Rhesus Monkey and Human Share a Similar Topography of the Corpus Callosum as Revealed by Diffusion Tensor MRI In Vivo

A recent study of the corpus callosum (CC) in humans revealed a new topographical arrangement of the cortical connectivity pattern. To explore the CC topography in nonhuman primates, we applied magnetic resonance diffusion tensor imaging and tract tracing techniques in individual rhesus monkeys in vivo. The results demonstrate that the CC topography of primates and humans is surprisingly similar. In particular, the relatively large representation and caudal extension of commissural frontal fibers in the CC is observed in both the monkey and human brain. If evolutionary changes in relative brain volumes are reflected in the arrangement of related fibers crossing the CC, the current study is in line with the fact that the relative volume of the frontal lobe did not significantly increase after the split of the hominin line from other primates.

Keywords: DTI, fractional anisotropy, frontal lobe, macaque brain

Introduction

The corpus callosum (CC) serves as the major connection between the 2 cortical hemispheres in placental mammals. In the rhesus monkey, the CC interconnects the frontal, parietal, temporal, and occipital cortices with more than 56 million callosal fibers (LaMantia and Rakic 1990b). Using light and electron microscopy LaMantia and Rakic (1990a, 1990b) described a mosaic-like fiber system in the CC of the rhesus macaque and a region-specific organization into 10 subregions along the anterior–posterior axis. The most anterior sectors 1–3 (in humans: genu) contain fibers from prefrontal associated cortices. They are characterized by the largest concentration of small myelinated and even unmyelinated axons with diameters of less than 0.5 μm. The CC regions that cover supplementary motor, premotor, and primary motor projections in sectors 4 and 5 (anterior midbody) as well as posterior parietal and temporal projections in sectors 8 and 9 (splenium or isthmus) contain medium and small myelinated axons with diameters between 0.5 and 2.5 μm. Sectors 6 and 7 (posterior midbody) comprising fibers from primary and secondary somatosensory cortices and sector 10 (posterior splenium) containing fibers from the visual cortex are characterized by large and very large fibers with diameters of 2.5 μm or more (LaMantia and Rakic 1990a). Interestingly, this pattern of regional differentiation derived from electron microscopy studies of monkey tissue (LaMantia and Rakic 1990b) agrees with corresponding findings of Aboitiz et al. (1992) reported for humans.

In a previous in vivo magnetic resonance imaging (MRI) study using diffusion tensor imaging (DTI) and fiber tractography, we were able to develop a new segmentation scheme of the human CC (Hofer and Frahm 2006), which is in clear contrast to the widely used Witelson scheme (Witelson 1989). Although differences occur mainly at the anterior tip and the broad midbody area, the DTI-based scheme fits well to histochemical data (Aboitiz et al. 1992). The reason for the increased anterior part of the new CC scheme may be the disproportional enlargement of the human frontal brain during evolution (Zilles et al. 1988; McBride et al. 1999). On the other hand, although a recent study confirmed that lesser apes (gibbons) and monkeys have a slightly smaller frontal cortex than humans when measured relative to overall brain size, the great apes (chimpanzees and gorillas) do not (Semendeferi et al. 1997, 2002). These results stimulated us to study and compare the topographic organizations of the CC in macaques and humans with a special emphasis on the representation of frontal callosal fibers.

DTI emerges as a unique method to obtain information about the neuroaxonal connectivity noninvasively (Basser and Pierpaoli 1996). It exploits structure-induced differences in the motion of water molecules that—in white matter—reflect the main orientation of fiber tracts. This is because the cellular microstructure of nerve fibers hinders the free diffusional displacement of water molecules for all movements except along the fiber direction. When fiber bundles yield coherently arranged structures, which scale to an image voxel, the MRI-observable diffusion properties become directionally dependent, which is anisotropic. Under the assumption that the resulting distribution of apparent diffusivities may be mathematically represented by a tensor, the principle axis of the corresponding diffusion ellipsoid coincides with the direction of the greatest diffusion coefficient, which then can be identified with the orientation of the underlying fiber bundle. Here, we used high-resolution anatomic MRI and a geometrically undistorted DTI technique without sensitivity to susceptibility differences (Nolte et al. 2000; Rieseberg et al. 2005) to study for the first time the CC of the rhesus monkey in vivo.

Methods

Animals and MRI Data Acquisition

MRI studies were conducted at 2.9 T (Siemens Tim Trio, Erlangen, Germany). Diffusion tensor MRI was performed at 1.5 mm isotropic resolution using diffusion-weighted single-shot stimulated echo acquisition mode DTI sequences and 5/8 partial Fourier encoding in combination with a projection onto convex subjects reconstruction algorithm (Rieseberg et al. 2005). The protocol comprised 24 diffusion gradient directions and b values of 0 and 1000 s mm$^{-2}$. A total of 36 transverse sections (1.5 mm thickness) covered the whole brain. The acquisition time per data set was approximately 7 min. To increase the signal-to-noise ratio, the acquisition was repeated 4 times. Anatomic images were based on a $T_1$-weighted 3D fast low angle shot MRI sequence (repetition time = 12.3 ms, echo time = 4.9 ms, flip angle 15°) with an isotropic resolution of 0.5 mm.
For MRI, 4 male rhesus macaque (*Macaca mulatta*, 4 years old) were premedicated using 0.01 mg kg\(^{-1}\) atropine. After induction by intramuscular injection of 15 mg kg\(^{-1}\) ketamine, general anesthesia was maintained by continuous intravenous propofol (10–15 mg kg\(^{-1}\)). While in supine position, the animal head was centered within the concavity of a 4-channel small shoulder coil (Siemens) using a homemade acrylic head holder. Animal experiments were approved by national animal welfare authorities, LAVES, Braunschweig, Germany (reference no. 509.4250/08-07.02).

**Fiber Tractography**

Before calculation of the diffusion tensor, the diffusion-weighted MRI data sets were interpolated to 0.75 mm isotropic resolution (half of the original voxel size) and smoothed with a 3D Gaussian filter (\(\sigma = 1.5\) mm). Estimates of axonal projections were computed by the fiber assignment by continuous tracking algorithm (Mori et al. 1999). Tracking terminated when the fractional anisotropy (FA) value was lower than 0.15 or the main diffusion directions in consecutive steps differed by more than 40° (empirically optimized thresholds). The DTI analysis relied on software developed in-house (M. Küntzel).

The fiber topography of the CC was determined by region-to-region tracking using regions-of-interest (ROIs) defined on color-coded maps of the main diffusion direction. The first ROI was always placed in a midsagittal plane to cover the entire CC. Different second ROIs were located close to the cortex in a transverse section to separate the transcallosal projections into prefrontal, premotor (and supplementary motor), primary motor, primary sensory, parietal, temporal, and occipital regions. To achieve a reliable identification of fiber bundles, we used a simple, robust, and objective method for defining anatomic landmarks. The prefrontal cortex included areas anterior to the arcuate sulcus (Petrides and Pandya 2002). Premotor and supplementary areas were defined half way between the arcuate and central sulcus in anterior-posterior direction. The primary motor cortex comprised the posterior half of the posterior bank of the arcuate sulcus and the anterior bank of the central sulcus, whereas the primary sensory cortex was assigned to the posterior bank of the central sulcus. The parietal cortex extended beyond the postcentral sulcus up to the parieto-occipital sulcus, including the intraparietal sulcus. The temporal cortex was below the ventral bank of the lateral fissure. The inferior occipital sulcus was the border between the temporal lobe and the visual cortex, and finally, the visual cortex was posterior to the parieto-occipital sulcus (Ramnani et al. 2006).

**Segregation of the CC**

We arrived at a segregation of the CC by introducing vertical subdivisions as described in a previous study (Hofer and Frahm 2006) for a better comparability. In the midsagittal section, a geometric baseline was defined by connecting the most anterior and posterior points of the CC. After fiber tracking, regions crossed by fibers belonging to defined cortical areas provided a natural segmentation (shown in individual CC maps). The transcallosal parietal, temporal, and occipital fiber bundles overlapped in this simple scheme and were not separated by vertical lines. They constitute the most posterior region of the CC.

**Fractional Anisotropy**

Quantitative measures of diffusion anisotropy were evaluated in the midsagittal and 2 directly neighboring CC sections. In every subject, we determined the mean FA values averaged across the 3 sections for each cortical region as defined by fiber tractography. To account for interindividual differences in absolute anisotropy and to facilitate group comparisons, the data were normalized to the mean of all FA values in the CC of the respective subject. We then calculated the mean and standard deviation of the normalized FA values of every CC region across subjects. Regional differences were tested for significance (Superior Performing Software System, SPSS Inc.) using analysis of variance combined with a post hoc test (Scheffe’s test for multiple comparisons) at a threshold of \(P < 0.05\).

**Results**

**Segregation of the CC**

The advantage of DTI-based fiber tractography over post-mortem tracer injection techniques is that it enables the reconstruction of a large spectrum of callosal fiber tracts projecting to the cortical hemispheres in a single rhesus monkey (Fig. 1). After identification and separation of tracts belonging to different cortical areas (Fig. 2), we could distinguish between CC regions that comprise prefrontal, premotor (combined with supplementary motor), primary motor, primary sensory, parietal, temporal, and occipital projections (Fig. 3). To compare the CC classification of monkeys and humans, we reconstructed the monkey CC in the same way as described previously for humans (Hofer and Frahm 2006; Fig. 4). In particular, a geometric baseline was defined by connecting its most anterior and posterior borders. We then differentiated 5 vertical partitions of the CC containing fibers projecting into the 7 aforementioned cortical areas. The most anterior region I covers the first fifth of the CC and contains fibers projecting into the prefrontal region. The rest of the anterior half of the CC, that is region II, comprises fibers projecting to premotor and supplementary motor cortical areas. Region III, the posterior half minus the posterior two-fifth, comprises fibers projecting into the primary motor cortex. Region IV constitutes the posterior two-fifth minus posterior one-fourth and contains primary sensory fibers. Parietal, temporal, and occipital fibers cross the CC through region V defined as the posterior one-fourth.

In the above scheme for rhesus macaque, the callosal frontal fibers cross the CC nearly just as far posterior as found for humans (Hofer and Frahm 2006). Because of the gross geometric definition of the substructures, minor changes in the extent of callosal frontal fibers in both species are negligible: in rhesus monkeys the frontal part covers three-fifth and in humans two-third of the CC.

**Figure 1.** Transcallosal fiber tracts from a single rhesus monkey overlaid onto individual anatomic images. (a, b) Oblique, (c) top, and (d) sagittal views of a 3D reconstruction of all callosal fibers comprising bundles projecting into different cortical regions. The colors correspond to the local mean diffusion direction: red = left-right, green = anterior-posterior, blue = superior-inferior. Arrows (white) indicate surface reconstructions of the central sulcus (black).
Regional Differences in FA

Quantitative measures of diffusion anisotropy were evaluated in the midsagittal and 2 directly neighboring sections of the CC. We observed higher values of the FA index in the anterior and posterior areas and the lowest FA values in the posterior middle region of the CC. When considering the FA values of all individual monkeys, the absolute FA values varied between subjects (Fig. 5, top), whereas the distribution of relative FA values along the CC turned out to be very similar (Fig. 5, bottom). This incidence was also observed in humans (Hofer and Frahm 2006). Accordingly, we determined means of normalized FA values averaged across animals for all

Figure 2. Segregated transcallosal fiber tracts of a rhesus monkey (a, c, e) and a human subject (b, d, f) overlaid onto individual anatomic images. (a, b) Oblique, (c, d) top, and (e, f) sagittal views of 3D reconstructions of all callosal fibers. In contrast to Figure 1, color distinguishes fibers projecting into specific cortical regions: green = prefrontal lobe, light blue = premotor and supplementary motor areas, dark blue = primary motor cortex, red = primary sensory cortex, orange = parietal lobe, yellow = occipital lobe, violet = temporal lobe.
topographically homogeneous ROIs that could be identified by DTI tractography. The FA values were found to be highest for prefrontal fibers (region I) as well as for fibers in premotor and supplementary motor areas (II), primary motor areas (III), and visual areas of the CC (most posterior part of V). The lowest anisotropy was observed in CC regions assigned to primary sensory (IV), parietal, temporal, and occipital lobes. Colors are identical to Figures 2 and 3. A, anterior; P, posterior.

The normalized FA values of several callosal regions were significantly different ($P < 0.05$) to each other (Table 1). In particular, this applies to the relatively low values found for parietal fibers—and in most cases also for primary sensory and temporal fibers—when compared with the high anisotropy observed in prefrontal, premotor and supplementary motor, and visual areas. Noteworthy, pronounced differences were seen for parietal, temporal, and visual fibers (all in V), which also form contiguous regions but are not simply separable by a geometric scheme with vertical subdivisions as shown in Figure 4.

### Discussion

In this study, we determined the fiber topography of the rhesus macaque CC and compared it with that of humans, especially with respect to the relative position of CC subregions. Extending other primate studies (LaMantia and Rakic 1990a, 1990b), we could analyze the monkey CC in vivo and with a DTI technique not hampered by geometric distortions due to tissue susceptibility differences (Nolte et al. 2000; Rieseberg et al. 2005). Because of the high spatial resolution of the anatomic and diffusion-weighted images, fiber tracts of the macaque CC were derived in anatomically correct appearance similar to what has previously been accomplished for the

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Table 1

| CC regions with significant differences and fractional anisotropy (FA) values |
|--------------------------------------------------------------------------|
| Region                      | Significant differences (FA) with CC regions projecting into          |
| Prefrontal                  | Sensory cortex, parietal region                                      |
| Premotor                    | Sensory cortex, parietal region, temporal region                     |
| Motor                       | Sensory cortex, parietal region, temporal region                     |
| Sensory                     | Prefrontal cortex, premotor areas, motor cortex                     |
| Parietal                    | Prefrontal cortex, premotor areas, motor cortex, visual areas        |
| Temporal                    | Premotor areas, motor cortex                                        |
| Visual                      | Parietal region                                                      |

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Figure 3. The midsagittal CC of 4 rhesus monkeys (a–d) and 1 human subject (e, see also, Hofer and Frahm 2008) with overlay of all discernable fibers projecting into specific cortical areas. The color scheme is identical to that of Figure 2.

Figure 5. (Top) Mean FA of water diffusion in the macaque CC ($n = 4$) for different regions as indicated in Figures 3 and 4. (Bottom) Relative values individually normalized to the mean value averaged across the entire CC and animals.

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Figure 4. Comparative fiber topography of a monkey (top) and the human CC (bottom) in a midsagittal section. Regions refer to fibers projecting into specific cortical areas: I = prefrontal lobe, II = premotor and supplementary motor cortices, III = motor cortex, IV = sensory cortex, V = parietal, temporal, and occipital lobes. Colors are identical to Figures 2 and 3. A, anterior; P, posterior.
human brain (Hofer and Frahm 2006). Thus, we could show that the topographic organization of the CC is almost identical in both species.

In general, water diffusion anisotropy as determined by MRI reflects a number of microstructural parameters such as the thickness of the individual myelinated axons, the density of respective fibers, and the presence of obliquely oriented fibers in an image voxel. In the human CC, Chepuri et al. (2002), Oh et al. (2005), and Hofer and Frahm (2006) could demonstrate that the anisotropy distribution of the water mobility measured by DTI is in general agreement with histologic data. High FA values could be assigned to regions containing densely distributed axons with relative small diameter. In contrast, regions carrying less densely packed fibers with relatively thick axons exhibited the lowest FA values. Here, we found a FA distribution along the macaque CC, which is comparable with that of humans. Because the observations by light microscopy fit well to the relative FA values observed here (Fig. 5), these findings establish a close relationship between the fiber composition of the CC (LaMantia and Rakic 1990b) and the FA distribution in the monkey brain. The data support the notion that the distribution of and differences in the CC fiber composition reflect the functional heterogeneity within the interhemispheric axon pathway.

In principle, DTI-based fiber tractography offers access to a comprehensive separation of transcallosal fiber projections in individual macaques in vivo—despite some general technical limitations discussed in more detail in Hofer and Frahm (2006). In fact, the reliability of diffusion-derived long association tracts in rhesus monkey has recently been tested and supported by a comparison with autoradiographic histologic tract tracing (Schmahmann et al. 2007). In the CC, DTI more easily identifies callosal fiber tracts that correspond to medial cortical areas, for example, in primary motor or primary sensory areas. Schmahmann and Pandya (2006) described the "homunculus" or motor cortex topography in the CC with the face representation in a most rostral location followed by the hand and leg. In other words, callosal motor fibers that originate from the cortical leg region in a most medially located motor area near the hemispheric fissure and close to the central sulcus should cross the CC in the most posterior part of the CC motor region. Because these motor fibers have been studied in the present work, the corresponding DTI results identified the most posterior callosal fibers of the frontal brain and thereby the posterior extension of its respective representation in the midsagittal CC.

In accordance with the similar topographic organization of the CC in both macaque monkeys and humans, the region associated with the frontal cortex covers up to three-fifth in the monkey and up to two-thirds in humans. Fibers originating in cortical areas posterior to the primary motor cortex (the postcentral region) pass the CC through the remaining posterior two-fifths in the monkey brain and one-third in humans. Thus, the frontal brain areas appear enlarged and occupy more space in the CC as previously described for both the macaque and human brain (Pandya et al. 1971; Pandya and Seltzer 1986; Witelson 1989; LaMantia and Rakic 1990a, 1990b). These discrepancies have to be rooted to the fact that postmortem tracer studies, which are performed sequentially area by area, do not allow for a common view onto a large spectrum of fibers within a single brain (including, for instance, callosal primary motor, primary sensory, and visual fibers).

Jancke et al. (1997) postulated an inverse relationship between the forebrain volume and relative size of the human CC. In this context, the decrease of interhemispheric connectivity with increasing human brain size should lead to hemispheric lateralization eventually mirrored in the CC architecture. In contrast, however, both the monkey and human CC appear very similar with respect to both the callosal fiber distribution and the position of topographic subregions, in particular the posterior border of the primary motor region.

Recent measurements of the forebrain volume in different primate species revealed that the frontal human cortices are not disproportionally larger than those of the great apes (Semendeferi et al. 2002). Although the absolute size of the human frontal cortex is larger than in other primates, the frontal cortex in humans and great apes occupies a similar portion of the cortex of the cerebral hemisphere. Furthermore, sectors of the frontal lobe (dorsal, mesial, and orbital) were very similar across different primates (Semendeferi et al. 1997), and—in relative units—different parts of the frontal cortex indeed occupy similar space in primates, most notably in the human and macaque. Generally speaking, the human frontal cortex is as large as expected for a primate brain of human size. With the assumption that subregions of the CC enlarge proportionally to cortical brain areas and volume, the primate (macaque) brain approaches the human brain regarding the relative posterior extension of the region reserved for frontal cortices. This general concept is reflected in the CC architecture and strongly supported by the present work.

In summary, using DTI-based fiber tractography, we reevaluated the arrangement of transcallosal fiber tracts in the macaque and found a close similarity with recent assessments in humans but striking differences to previous classifications. Most importantly, under the assumption that regional position of the cortical hemisphere is mirrored in the topographic arrangement of the CC—with particular reference to the posterior extension of the regions assigned to the frontal cortices—we fully agree with Semendeferi et al. (2002) that the traditional notion that disproportionally larger frontal lobes are the hallmark of hominid brain evolution is not supported.

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Notes
Conflict of Interest None declared.
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