Morphometry of the human cerebral cortex microcirculation: General characteristics and space-related profiles

Frederic Lauwers, a, b, ⁎ Francis Cassot, a Valerie Lauwers-Cances, c Prasanna Puwanarajah, d and Henri Duvernoy e

a Functional Neuroimaging Laboratory, INSERM U825, CHU Purpan, 31059 Toulouse-cedex 3, France
b Department of Anatomy, Faculty of Medicine, Toulouse-Purpan, 133 Route de Narbonne, 31062 Toulouse-cedex, France
c Department of Epidemiology and Public Health, Faculty of Medicine, 37, Allee Jules Guesde, 31073 Toulouse Cedex, France
d Nuffield Department of Surgery, John Radcliffe Hospital, Oxford OX3 9DU, UK
e 12 Chemin des Relançons, 25 000 Besancon, France

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Studies on human brain microcirculation have thus far yielded few quantitative data, preventing the closest possible interpretation of functional imaging methods such as fMRI and PET that necessarily rely on robustly delineated morphology of haemodynamic systems. Inadequate data in this area can lead to severe underestimation of the spatial specificity of the BOLD response. We took thick sections of Indian ink injected human brain and, using confocal laser microscopy and a novel three-dimensional computer-assisted method we extracted and analyzed hundreds of thousands of vascular segments within a large area of cortex. From this database the global densities, the statistical distributions of diameters and lengths were analysed, separating the tree-like and the net-like parts of the microcirculation. Furthermore, our analysis included variations in volume density along the cortical depth and along vectors parallel to the cortical surface. These morphometric parameters are all key requirements for a sound model of cerebral microcirculation.

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Introduction

Detailed information on microvascular network anatomy is required for understanding several aspects of circulatory system function. Distribution of pressure and wall shear stress in microvessels (Pries and Secomb, 2005) and transport and exchange of oxygen and other materials in physiological systems (Beard and Bassingthwaighte, 2001; Pittman, 1995) are tightly coupled to microvascular architecture. The anatomy of the microcirculation influences the regulation of blood flow, angiogenesis and remodeling (Carmeliet, 2005; Le Noble et al., 2005) and the acute or chronic structural adaptation of vascular beds in response to functional demands of the tissue (Pries et al., 2003), as well as the blood flow response to physical or neural activity. The anatomy of the microcirculation also influences the interpretation of hemodynamically based functional imaging methods (Arthurs and Boniface, 2002; Heeger and Ress, 2002; Hyde et al., 2001; Logothetis and Wandell, 2004; Turner, 2002). Investigators in all these fields have pointed out the crucial influence of network structure, that "future progress will require not only investigation of molecular and cellular mechanisms but also network-level studies that show how these mechanisms are integrated in a physiological context" (Zakrzewicz et al., 2002).

However, for technical reasons, microanatomical studies in humans are rare (Brett et al., 2002) and there exist in the literature very few quantitative data concerning human microvascular cerebral networks. Henry Duvernoy has delineated the main morphological features of arteries and veins from the pial network to the deep cortical layers and in this work describes what is essentially a functional vascular unit (Duvernoy et al., 1981). However, topologic data are still needed. Only 3D morphometry is able to obtain reliable data on the highly complex and fully three-dimensional vascular networks (Minnich et al., 2001) as the human brain microcirculation.

Recently some 3D computer-assisted methods have been introduced and applied to microvascular cerebral network analysis (Cassot et al., 2006; Dickie et al., 2006; Heinzer et al., 2006). However, except for the preliminary results on the morphometry and topology of the human brain microcirculation that we have presented recently (Cassot et al., 2006), these methods are yet to yield significant quantitative data.

* Corresponding author. Department of Anatomy, Faculty of Medicine Toulouse-Purpan, 133 Route de Narbonne, 31062 Toulouse-cedex, France. Fax: +33 561 49 9524.
E-mail addresses: flaauwers@toulouse.inserm.fr (F. Lauwers), fcassot@toulouse.inserm.fr (F. Cassot), lauwers@cict.fr (V. Lauwers-Cances), ppuwanarajah@doctors.org.uk (P. Puwanarajah), henri.duvernoy@wanadoo.fr (H. Duvernoy).
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In the present study, a relatively large volume of human cerebral cortex was “scanned” from both sides of a sulcus. More than three hundred thousands vascular segments were digitized. From this database we analyzed the global densities, the statistical distributions of diameters and lengths, separating the tree-like and the net-like parts of the vascular network. This study emphasizes the spatial variations of the volume density throughout both the cortical depth and parallel to the surface of the brain. Finally, we discuss implications for functional imaging as the ability for functional magnetic resonance imaging (fMRI) at high fields to reveal the microarchitecture of the brain cortex remains controversial (Duong et al., 2001; Logothetis, 2000). On another hand, modulation of fMRI signals as a function of cortical depth has been analyzed in a variety of recent studies in human, monkeys and cats using different fMRI techniques (gradient-echo (GE) and spin-echo (SE) blood oxygenation-level dependent (BOLD) and cerebral blood volume (CBV)-weighted approaches) at different high magnetic fields (3 to 9.4T) (Goense et al., 2007; Harel et al., 2006; Ress et al., 2007; Smirnakis et al., 2007). It appears that spatial specificity of fMRI signals at an intracortical level could profit from precise quantitative evaluation of the vascular network suggested by the present anatomic study.

Materials and methods

The procedures used for image acquisition, mosaic construction and vessels segmentation have been described in detail elsewhere (Cassot et al., 2006; Fouard et al., 2006) and are outlined briefly below. The central features of these procedures are illustrated in Fig. 1. New methodological aspects are described in detail.

Image acquisition

Digital three-dimensional images of the network were obtained from thick sections (300 μm) of India ink-injected human brain (Duvernoy et al., 1981) by confocal laser microscopy. The secondary cortex next to the collateral sulcus in the right temporal lobe was the anatomical region chosen for this study because of the outstanding quality of injection in this area. A linear encoder was used to quantify the exact displacement of the microscope table allowing precise movement of the specimen with an accuracy of approximately 5 μm. The entire volume of the cortex on both sides of the sulcus (fusiform and parahippocampal gyrus) was digitized on 3 adjacent coronal sections named S1, S2 and S3.

Each elementary block contained 70 sections (512 × 512 pixels), each one separated by a distance of 3 μm. Data for all the blocks were stored on a hard drive for subsequent analysis.

Mosaics construction

The coordinates of the left upper corner measured by the positioning system were registered as well as the voxel size (typically 1.22 × 1.22 × 3 μm).

The individual bricks covering a large zone were then added in a special data object called an image mosaic, which simply stores links to files on disk. The data blocks gathered in a mosaic were then realigned either automatically using a correlation technique (Cassot et al., 2006) or semi-automatically using projection views of the blocks and the ImagePro imaging software (Media Cybernetics, Silver Spring, MD, USA), ensuring that the vessels in the overlapping area matched perfectly. Ten mosaics were built according to their location with respect to the collateral sulcus and the pattern of the cortex studied and called “lateral” (fusiform gyrus), “medial” (para-hippocampal gyrus), “bottom” (junction between both gyri) and “top” (surface of the fusiform gyrus). The boundaries of six mosaics were chosen to include the rectilinear part of the cortex. Three mosaics were isolated in the crux of the fold where the cortex undergoes a folding pattern in extension and the vascular trees diverge from the sulcus root to the white matter. The top of the fusiform gyrus was also digitized in one section (S3). In this area, the folding pattern is inverted (contraction) and the main vessels converge from the cortical surface to the white matter.

Each image mosaic contains a large quantity of data, typically several gigabytes and cannot be loaded and processed at once in the memory of a standard personal computer. Adapted methods were developed to adequately process such images (Cassot et al., 2006).

Vessels segmentation

First, a large disk data object was created. In this way, the 3D visualization software Amira (Mercury Boston USA) can manage a volume of data larger than the computer's main memory. Furthermore, it allows the extraction of subvolumes and the subsequent visualization of these using standard or advanced 3D visualization techniques.

The next stage is to extract vessel centre lines. Indeed, centre lines are compact representations of data; they give direct information on network topology and vessel directions, lengths, and junctions. Moreover, if augmented with a distance map, they also give information on vessel radii and density. To do so, we developed algorithms adapted to large disk data sets, the main feature and advantage of which being that they process data locally while preserving global properties. Note that the distance map measures the distance between any point inside a vessel to the nearest vessel wall in any direction. For a centre line point this provides a good estimation of the vessel radius in a particular point of its trajectory.

Finally, this algorithm gives a representation of the vascular network as a set of cylinders centred at the centre line points, the radii of which correspond to the distance map values at these points. This skeleton or lineset can be visualized and superimposed on different types of 3D visualization of the original confocal microscope images (iso-surface, volume rendering, projection views with a colour scale) for representation and control purposes. In addition, the lineset can be edited. For further processing of the morphometric data and analysis of the topology, the lineset is stored in an ASCII file. Each line of this file corresponds to a centre line point and contains the x, y, z coordinates of that point, and the radius of the vessel at this point. The points of a vessel segment, i.e., a blood vessel between two successive points of bifurcation, are listed successively, and vessel segments are separated.

Morphometric analysis and density profiles

Subsequently, the established lineset yields the following information on the brain microvascular network: statistical distributions of vascular length and diameter; volume and surface densities; segments orientations; diameter ratio.

The topological structure of the cerebral arterioles and venules is tree-like, while that of the capillaries is net-like. Because of this essential difference in nature, the topology of these branching or
Fig. 1. (A) Three adjacent coronal sections (S1, S2 and S3) of Indian ink-injected human brain used for data acquisition. The region of interest is located around the collateral sulcus in the right temporal lobe (black rectangle). (B) Depth coded projection of the zones is reconstructed by confocal microscopy. The 10 mosaics are outlined (lateral=blue, medial=red, bottom=yellow, top=green). Three mosaics are cut out from each section according to the cortical pattern, a fourth one is defined on S3 in the superficial gyral area (para-hippocampal gyrus). (C) 3D volume rendering of a selected zone (white rectangle) after alignment of the 3 sections (bottom centre). Vascular skeleton of the same zone on S1 (bottom right). 3D reconstruction of this part of S1 skeleton (bottom left). Scale bar=1 mm.
mesh structures must be analyzed separately. A tree-like network is a branching, unmeshed structure while a net-like one refers to a meshed pattern. To extract a vascular tree from the skeleton, we identified its origin in the sulcus and traced automatically all the paths from this origin through vessels in which resistance to flow is lower than a prescribed value. Tree-like topology needs specific tools and will not be addressed in this study (Cassot et al., 2006).

The net-like “capillary” network was then separated from the tree-like structure by subtracting all the arterial and venous trees from the skeletons. Despite this, very small trees remained after the subtraction. To achieve a complete separation between the tree-like and the net-like network, all the remaining vessels for which the diameter was above a prescribed value were eliminated. This diameter threshold was fixed to 10 μm. Vascular volume and its spatial distribution can be computed directly from the lineset data. Values of the microvessel volume density (MVD) were derived on a frame (3D grid) with a box size of 100 × 100 × 100 μm side lengths. They were defined as the ratio of the total volume of the vessels contained in each box to the volume of the box. These measures were applied to two populations: the complete network, including tree-like vessels, and the capillary network, excluding these vessels.

To verify and quantify more precisely the regional differences in microvascular profiles we rotated the skeletonized vascular network so that the x axis was parallel to the sulcus. The grid of cubic boxes on which microvessel densities were computed was defined such that their centres were located at planes y=yn, where yn=nΔy with Δy=50 μm. On this set of overlapping boxes, a map of MVD was drawn and the mean values of these densities were derived for each yn.

The vascular cortex was defined from the sulcus surface to the point where the vascular density was less than 50% of the mean value and the cortical thickness was then measured. By dividing the distance from the sulcus in the normal direction y by the cortical thickness, density profiles were derived on the normalized cortical depth and interpolated using cubic spline interpolation with a normalized depth step of 2%. For the curved zones, the same kind of analysis and normalization was applied using polar coordinates. Mean density profiles and corresponding standard deviations were then estimated for all mosaics.

For rectilinear (non curved) zones only, the density variations along lines parallel to the sulcus (longitudinal profiles) were also analyzed. For this purpose, the cortex was divided into four areas depending on the following values of the normalized cortex depth: a superficial layer (from 0% to 10%), a middle layer (from 10% to 50%), a deep layer (from 50% to 80%) and a subcortical layer (from 80% to 100%). An autocorrelation function analysis was performed on the mean longitudinal profiles for each layer in order to emphasize possible periodic behaviour.

Vessels orientation

The orientation of the vessels was analyzed on the same grid. These orientations were defined by the angle (θ) of the local vessel direction with the x axis (parallel to the sulcus) and the angle (φ) with the z axis (normal to the brain section surface). These angles range between 0° and 180° (respectively 0° and 90°). Histograms of these angles distributions were constructed for each box previously defined. We normalized the histograms with respect to the cortical depth and defined a mean orientation histogram along the normalized cortical depth. Finally, profiles of the mean orientation and of the circular standard deviation or angular dispersion were estimated as a function of the cortical depth (Zar, 1999). Note that this analysis was restricted to areas of the rectilinear zones of approximately 4 mm extension in the x-direction.

Statistical analysis

Data were normalized using appropriate transformation and we compared means between groups by analyzing variance and by t-testing. Because of the number of segments observed, statistical significance was achieved for differences that in themselves did not represent features of clinical significance, comparisons between sections and between regions were made using random subsamples of appropriate size extracted from the complete network. The minimum significant difference that we wished to detect was equal to the accuracy of the technical process (=1 μm) and sample size of the subsamples were estimated to a power of 90% and an alpha error of 5 percent. We report the median p value for the comparisons made from each subsample and the number of tests which remained significant after controlling of the false positive rate in multiple testing (Benjamini and Yekutieli, 2001).

Results

Global morphometry

From 10 mosaics built from 3 adjacent sections of the collateral sulcus area (Fig. 1), the automatic segmentation process extracted 359,242 vascular segments. This extensive database was studied globally, region by region (medial, lateral, bottom of the cortex, and collateral sulcus area). The number of vascular segments and their volume, surface, and density were computed for each layer.

Table 1: Morphometric parameters according to the localization of the mosaics (mean values of the three sections for lateral, medial and bottom and mean values for one section (S3) in top)

<table>
<thead>
<tr>
<th></th>
<th>Sample volume (mm³)</th>
<th>Number of segments</th>
<th>Number of segments/mm³</th>
<th>Total length/mm³</th>
<th>Vascular surface/mm³</th>
<th>Vascular volume (% of total volume)</th>
<th>Volume/surface (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral</td>
<td>14.57</td>
<td>144675</td>
<td>9929.65</td>
<td>501.82</td>
<td>11.49</td>
<td>2.63</td>
<td>2.3</td>
</tr>
<tr>
<td>Medial</td>
<td>9.555</td>
<td>100729</td>
<td>10542.02</td>
<td>527.21</td>
<td>12.85</td>
<td>3.02</td>
<td>2.4</td>
</tr>
<tr>
<td>Bottom</td>
<td>6.93</td>
<td>51792</td>
<td>7473.59</td>
<td>411.60</td>
<td>10.19</td>
<td>2.43</td>
<td>2.4</td>
</tr>
<tr>
<td>Top</td>
<td>4.41</td>
<td>62046</td>
<td>14069.39</td>
<td>613.17</td>
<td>12.63</td>
<td>2.66</td>
<td>2.1</td>
</tr>
<tr>
<td>S1</td>
<td>9.65</td>
<td>90177</td>
<td>9344.77</td>
<td>471.85</td>
<td>10.73</td>
<td>2.39</td>
<td>2.2</td>
</tr>
<tr>
<td>S2</td>
<td>10.545</td>
<td>92503</td>
<td>8772.21</td>
<td>493.67</td>
<td>11.72</td>
<td>2.75</td>
<td>2.3</td>
</tr>
<tr>
<td>S3</td>
<td>15.27</td>
<td>176562</td>
<td>11562.67</td>
<td>533.48</td>
<td>12.40</td>
<td>2.86</td>
<td>2.3</td>
</tr>
<tr>
<td>Total</td>
<td>35.465</td>
<td>359242</td>
<td>10129.48</td>
<td>504.88</td>
<td>11.74</td>
<td>2.70</td>
<td>2.3</td>
</tr>
</tbody>
</table>

The same data were computed globally (lateral, medial and top for S1 and S2; lateral medial, top and bottom for S3) for each section in order to assess the reproducibility of the method and potential variations along the z axis.
sulcus and top of the gyrus) and section by section. These results are compiled in Table 1. In 35.5 mm³ of cortex, the end-to-end vascular length was around 500 mm/mm³, vascular surface was measured at 11.74 mm²/mm³ and vascular density at 2.7 % of the total volume. The relationship between surface and volume was also given as it remained remarkably constant from a sample to another (2.3 μm).

**Length and diameter (Fig. 2)**

To examine the frequency distributions of diameter and length the global population was cleared of all unconnected segments and all terminal segments. This permitted the creation of a new population which contained only complete vascular segments included between 2 nodes. Distributions were rather asymmetric with a large positive skewness and a leptokurtosis (Figs. 2A, C). However, for these networks the logarithm of the length and the inverse of the square root of the diameter showed normal distribution with similar mean and median, skewness close to zero and kurtosis close to 3 (Figs. 2B, D).

There was no significant difference between diameters of the three different sections (section 1 versus 2, \( p_{\text{median}} = 0.31 \), 0/302 tests were significant; section 2 versus 3, \( p_{\text{median}} = 0.51 \), 0/314 tests were significant, section 1 versus 3, \( p_{\text{median}} = 0.23 \), 0/302 tests were significant). This result illustrated the reproducibility of the segmentation method. On the other hand, a significant difference was found between diameters of the vessels within the top region and vessels within the other regions (medial \( p_{\text{median}} < 10^{-3} \), 226/239 significant tests, lateral \( p_{\text{median}} < 10^{-3} \), 117/239 significant tests and bottom \( p_{\text{median}} < 10^{-3} \), 197/201 significant tests); this difference was particularly clear between the top and the bottom where the network geometry is influenced by opposite cortex plicatures.

These are findings from a mixed population including tree-like vessels and the net-like capillary network. To separate both populations and further characterize the capillary vessels, all the tree-like vessels, extracted according to a resistance criterion, were eliminated from the sample. Then the diameter distributions of capillaries network appeared strictly normal (Fig. 2E). In this capillary sample, mean diameter and length were respectively, 6.47 μm and 52.95 μm, characterizing the mean capillary dimensions.

<table>
<thead>
<tr>
<th>Complete network</th>
<th>Capillaries</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diam (μm)</strong></td>
<td><strong>1/√D (μm)</strong></td>
</tr>
<tr>
<td>Mean</td>
<td>7.82</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>3.52</td>
</tr>
<tr>
<td>Median</td>
<td>7.19</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>5.69/9.60</td>
</tr>
<tr>
<td>Skewness</td>
<td>2.96</td>
</tr>
<tr>
<td>Kurtosis</td>
<td>20.62</td>
</tr>
</tbody>
</table>

Fig. 2. Characteristics of length (L) and diameter (D) distributions of the vascular populations under consideration. For the complete network, the diameter of vessels segment was best normalized by the inverse of the square root function (A, B) and the natural logarithm function best approximated the segments lengths (C, D). The diameter distribution of the capillaries segment appeared spontaneously near normal (E).
Vascular density (Figs. 3 and 4)

We normalized the cortical depth of the areas to allow comparison between them, as cortical thickness differed conspicuously from the medial part of the sulcus to its lateral one. In the lateral part, the mean cortex depth was 2478 μm, versus 1955 μm in the medial part. Despite this difference, the normalized density profiles were very similar, suggesting a degree of scale invariance of the microvascular topology from one gyrus to another (Fig. 3E).

Bearing in mind this difference, we divided the cortex into 4 vascular layers ranging from the surface to the white matter junction (Fig. 4):

- from the surface to 10%: a clear band devoid of capillary followed by a rough increase on vascular density;
- from 10% to 50%: vascular density increases slowly to a maximum;
- from 50% to 80%: vascular density decreases slightly; and

Fig. 3. Vascular density profiles with and without the tree-like network versus the cortical depth: fusiform gyrus (A), para-hippocampal gyrus (B), junction of both gyri at the bottom of the collateral sulcus (C) and top of the fusiform gyrus (D). The vertical bars represent the standard deviation. Whatever the vessels types and the regions, density distributions were different between the four vascular zones defined (p=0.0001). Comparison of capillary density profiles region by region and quantitative definition of the high density stripe in the middle third of the cortex (E). In the 6 mosaics where the cortex is rectilinear (lateral and medial), the relative contribution of tree-like and net-like vessels on the cortical depth shows that capillaries are predominant (grayed zone) in the high density area (F).
- from 80% to the white matter: rough decrease until the white matter junction.

We defined a high capillary density area located approximately in the middle third of the cortex. This topology was especially clear along the banks of the sulcus but slightly different in the plicature zones. At the top of the gyrus, the maximum density area gave 2 distinct peaks, the first one ranging from 32% to 48% and the second one from 48% to 64%. At the bottom of the sulcus the first vascular layer looked wider, ranging from the surface to 16%, then the profile was directly comparable to the other ones with lower values and a maximum reaching only 1.6 (Fig. 3E). This finding could be explained by the cortical depth that was affected by extreme variations in this region as aforementioned. On another hand, these differences might be due to the variation of the cellular layers. Considering the entire vascular population, this high vascular density zone is shifted towards the cortical surface and ranges from 10% to 50% in all the regions studied, except in the gyrual part where the density profile is probably affected by the converging orientation of the vessels.

Analysis of the relation between vascular volume density and the cortex depth (according to the segmentation chosen: zone 1 from 0% to 10%, zone 2 from 10% to 50%, zone 3 from 50% to 80% and zone 4 from 80% to 100%) was performed using Kruskal–Wallis test. In all capillary samples, the density distributions were statistically different between zones 1 and 2 and zones 3 and 4 ($p<0.001$). There was no statistically significant difference between zones 2 and 3 except in the top of the parahippocampal gyrus. When considering the whole population, density distributions were significantly different between all zones with some exceptions; there was no significant difference between zones 1 and 2 in medial and bottom samples probably because of recruiting of large perforating trunks in region 1 by the local calculation of the vascular volume. There was no significant difference between 2 and 3 in the top of the parahippocampal gyrus where arteries and veins converge towards the white matter with an increase of vascular density into the deep cortical layers.

When we addressed the variations of the vascular density along the $x$ axis in the medial and lateral mosaics we found respectively a periodicity of 6–700 μm and 400 μm. This periodicity can be superimposed onto the main arteries which cross over the cortex perpendicularly to the surface (Fig. 4).
Other features of note are as follows:

- The significant heterogeneity of the capillary vascular volume density through the cortex. Besides the relatively smooth variations observed along the cortex depth, density maps showed irregular “blobs” of high density, mostly located within the middle layers of the cortex (Fig. 4).

- The remarkable uniformity of the mean value of the vessel diameter throughout the depth of the cortex.

- The uneven breakdown of the vascular volume between tree-like (or large vessels) and net-like ones (or capillaries) whose ratio oscillates around one, the volume of large vessels being predominant in the superficial (<42%) and deep (>86%) cortical layers while the volume of capillaries was higher in the middle third of the cortex (Fig. 3F). This finding was illustrated from the six mosaics where the cortex is rectilinear (medial and lateral parts of the collateral sulcus on the 3 sections).

After identification of venous and arterial vessels from morphological criteria, we could construct density maps of the veins only. We obtained a differential density map by subtracting the venous trees from the complete network (Figs. 5A, B). This has been done on 5-mm rectilinear zones of the lateral part of the sulcus on the 3 sections. This gave a 3D representation of the density distribution from which we derived an average 2D $xy$-projection of the selected zone and computed the corresponding power spectrum representation $P(k_x, k_y)$ using the Fast Fourier Transform algorithm. Finally we, plotted in bi-logarithmic coordinates the averaged power spectra of the density map versus $k_{xy} = \sqrt{k_x^2 + k_y^2}$, the wave number in the $(x,y)$ direction (Fig. 5D). We applied this procedure to both the venous and differential density maps. It appears that the venous map is sparse while the differential one is dense. The bi-logarithmic plots of the averaged power spectra of these density maps show that attenuation of the high-frequency components starts at lower frequencies and is much more important for the venous map than for the differential one.

**Capillary orientation**

Segment orientation profiles are shown in Fig. 6. This part of the study concerned the capillary network. The most frequent orientation was nearly perpendicular to the sulcus surface (80°). This mean angle remains approximately constant between 10% and 60% of the cortical depth. Near the sulcus, the orientation profile rapidly changes from 0° to 80°. The mean orientation returns to an approximately zero value in the white matter indicating that the vessels are oriented parallel to the surface. The dispersion angle decreases from 50° to 40° in the superficial layer and increases slowly from 40° to 55° in the remaining depth of the cortex. These values indicate a relatively high scatter of the distribution of...
capillary orientation. However, the orientation is not random, as is assumed in all fMRI models.

Discussion

From a novel 3D approach of the cerebral microcirculation, we analyzed quantitatively the vasculature of a large volume of human cerebral cortex. To our knowledge, we thus provide the first quantitative database dedicated to the microcirculation of this organ.

We used a classical anatomical technique that capitalizes upon a number of advantages, namely a complete filling of the whole network by a low-viscosity contrast agent that allows a good “penetration” of the confocal imaging system (Dickie et al., 2006), as well as preservation of the anatomical context throughout the procedure (precise localization of the cortex studied, its orientation and geometry). This last issue is of great importance as functional imaging procedures are based on the microvascular anatomy of the cortex and must be allied to anatomic images for appropriate interpretation.

One of the limits of our method is the weak thickness of the sample. This problem is, in fact, characteristic of the anatomical technique and well known to all confocal microscope users. In vivo animal studies using biphotonic confocal microscopy are actually restricted to superficial cortical layers. Vascular casts give better results but the anatomical information and orientation are usually lost. This problem persists with synchrotron radiation microcomputed tomography using isolated samples (Plouraboue et al., 2004). An approach combining vascular casts and synchrotron tomography (Heinzer et al., 2006) has been recently proposed to visualize large vascular networks from the pial vessels to the cortical capillary bed in the mouse brain but its use in studying the human brain appears distant at present.

Our study addressed this problem by sampling three contiguous sections corresponding to approximately one mm of cortex in the sagittal plane. The mosaics were aligned according to the pattern of the gyrus and the main vessel's crossing branches. Morphometric data were compared and no significant difference could be found, suggesting that vascular architecture remains similar in this direction.

The mounting of a large number of individual bricks in each mosaic and the original segmentation method adapted to this large quantity of data enabled us to follow with a high resolution the vascular network within the large “field of view” required by the local functional architecture (Vaznetta et al., 2005) (of the order of 10 square millimeter of cortex).

Diameter and length

From the vast quantity of data collected, we found that the mean capillary size, averaged from various geometrically different zones, was estimated at 6.47 μm in diameter and 52.95 μm in length. These values, collected from the 10 mosaics, were remarkably constant from one sample to another as illustrated by the V/S ratio which closely links the mean diameter (∼D/4) and range around 2.3 μm in all the mosaics. Pawlik et al. (1981) has reviewed capillary morphometry in mammalian neocortex (cat and rat) and found diameters ranging from 4.2 to 7 μm and segment lengths around 110 μm. Using histological section of rat brain, Schlageret al. (1999) found diameters ranging from 4.6 to 5 μm in different cortical areas. Others animal studies (Hudetz et al., 1993; Ivanov et al., 1981; Mironov et al., 1994; Motti et al., 1986) report capillary segments length of 10–300 μm Most of these studies have excluded large vessels by various methods which are invariably arbitrary or unclear. Essentially, there is no clear consensus on what a capillary is, anatomically, histologically or hemodynamically and the boundary between tree-like structures and the net-like capillary networks remains unclear. In our study, we have shown that the whole network was connected in that it could be completely perfused from any input. This continuous network idea has been postulated for years (Duret, 1874; Lazorthes and Gouaze, 1976) and is clearly verified in the present study. However, tree-like structures were identified and subtracted using a flow resistance criterion. The diameter distributions of the remaining capillaries networks were normal, a notion already proposed by Pawlik et al. (1981) who used the symmetry of the diameter distribution to define the upper limit of capillaries diameters. This limit was set to 10 μm in our study, taking into account the mean diameter of precapillary arteries and postcapillary veins. For the complete network, the length of vessels segment was best approximated by the log-normal distribution, and the inverse square root of the diameter of the segments was the function that best fitted with a normal distribution. These features have been variously noted by other authors (Hudetz et al., 1993; Pawlik et al., 1981) using smaller populations and remain vital for an adequate model of cerebral microcirculation.

Global vascular density

As noted above, the preparation can modify the diameter and length of a vascular segment due to shrinkage of the sample. However, these parameters vary in vivo too, and the outcome of the sample preparation should not influence other parameters, such as vascular density and frequency distributions of vascular length and diameter, since this shrinkage is global and homogeneous.

Most of the data found in the literature are extrapolated to expand a result to 1 mm³ of brain parenchyma; the values derived here were computed and averaged using over 35 mm³ of cortex. Vascular density can be analyzed a number of ways. Total vascular length by mm³ is a universally utilized parameter in two-dimensional morphometric studies of histological sections. We have used this method to compare our results with the findings of others.

The mean vascular length by mm³ was evaluated globally within the entire analyzed volume at around 500 mm/mm³. This parameter has been estimated in cat and rat cortex from 1000 mm/mm³ (Craigie, 1921; Hudetz, 1997; Pawlik et al., 1981) to 215 mm/mm³ (Tata and Anderson, 2002). Few quantitative studies have recorded this parameter in normal human brain. Kreczanski et al. (2005) recently reported a value of 400 mm/mm³ for the human frontal cortex. These variations can certainly be explained by the large discrepancies between methods, specimen and cortical areas, but microvascular density is itself an extremely variable parameter when analyzed on large cortical volume. This heterogeneity is a conspicuous and intrinsic feature of cerebral microcirculation which should be systematically taken into account.

Regarding the different geometrical regions examined here, significant variations of density were found, with bigger values near the surface of the brain at the top of the gyrus (613.17 mm/mm³) and lower values in the bottom of the sulcus (411.6 mm/mm³). A possible explanation for these findings is the different direction of perfusion in these areas. The cortex is curved in extension at the bottom of the sulcus with an inherent decrease of vascular density, whereas it is in contraction at the top of the gyrus with an increasing vascular density.
These variations were also found in surface and volume density analysis, calculated here without any extrapolation as real three-dimensional vascular parameters.

Vascular surface per mm$^2$ was measured as a relevant criterion of the exchange surface; the mean value was 11.74 mm$^2$/mm$^3$. This differs considerably from results from animals’ studies. In Pawlik et al. (1981) review, (2D) values obtained by histology range from 6.2 to 9.7, its own evaluation by in vivo transillumination giving 15.3 mm$^2$/mm$^3$. Risser et al. (2007) recently proposed values around 4.5 mm$^2$/mm$^3$ in a 3D study using synchrotron tomography in small primate cortex. These findings assume major discrepancies between animal and human cortical microcirculation or incomplete recruitment of the microvasculature since the anatomical preparation used in our study should generate an under-estimation of quantitative data.

Vascular volume per mm$^3$ gives the most robust estimation of cortical blood volume as it is quantitatively evaluated in vivo using imaging techniques in animal models. In our study, values range from 2.43% to 3.02% depending on the area studied.

Space-related density of vessels

The idea of a vascular module fashioned on a neuronal unit and its implication in functional imaging techniques has been vigorously defended by a number of authors (Grinvald et al., 2000; Woolsey et al., 1996) who regard it as the cornerstone of the “awake human brain functional study”. As fMRI studies actually reach a columnar organization with a rather weak spatial resolution it is essential to demonstrate it from an anatomical point of view. It remains a challenge to understand the true three-dimensional nature of the cortical vascular architecture especially in its tree-like component. Microscopic studies are for the most part limited by the small size of the samples less than the estimated size of one column (200–1000 µm wide). Synchrotron tomography isolates large samples where vascular columnar organization appears spontaneously (Heinzer et al., 2006; Risser et al., 2007) but no quantitative evaluation has been performed in this area. This justifies the large cortical volume digitized in our work and the mosaic construction in order to observe a periodic organization of the vasculature throughout the gyri chosen. The disposition of intracortical vessels in a regular pattern has been described by several authors (Duret, 1874; Duvernoy et al., 1981; Lazorthes and Gouaze, 1976); Duvernoy has proposed a circular vascular unit centred on a vein and surrounded by an arterial ring. The diameter of such a vascular column ranges from 0.75 to 4 mm depending on the size of the central vein. In this paper we did not focus on vascular trees (data being generated by our group is currently pending analysis), but we assumed that the main periodicity of vascular structures is given by arterial vessels that were similar in size and shape throughout the cortex studied. Assessment of the variation of vascular density in z-direction along the rectilinear parts of the cortex revealed a clear periodicity in the middle and deep layers. In our work, this periodicity illustrates an arterial columnar segmentation of the cortex and is far from able to demonstrate an arteriovenous modular morphology as proposed by Duvernoy and others. Venous vessels are less typical in their shape, diameter and intra-cortical penetration and more heterogeneous in their distribution and rhythm (Duvernoy et al., 1981; Reina-De La Torre et al., 1998).

In contrast, many authors emphasize a greatest capillary density in the middle cortical layers characterizing sensory cortex. This depth-related density of vessels was quantitatively demonstrated by the technique presented here. Potentially, the high vascular density layer could therefore include layer IV in totality and overlap adjacent layers. This correlation would need further work, including a thorough description of the cyto-architecture of the cortex studied. Masamoto et al. (2004) has recently specified successive depth variations of microvascular distribution in rat cortex and the findings here are consistent with the depth-profiles proposed. He showed that the highest density of microvessels varies slightly from one subfield to another across the middle layers, supported by our findings in human cortex where the global geometrical variations of gyri correlate to the variations of the depth vascular profile in accordance with cytoarchitectonic changes from the top of the gyrus to the bottom of the sulcus (Duvernoy et al., 1981). Insofar as we found that there was no depth-related variation in diameter distribution, variations of either global vascular density or capillary density are probably due to disparities in the number of vascular segments or vascular lengths.

Therefore, by mapping vascular densities in all mosaics using a colour-coded representation, most of high density fields are included in the middle layers of the cortex and are not sporadically distributed. Further study could interpret these vascular “blobs” as areas of the greatest functional activity since correlations of this nature have been well documented by Zheng et al. (1991) in primate visual cortex. In summary, analyzing space-related variations of vessel density reproducibly suggests the presence of a high-density stripe in the middle third of the cortex thickness, with a relative periodic organization in a plane parallel to the cortical surface. Despite the lack of direct cellular information about our samples, these results give a first 3D quantitative description of human cortical microcirculation, confirming previous qualitative postulations.

Implications in functional imaging

The vascular source of the signal in functional imaging methods is a point of controversy that clouds the interpretation of these images and the underlying neuronal activity (Logothetis, 2000; Vanzetta et al., 2005). Many investigators have pointed out the crucial influence of vascular network substrate for interpreting the BOLD signal (Logothetis and Wandell, 2004). However, the relationships between neural activity, hemodynamic changes and the signal recorded remain largely debated, all the more because they are interpreted at an intracortical level.

Recent reports (Duong et al., 2001; Zhao et al., 2005) have demonstrated that CBV-weighted fMRI could reach a spatial specificity to the columnar functional architecture. It is tempting to hypothesize that the periodic pattern of the density maps observed along the cortical surface relates to the functional columnar organization of the cortex. In this case, an activated single column could be resolved only if the neighbouring ones are not activated. This would imply a distance equal to twice this period (~0.7 mm) to resolve two adjacent columns and hence a columnar resolution of 1.4 mm, which is exactly the value found by Zhao et al. (2005). Of course, this speculation would need further demonstration.

On another hand, the absolute values of vascular density across cortical thickness vary in a small range that seems insufficient in itself to explain either the hemodynamic response or the laminar profile observed in other fMRI studies (Ress et al., 2007; Zhao et al., 2006). However, in agreement with Ress et al. (2007) who
demonstrated that “laminar activity profiles measured in healthy human gray matter have a self-similar character with respect to gray matter thickness”, we can underline the self-similarity of space-related density profiles in all the regions studied and despite the important variation of cortical thickness from one region to another.

Relatively higher dispersion of the orientation of vascular segments near the cortical surface and into the subcortical layer may be a possible source of variations in the BOLD signal, and one that is yet to be considered. Another point of contention is the relative contribution of capillary and large vessels in different functional mapping techniques. fMRI modalities at high field approach a better resolution within the gray matter where both populations of vessels are unevenly distributed. We found that their respective profiles were inverted with large vessels having a greater contribution to the vascular volume in the upper layers of the cortex and a greater contribution of the capillary compartment in the middle third of the cortex. This relative distribution might play a role in the laminar profiles of functional activity recently observed. Many of these fMRI profiles bear a clear relationship with the capillary density profiles presented here. Reports show the global mean laminar profile of relative BOLD amplitude in human brain measured at 3T (Fig. 5A in Ress et al., 2007), of the CBV signal modulation profile (Fig. 2D in Smirnakis et al., 2007) and the BOLD functional contrast-to-noise ratios (ICNR) (Fig. 2E in Smirnakis et al., 2007) profile in macaque area V1 at 4.7T, of the CBV-weighted profile (Fig. 5 in Harel et al., 2006).

High-resolution SE-BOLD signals also have been shown to peak at cat cortical layer IV (Harel et al., 2006) and at the level of Gennari line of macaque area V1 (Fig. 2E, F in Goense et al., 2007) which lies between cortical layers IVA and IV (C in the same paper) a striking similarity between our capillary density profiles and the image intensity profile derived from SE-anatomical scans; (Fig. 2J). However, SE profiles, even at high-resolution, also show secondary superficial peaks at the cortical surface. Nevertheless, this peak is much lower than the large ones observed in the superficial layers, even outside the gray matter, in GE-BOLD experiments at 3T in human (Ress et al., 2007), or at 4.7T in monkeys (Goense et al., 2007) and at 9.4T in cats (Harel et al., 2006). The normalized BOLD depth profiles in human, the signal modulation profiles in monkeys and the GE profiles in cats are shifted towards the superficial cortical layers as are our total vascular density profiles. This coheres with the expectations that “at high magnetic fields, GE-BOLD functional maps are expected to display contributions from large blood vessels, as at low magnetic fields, but also from the microvasculature (capillaries and post-capillary venules), whereas HSE-BOLD maps should be dominated by contribution from the capillaries” and that in CBV imaging “the presence of MION particles significantly suppresses baseline signals from large vessels” (Harel et al., 2006). However, the surprising observations that, under large surround stimulation conditions, CBV maps can extend along the cortical surface to cover large (>10 mm) regions of the cortex that are devoid of visual stimulation, significant BOLD modulation and likely neuronal activity (Smirnakis et al., 2007) indicates that even such maps should be interpreted with the basal hemodynamic mechanisms and the role of the underlying vascular substrate borne carefully in mind. This clear similarity between the density profiles and the laminar profiles derived from high-resolution fMRI does not demonstrate a causal relationship but emphasizes that among the many factors which determine the fMRI signal, the anatomically and hemodynamically based ones play a role which may have been underestimated until now.

The database provided in this study allows better modelling of the hemodynamic response efficiency and its interaction with neural activation via complex mechanisms of regulation, which could improve the interpretation of fMRI measurements. A number of mathematical models have been proposed for this purpose. Some of them (such as the popular balloon model) have been questioned by recent experimental findings (Vanzetta et al., 2005). Direct flow simulations on complex realistic microvascular network of the human cortex have also been proposed recently (Lorthois et al., 2006).

Various studies (Shmuel et al., 2007; Turner, 2002) have investigated either experimentally or theoretically the specificity of fMRI measurements. Turner (2002) proposed a theoretical method to estimate the extent of the change in oxygenation of venous blood occurring without dilution along veins (the draining vein problem). This estimation depends in part upon the mean values of the capillary diameter and length (as well as the vascular length by unit volume). Using the parameters estimated in this study, the spatial extent of draining vein contamination is predicted to be three times greater than that estimated by Turner (Supplementary material).

Comparison of the power spectra derived from the venous and differential density maps shows that the venous network acts as low-pass filter which produces a large attenuation of the high-frequency components. Below some critical wavelength, for both the venous and differential maps, the Fourier spectrum saturates (cutoff frequency). We note also that this cutoff frequency is lower for the venous than for the differential map. Since the higher frequency components in the frequency domain correspond to the smaller scales in the spatial domain this low-pass filtering effect in the frequency domain results in an alteration of the resolution power and a point spreading effect in the spatial domain.

In many fields of work, mapping the brain microcirculation remains an extensive but essential task. In the area of tumour angiogenesis, characterizing a normal or normalized vascular network is an unavoidable step in identifying any pathological change or therapeutic anti-angiogenic effect (Jain, 2005). Vascular density and fractal dimension are reliable parameters for such a purpose. Further uses include the validation of a non-invasive measurement of the index of capillary diameter as proposed with the Vessel Size Index measured in vivo through MRI techniques (Tropres et al., 2001).

We present here some central morphometric features of the microcirculation within the human brain. The data gathered will allow further study of the branching vessels, arteriovenous relationships, extra-vascular distances and fractal dimensions, leading towards a better understanding of the cerebral vascular bed in a functional context.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2007.09.024.
References


