histological preparation techniques, microscopy analysis, and image segmentation algorithms we employed, and therefore differ from previous studies that have measured GLI or GLR using different brain samples and image analysis methods.

By sampling a wide range of regions in both hemispheres, including the frontopolar cortex and language-related areas, for which previous research has reported human-specific specialization of neuropil space (Buxhoeveden et al., 2001a; Schenker et al., 2008; Semendeferi et al., 2011), our study provides the most comprehensive comparative analysis of neuropil distribution to date. Our main aim was to use a technique comparable to previous studies to re-examine more systematically the hypothesis that the neuropil compartment of the neocortex shows region-specific modifications in humans as compared to chimpanzees. The current results support the potential that the interconnectedness among neurons in particular areas of the prefrontal cortex of humans is relatively increased as compared to chimpanzees, suggesting that evolutionary modifications of dendrites, axons, or synapses within these regions might be important for distinctively human cognitive functions. Our results, however, were not consistent with prior research suggesting that lateralization of neuropil and minicolumn spacing is uniquely present in human cortical language regions.

The prefrontal cortex is responsible for many higher cognitive abilities underlying the uniquely human capacity for symbolic thought, language, and creativity (Damasio, 1985). Early clinical observations conducted by Luria (1966) noted that lesions in the prefrontal cortex result in, “lack of continuous comparison between the plan of action and the results actually attained..., [and] gross changes in the affective sphere leading to disturbances of character and personality”. Modern neuroimaging research, moreover, has demonstrated that the prefrontal cortex is involved in decision-making, problem solving, mental state attribution, and temporal planning (Badre et al., 2010; Barbey et al., 2009; Cappa and Grafman, 2004; D’Esposito, 2007; Forbes and Grafman, 2010).

Given its fundamental role in higher cognitive functions, the prefrontal region has been the subject of intense interest for understanding the evolutionary origins of human psychological specializations and is often cited as being disproportionately enlarged or somehow anatomically modified (Deacon, 1997; Preuss, 2004; Rilling, 2006; Schoenemann et al., 2005; Semendeferi et al., 2001; Smaers et al., 2010; Smaers et al., 2011). Comparative analyses of the allometric scaling of prefrontal cortex size, however, have yielded conflicting results, with some studies suggesting a significant increase in humans (Blinkov and Glezer, 1968; Schoenemann et al., 2005; Semendeferi et al., 2001), while others conclude that there is only moderate, if any, enlargement beyond what is expected for human brain size (Holloway, 2002; McBride et al., 1999; Sherwood et al., 2005; Uylings and van Eden, 1990).

Several studies have also investigated whether the prefrontal cortex of humans displays evolutionary changes in histological architecture, connectivity, gene expression, or function (reviewed in Preuss, 2011; Rilling, 2008; Sherwood et al., 2008). This research has demonstrated human-specific alterations of innervation by neuromodulatory axons (Raghanti et al., 2008a; b; c), elevated expression of genes related to aerobic metabolism, synaptic plasticity and activity (Cáceres et al., 2003; Cáceres et al., 2007; Fu et al., 2011; Uddin et al., 2008; Uddin et al., 2004), and increased connectivity of Broca’s area with posterior language regions via the arcuate fasciculus (Rilling et al., 2008). Additionally, von Economo neurons are more numerous in humans than in apes (Allman et al., 2011). Von Economo neurons are a class of large, spindle-shaped neurons found predominantly in layer V of anterior cingulate and frontoinsular cortices, which are hypothesized to be involved in the integration of autonomic information in the guidance of social behavior (Nimchinsky et al., 1999; Seeley et al., 2011; Stimpson et al., 2011).

Taken together with the findings of Semendeferi et al. (2001; 2011), our results point to further evolutionary changes of the human prefrontal cortex, indicating that the frontopolar cortex and frontoinsular cortex are distinctive as compared to apes in having a relatively greater fraction of neuropil space than other regions. It is unlikely that this human-specific pattern is simply a result of connectional scaling to maintain proportional connectedness among neurons with brain size enlargement (Ringo, 1991), as previous studies have shown a lack of correlation between measurements of the neuropil space and brain size for areas 10, 13, and 4, as well as visual areas among hominoids (de Sousa et al., 2010b; Sherwood and Hof, 2007). Instead, we suggest that this marked difference in neuropil might reflect increases in dendritic length, axonal volume, or synapse density that are specific to these regions. This possibility is consistent with Golgi impregnation studies showing that the human prefrontal cortex is characterized by pyramidal neurons in layer III with greater dendritic complexity and higher spine densities compared to other cortical areas (Elston et al., 2001; Elston et al., 2006; Jacobs et al., 2001; Travis et al., 2005). Cytoarchitectural analyses also report relatively greater spacing between minicolumns in the prefrontal cortex of humans (Buxhoeveden et al., 2006; Casanova et al., 2006). Seldon (1981a; b) has suggested that more neuropil space and greater distance between minicolumns results in less

overlapping of dendritic trees, facilitating more segregated and specialized modular function. Because the density and distribution of GABAergic inhibitory interneurons in the human prefrontal cortex (areas 9, 32, and 44) does not differ significantly from other primates (Sherwood et al., 2010a), we suggest that the increased neuropil of these prefrontal regions in humans may be due mostly to changes in the morphology of pyramidal neurons that participate in association projection systems (Elston, 2007).

Our results from chimpanzees, demonstrating similarity in neuropil fraction among prefrontal and other cortical regions, might appear to contrast with previous neuromorphological studies of the basilar arbors of layer III pyramidal cells of macaque monkeys, showing greater dendritic complexity and spine density in prefrontal compared to occipital and temporal areas (Elston et al., 2001). It should be noted, however, that these studies analyzed a specific component of the neuropil from a small subset of layer III neurons, whereas our method provides an overall estimate of neuropil space sampled from the whole cortex. For these reasons, further comparative studies of pyramidal neuron morphology in chimpanzees and other great apes are needed to determine the precise anatomical basis of the species differences in the distribution of neuropil observed in the current study.

In contrast with previous findings indicating that humans have significant asymmetry of neuropil in the cortex of the planum temporale, Broca's area, and visual cortex (Amunts et al., 2007; Amunts et al., 1999; Buxhoeveden et al., 2001a), we did not find evidence of neuropil asymmetry in any cortical area of either humans or chimpanzees. The previous studies of planum temporale sampled the neuropil only in layer III, whereas our analysis sampled across all layers. Therefore, one possibility is that a localized asymmetry within layer III was masked by our analysis of the whole cortical thickness. It is also important to note that all previous research employed very small sample sizes of 10 or fewer individuals per species. In addition, other studies, including ours, have not examined these microstructural variables in brains obtained from humans with documentation of handedness or language dominance. Given the ~90% prevalence of right-handedness and more than 95% prevalence of left hemisphere language dominance in humans (Knecht et al., 2000), however, it is reasonable to assume that if anatomical asymmetries are linked to the cortical areas underlying these lateralized functions they would be detectable in a typical human sample. Normal interindividual variation in functional and anatomical asymmetry, nonetheless, might be represented differently in the composition of the sample from each study. These issues await resolution with studies of larger samples of human brains with accompanying documentation of behavioral and functional lateralization.

Because we had behavioral data on handedness associated with the chimpanzee brains in this study, we also examined whether there was a correlation between neuropil asymmetry and interindividual variation in manual laterality, assessed through a bimanual coordinated feeding task and a gestural communication task. Our results revealed no association between hemispheric asymmetry of neuropil and handedness. Therefore, it is likely that the neural organization supporting lateralized behaviors depends on hemispheric bias in some other aspect of microstructure. Indeed, previous research has demonstrated that asymmetry in the density of parvalbumin-immunoreactive interneurons and synaptophysin-immunoreactive puncta in the primary motor cortex were significantly correlated with handedness on the task of bimanual coordination in chimpanzees, while NF showed no association with behavioral lateralization (Sherwood et al. 2007b; Sherwood et al. 2010b).

In conclusion, our findings suggest that significant modifications of neuropil within particular prefrontal cortical areas accompanied the evolution of the human brain. Such anatomical specializations might potentially provide increased capacity for enhanced

neuronal integration to support executive cognitive functions. Further studies will be needed to characterize these changes fully to determine whether they are due to alterations in pyramidal neuron morphology, the extent of axonal innervation, or synaptic density, and to assess their specific role in the emergence of human functional specializations.

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A - Human

B - Chimpanzee

Figure 1.

The cytoarchitecture of the six neocortical regions sampled in (A) humans and (B) chimpanzees. Layers are indicated by roman numerals. Scale bar = 500 μ m.

Figure 2.

An illustration of the image collection and conversion method used for measurement of the neuropil fraction (NF). (A) A representative section of the primary motor cortex (area 4) showing the location of sites that were selected by fractionator sampling for image collection. Images that were selected by fractionator sampling that included regions outside of the cortical gray matter were excluded from the analysis. (B) A grayscale image of layer III that was sampled for analysis. (C) The converted binary image that was used to measure the neuropil fraction (NF). Scale bar in B = 80 μ m. PoG, postcentral gyrus; PrG, precentral gyrus.

Figure 3. Regional variation in measurements of neuropil fraction (NF) in 12 chimpanzees and 12 humans according to the methods used in this study. Bar graphs of the mean neuropil fraction by cortical area for (A) chimpanzees and (B) humans. Error bars indicate standard error.

Figure 4. Hemispheric variation in neuropil fraction. Box plots of the asymmetry quotients (AQ) for (A) chimpanzees and (B) humans calculated from the neuropil fraction in each cortical area.

Table 1

Specimens used in this study

Chimpanzees	Age	Plane of section	Humans	Age	Plane of section
Female	13	Coronal	Female	11	Coronal
Female	35	Coronal	Female	32	Coronal
Female	42	Coronal	Female	40	Horizontal
Female	44	Coronal	Female	67	Coronal
Female	45	Coronal	Female	75	Horizontal
Female	48	Coronal	Female	97	Coronal
Male	17	Coronal	Male	19	Coronal
Male	18	Coronal	Male	20	Horizontal
Male	25	Coronal	Male	25	Coronal
Male	35	Coronal	Male	40	Horizontal
Male	40	Coronal	Male	50	Coronal
Male	41	Coronal	Male	86	Coronal
Mean	34		Mean	47	
SD	12		SD	28	
CV	36		CV	60	

* Age in years

Table 2

Results of repeated-measures ANOVA of neuropil fraction in chimpanzees

	Between groups df	F	P
Hemisphere	1	1.90	0.17
Area	5	5.02	0.00
Sex	1	0.21	0.65
Hemisphere * Area	5	0.30	0.91
Hemisphere * Sex	1	0.02	0.88
Area * Sex	5	2.13	0.07
Hemisphere * Area * Sex	5	0.10	0.99

Table 3

Results of repeated-measures ANOVA of neuropil fraction in humans

	Between groups df	F	P
Hemisphere	1	0.00	0.99
Area	5	10.77	0.00
Sex	1	1.32	0.25
Hemisphere * Area	5	0.25	0.94
Hemisphere * Sex	1	0.09	0.77
Area * Sex	5	1.16	0.33
Hemisphere * Area * Sex	5	0.47	0.80

Table 4Results of one sample *t*-tests of neuropil fraction asymmetry quotient

Species	Area	Mean	<i>t</i>	<i>P</i>
Chimpanzee	10	−0.03	−1.53	0.15
	45	0.00	−0.17	0.87
	FI	−0.05	−2.34	0.04
	4	−0.01	−0.42	0.68
	41/42	−0.03	−1.23	0.24
	22	0.00	−0.044	0.97
Human	10	−0.01	−1.01	0.33
	45	0.01	1.08	0.30
	FI	0.01	1.16	0.27
	4	0.00	−0.34	0.74
	41/42	−0.01	−0.67	0.52
	22	−0.01	−0.67	0.51

* None of the results were statistically significant after Bonferroni correction

Table 5

Results of repeated-measures ANOVA of neuropil fraction asymmetry quotient

	Between groups df	F	P
Species	1	4.39	0.04
Sex	1	0.16	0.69
Area	5	0.77	0.57
Species * Sex	1	0.01	0.93
Species * Area	5	1.08	0.37
Sex * Area	5	0.72	0.61
Species * Sex * Area	5	0.41	0.84