Hemispheric and Gender Related Differences in the Midcingulum Bundle: A DTI Study

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Abstract: The midcingulate cortex and therefore the underlying midcingulum bundle (MCB) as well play a major role in attention. Although a specific structure's function does strongly depend on its neuroanatomical characteristics, research assessing the morphological variability of the midcingulate region is rather sparse. The present study examined the micro- and macrostructure of the MCB in both hemispheres by means of diffusion-tensor imaging. Besides, effects of gender (Female = 40, Male = 39) and handedness (Left-handers = 45, Right-handers = 34) were assessed as well. Measures of fractional anisotropy, mean diffusion, as well as the white matter volumes of the MCBs were assessed. By integration of multi-modal images, the MCB was isolated and confounding with callosal fibers was avoided. Evidence was found indicating differences between hemispheres and gender regarding both volume and microstructural characteristics of the MCB. Interestingly, gender-related effects seem to be substantially associated with variations in individual brain volumes. Handedness did not emerge as relevant factor in the analyses. These findings might indicate a higher functional connectivity of the left MCB and in males as compared to females. *Hum Brain Mapp* 30:383–391, 2009. © 2007 Wiley-Liss, Inc.

Key words: cingulum bundle; DTI; ACC; anterior cingulate; handedness; gender

INTRODUCTION

The midcingulate cortex (MCC) seems to be involved in higher-level cognitive processes such as conflict monitoring, response selection, error monitoring, and reward-based decision making [Devinsky et al., 1995]. There are plenty of studies which have focused on the functional characteristics of this region. Nevertheless, a unifying theory accounting for relevant findings still has to be

developed. A conclusive interpretation of the data might additionally be hampered by the insufficient description of germane regional morphologic attributes. Although the anatomic features of the MCC have been described on the macro- and microstructural level by some groups [Huster et al., 2007; Paus et al., 1996a,b; Vogt et al., 1995, 2003; Yücel et al., 2001], research assessing pertinent characteristics of the underlying white matter tract is comparably sparse. Along its rostro-caudal course the cingulum bundle also carries associative fibers, which are originating and terminating in other than cingulate regions [Schmahmann et al., 2007]. Nevertheless, it has to be assumed that the functionality of the MCC heavily relies on characteristics and the integrity of the underlying midcingulum bundle [MCB; Benes, 1993]. A functional relevance of the MCB is further supported by studies on psychopathological phenomena such as schizophrenia, where alterations of its neuroanatomical attributes are found (see later).

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Some studies have been conducted to describe the overall pattern of cingulate connectivity as observed in dissected monkey brains [Mufson and Pandya, 1984; Schmahmann et al., 2007; Vogt et al., 1987]. However, concerning human subjects and inter individual or hemispheric differences in cingulum bundle morphology, there is a clear lack of relevant surveys. Of course, direct assessments of macro- and microstructural white matter characteristics are seldom done because of the highly invasive nature of such examinations. On the other hand, there are obvious difficulties in the extraction of this fasciculus by means of magnetic resonance imaging (MRI) as well. Using conventional anatomical MRI (T1- or T2-weighted), the MCB lacks clear boundaries to adjacent fiber tracts. This holds especially true for its ventral periphery, where the white matter of the cingulum bundle and callosal fibers seem to fuse. Such potential confounding can be overcome by use of diffusion tensor imaging (DTI). This method acquires information about the random translational motion of molecules (normally water molecules are studied). Because in brain tissue this so-called Brownian motion is interfered by tissue components such as cell membranes, macromolecules, or fibers, DTI provides information about microstructural characteristics. Molecular mobility in different directions is normally not affected to the same degree. Anisotropic molecular displacement can be detected by measuring diffusion in several directions. To describe the underlying neuronal architecture for a given voxel, measures of mean diffusion and anisotropy for these molecular processes can be reported. Because fiber tracts differ in their directionality and DTI is sensitive to these differences, this method can be used to avoid confounding of adjacent white matter fasciculi and to describe their characteristics at the microscopic level.

Only few studies using DTI to describe MCB characteristics in healthy subjects have been conducted. Nevertheless, effects of specific demographic factors on relevant white matter features in this region are to be expected for at least two reasons. First, it already has been shown that both gender and handedness are associated with variability in regional fissurization and gray matter volume [Huster et al., 2007; Paus et al., 1996a,b; Vogt et al., 1995; Yücel et al., 2001]. Second, the MCC contains three cingulate motor areas which seem to be organized in a somatotopic manner, including separate or dissociable representations for the arms and hands [Picard and Strick, 2001; Strick et al., 1998]. Gong et al. [2005a,b] assessed hemispheric differences as well as possible effects of gender and handedness in two distinct studies. Summarizing, a left-greater-than-right asymmetry of anisotropy was found in the MCB. Regarding gender differences, males showed a trend towards higher fractional anisotropy (FA) when compared to females. Additionally, righthanders differed from left-handed subjects, the former showing bilaterally higher anisotropy. Using a voxel-based morphometric approach to DTI data, Büchel et al. [2004] compared left- and right-handed subjects, but did not find differences between

groups in cingulate white matter. Therefore, further research is needed to clarify the effects of handedness and gender on MCB characteristics. Whether there is an interaction of gender and handedness has yet to be determined, since Gong et al. assessed relevant effects in two separate samples.

Further support for hemispheric differences in the MCB stems from studies on patients with schizophrenia. When side differences were examined, higher white matter anisotropy in the left as compared to the right midcingulum fibers was found in controls and in patients [e.g., Kubicki et al., 2003; Park et al., 2004; Wang et al., 2004]. These studies also observed a bilateral reduction in anisotropy and a diminished asymmetry in patients. Altered patterns of neuroanatomical properties of the MCB in schizophrenics as measured by DTI argue in favor of a general functional relevance. Moreover, Nestor et al. [2004], using DTI to control for possible confounding with callosal fibers, found a substantial association between executive functions related to performance monitoring and volume of the left but not the right MCB.

Although the MCB presumably is of utter importance for cognitive functioning, relatively little is known about morphologic characteristics of this fasciculus. The lack of obvious boundaries between fibers of the MCB and other tracts in conventional structural MRI might be one reason. This problem can be solved by use of DTI. We combined both structural MRI and DTI to avoid confounding of cingulate and callosal fibers in the midcingulate region. Measures of mean molecular displacement and diffusional anisotropy were derived to assess the microstructural attributes of the MCB. Microstructural and volumetric properties were evaluated with respect to hemispheric differences as well as effects of gender and handedness.

METHODS

Subjects

A total of 89 female and male students from the University of Trier (Germany) underwent the scanning protocol as described later. To assess the effects of handedness, nominal left- and right-handed persons were recruited. Written informed consent was obtained from all subjects prior to study participation. Nominal handedness was validated using a modified version of the Edinburgh Handedness Inventory [EHI; Oldfield, 1971; for a detailed description of modifications see Sequeira et al., 2006] in which hand preference was assessed for nine relevant activities. For each item, hand preference was recorded as always left, mostly left, either, mostly right, and always right. The answers were scored from -2 for "always left" to +2 for "always right," yielding a total score which varied from -18 to +18. Mean EHI scores and their standard deviations were as follows: Lefthanders = -14.22 (± 4.11); Righthanders = 16.15 (± 3.18). Data sets showing an intolerable degree of MRI-related artifacts were excluded from

the analysis, as were participants showing noncorrespondence of psychometric and nominal hand preference (2 cases only). Divergent information regarding the subjects' handedness could indicate an ambiguous laterality in this respect and might dilute the distinction between right- and left-handed participants. As queried by self reports, none of the participants indicated a history of neurological or psychiatric disorders. The final sample consisted of 45 left- (24 females, 21 males) and 34 righthanders (17 females, 17 males). The age of the subjects ranged from 19 to 34 years (mean 23.8 ± 3.45 years) and did not differ significantly between subgroups.

MRI Protocol

All scans were performed on a 1.5 Tesla Philips Gyroscan Intera system (Philips, Netherlands). Movement-related artifacts were minimized by stabilization of the participant's head with foam cushions and an elastic forehead strap. Anatomical images were obtained using a Fast Field Echo sequence. In total, 160 contiguous T1-weighted ($TR = 11.64$ msec, $TE = 3.3$ msec) 1 mm thick slices were collected in the axial plane. Given a congruent field of view (FOV; 256×256 mm) and in plane matrix (256×256), resulting voxels were isotropic ($1 \times 1 \times 1$ mm). Diffusion-weighted images were acquired with a multishot echo-planar imaging sequence ($TE = 98$ msec) in six non-collinear directions (weighting factor: $b \approx 600$ sec/mm²). Thirty-five slices (3 mm thickness, no gap) were obtained in the axial plane. The recording matrix was defined as 128×128 with a field of view of 200×200 mm. In addition, a reference image ($b = 0$ sec/mm²; B0-image) was recorded. Motion-related artifacts were reduced via cardiac triggering and navigator echo motion correction.

DTI Parametrization

Postprocessing of the images was conducted via routines written in MATLAB (Version 6.1, The MathWorks, Natick, USA). First, the six independent elements of the diffusion tensor were determined using multivariate linear least square estimation. The three eigenvectors and the corresponding eigenvalues were then obtained through diagonalization of the diffusion tensor. To evaluate the microstructural characteristics of the MCB, measures of mean diffusion (MD) and FA were calculated voxelwise [Le Bihan et al., 2001]. MD represents the mean of the three eigenvalues, with lower scores indicating a lower degree of overall diffusion. To characterize the anisotropy of molecular displacement, FA maps were obtained according to Basser and Pierpaoli [1996]. FA measures the fraction of diffusional processes that can be described as anisotropic (Brownian motion is higher in one as compared to the other directions). FA ranges from 0 to 1, where the latter would indicate perfect anisotropy. Because FA demonstrated lower sensitivity to noise in several studies, it was

given preference over other indices of anisotropy [e.g., Hasan et al., 2004].

Midcingulum Bundle Extraction and Statistical Analysis

Using the algorithms as implemented in SPM2 (Wellcome Institute, University College, London, UK), parameters for the coregistration of MD and FA maps to the anatomical images were estimated from the B0-images and subsequently applied. This operation was repeated for those ADC (apparent diffusion coefficient) maps displaying diffusional processes in an anterior-to-posterior direction (ADC_{a-p}). Importantly, voxels covering regions where maximal diffusion anisotropy is expected in a direction perpendicular to ADC_{a-p} will show extreme low intensity values. This is exactly the case with voxels containing callosal fibers. Using these coregistration procedures, all images were finally represented in native space with a resulting voxel size of $1 \times 1 \times 1$ mm. Although some images were interpolated to a higher degree of resolution, given the nature of this calculation no spurious information is added to the parameter maps. White matter maps were computed from anatomical T1-weighted images using the segmentation algorithm as implemented in SPM2.

To prevent confounding of cingulate and callosal fibers and to restrict the analysis to white matter, FA and MD images were overlaid with thresholded white matter as well as ADC_{a-p} maps. Specifically, voxels with a low white matter probability (<0.8) were excluded from the analysis as were voxels exhibiting a low coefficient in images sensitized to diffusional processes in an anterior-to-posterior direction (<225 ; according to $\sim 20\%$ of average ventricular image intensity). Voxels surviving this procedure (a) have a high probability of displaying white matter only, and (b) do not include fiber tracts with a course from left to right (as those of the corpus callosum).

Finally, a region of interest (ROI) approximating the MCB was defined on an average of the normalized T1-weighted images, with a rostral border above the most anterior extension of the genu and a posterior delineation at about 10 mm caudally to a line perpendicular to the AC-PC plane and passing through the anterior commissure. Dorsal and ventral delineations were defined by the upmost position of the paracingulate sulcus found in these subjects and the undermost callosal sulcus, respectively. These areas were marked on eight consecutive sagittal slices in each hemisphere leaving only the midsagittal slice out and leading to symmetric ROIs for the left and right fasciculi of the midcingulate region. Inverted deformation fields were computed from the spatial normalization parameters and subsequently applied to these ROIs. Finally, these modulated ROI-images were dichotomized resulting in binary representations of ROIs in the same native space as the anatomical images. At last, the ROIs were applied to the parameter maps, which had been processed as

Figure 1.

Extracted MCBs. Depicted are the automatically generated regions (green) of the left and right MCBs of a subject as resulting from the extraction procedure. For illustration purposes these extractions are overlaid on the corresponding segmented white matter images.

described in the previous paragraph, thereby excising those voxels belonging to the bundle underlying the MCC.

Estimates for MCB volumes of the left and right ROIs were computed by counting the number of voxels which survived the thresholding steps as described earlier. An example for a by this means extracted MCB is given in Figure 1. Arithmetic means of MD and FA were calculated from the processed parameter maps. The approach taken here is comparable to the frequently applied method of segmentation by manual drawing of ROIs on color-coded eigenvector maps [e.g., Kubicki et al., 2003; Wang et al., 2004] with both methods taking advantage of the direction-specific tensor information. Nevertheless, the here presented approach for automatic segmentation is not prone to examiner induced variability and has already successfully been applied to study asymmetries of the corticospinal tract [Westerhausen et al., 2007].

To derive estimates of brain volume for additional inclusion in the statistical analyses as covariate, T1-weighted anatomical images were first normalized and segmented. Then, white and gray matter images were masked to retain the hemispheres while excluding the cerebellum and brainstem. Using the parameters from spatial normalization inverted deformation fields were computed and applied to the masked and normalized images. This procedure resulted in representations of the segmented hemispheres in native space. Bilateral hemispheric volume was calculated by summation of gray and white matter voxels.

The statistical analyses were done by repeated measures univariate and multivariate analysis of variance (ANOVA, MANOVA) for the volumetric and microstructural parameters, respectively. In addition to the MANOVA, univariate tests were also computed for MD and FA. To quantify the size of an effect the proportion of systematic or explained variance (ω^2) was calculated. This coefficient spans the range between zero and one.

Figure 2.

MCB volume: effects of gender and hemisphere. Means and standard deviations of left and right midcingulum bundle (MCB) volumes for males and females. Units are number of voxels ($1 \times 1 \times 1$ mm).

RESULTS

Midcingulum Bundle Volume

A repeated measures ANOVA was computed to examine the impact of hemisphere (left/right), handedness (left-/right-handers), and gender (female/male) on MCB volumes. A statistically significant effect of hemisphere emerged ($F_{(1,75)} = 5.57$, $P < 0.03$). The left MCB showed a larger volume as compared to the right. Furthermore, the MCB in male subjects was significantly larger than in female participants ($F_{(1,75)} = 9.94$, $P < 0.01$). Figure 2 shows means and standard deviations for the MCB volumes. After entering brain volumes as covariate to the statistical analysis neither main effects nor interactions did reach statistical significance. As is to be expected, the covariate significantly altered the pattern of statistical results ($F_{(1,74)} = 25.29$, $P < 0.001$, $\omega^2 = 0.23$). Effect sizes for the

TABLE I. Effect size estimates for midcingulum bundle microstructure

Comparison	Effect sizes					
	Vol	MD	FA	cVol	cMD	cFA
Hem	0.03	0.00	0.55	0.00	0.00	0.00
Hand	0.00	0.00	0.00	0.00	0.00	0.00
Sex	0.10	0.00	0.22	0.00	0.05	0.14
Hem $\times$ Hand	0.02	0.00	0.00	0.01	0.00	0.00
Hem $\times$ Sex	0.00	0.01	0.00	0.00	0.01	0.00
Hand $\times$ Sex	0.00	0.00	0.00	0.00	0.01	0.01
Hem $\times$ Hand $\times$ Sex	0.02	0.00	0.00	0.01	0.00	0.00

Vol, volume; MD, mean diffusion; FA, fractional anisotropy; c, corrected for brain volume.

Listed are effect sizes (ω^2) for each single effect as computed from univariate tests. Statistically significant effects are denoted bold.

TABLE II. Means and standard errors of MCB characteristics

			Vol		MD		FA	
Lefthanders	Male	Left	1111.90	(52.68)	0.800	(0.010)	0.657	(0.009)
		Right	991.77	(51.32)	0.799	(0.010)	0.616	(0.008)
	Female	Left	938.21	(51.52)	0.792	(0.010)	.626	(0.009)
		Right	854.52	(50.19)	0.801	(0.010)	0.584	(0.008)
Righthanders	Male	Left	1081.29	(59.93)	0.793	(0.012)	0.664	(0.010)
		Right	1133.82	(58.38)	0.790	(0.011)	0.629	(0.009)
	Female	Left	987.17	(59.93)	0.809	(0.012)	0.617	(0.010)
		Right	909.88	(58.38)	0.814	(0.011)	0.570	(0.009)
Lefthanders			974.11	(32.72)	0.798	(0.007)	0.621	(0.006)
Righthanders			1028.01	(37.63)	0.801	(0.008)	0.620	(0.007)
	Male		1079.70	(35.43)	0.795	(0.007)	0.642	(0.006)
	Female		922.45	(35.10)	0.804	(0.007)	0.598	(0.006)
		Left	1029.65	(28.08)	0.798	(0.006)	0.641	(0.005)
		Right	972.50	(27.35)	0.801	(0.005)	0.600	(0.004)

Vol, volume; MD, mean diffusion; FA, fractional anisotropy.

Shown are the mean values and their standard errors (in parentheses) for assessed macro- and microstructural features. Statistically significant differences are denoted bold (corresponding to main effects of gender and hemisphere for Vol and FA).

effects of interest are reported in Table I; means and standard deviations are given in Tables II and III.

Microstructural Characteristics of the Midcingulum Bundle

A repeated measures MANOVA with MD and FA as dependent variables was computed to assess the microstructural attributes of the MCB. Again, we tested for the impact of hemisphere (left/right), handedness (left-/right-handers), and gender (female/male). Overall, the analysis revealed statistically relevant effects of hemisphere ($F_{(2,74)} = 101.83$, $P < 0.001$) and gender ($F_{(2,74)} = 13.21$, $P < 0.001$). Additional univariate statistics indicated the FA to

be more discriminative than the MD parameter. Specifically, FA was substantially higher in the left hemisphere ($F_{(1,75)} = 197.49$, $P < 0.001$) and in male subjects ($F_{(1,75)} = 22.89$, $P < 0.001$). Means and standard deviations of FA are depicted in Figure 3. Running a repeated measures multivariate analysis of covariance (MANCOVA) and controlling for differences in brain volumes a main effect of the covariate reached statistical significance ($F_{(2,73)} = 3.89$, $P < 0.03$). This effect was more pronounced with MD as compared to FA as dependent variables ($\omega^2 = 0.06$ and $\omega^2 < 0.01$, respectively). Beyond this, a main effect of gender was observed ($F_{(2,73)} = 7.08$, $P < 0.01$). Specifically, male subjects showed lower values of MD but a higher degree of FA as compared to females as indicated by the univariate tests ($F_{(1,74)} = 5.13$, $P < 0.03$; $F_{(1,74)} = 14.27$, $P < 0.001$; test statistics for MD and FA, respectively). Effect

TABLE III. Corrected means and standard errors of MCB characteristics

			cVol		cMD		cFA	
Lefthanders	Male	Left	1035.83	(51.75)	0.791	(0.011)	0.658	(0.010)
		Right	905.68	(48.50)	0.789	(0.010)	0.617	(0.009)
	Female	Left	1030.19	(52.41)	0.803	(0.011)	0.625	(0.010)
		Right	958.59	(49.11)	0.813	(0.010)	0.583	(0.009)
Righthanders	Male	Left	977.10	(60.66)	0.780	(0.013)	0.665	(0.011)
		Right	1015.93	(56.32)	0.777	(0.012)	0.630	(0.011)
	Female	Left	1065.38	(58.15)	0.818	(0.012)	0.617	(0.011)
		Right	998.37	(54.48)	0.823	(0.012)	0.570	(0.010)
Lefthanders			982.57	(28.49)	0.799	(0.007)	0.621	(0.006)
Righthanders			1014.20	(32.82)	0.800	(0.008)	0.620	(0.007)
	Male		983.64	(36.24)	0.784	(0.008)	0.643	(0.007)
	Female		1013.14	(35.43)	0.814	(0.008)	0.599	(0.007)
		Left	1027.13	(25.65)	0.798	(0.005)	0.641	(0.005)
		Right	969.65	(24.04)	0.901	(0.005)	0.600	(0.004)

Vol, volume; MD, mean diffusion; FA, fractional anisotropy; c, statistics corrected for brain volume.

Shown are mean values and their standard errors (in parentheses) for assessed macro- and microstructural features after correction for total brain volume. Statistically significant differences are denoted bold (corresponding to main effects of gender for MD and FA).

Figure 3.

MCB anisotropy: effects of gender and hemisphere. Means and standard deviations of fractional anisotropy for both gender and hemisphere.

size measures, means and standard deviations for the statistical tests with and without brain volumes as covariate can again be found in Tables I–III.

DISCUSSION

In this study, macro- and micro-structural characteristics of the MCB were assessed by means of DTI. Hemispheric differences as well as effects of gender and handedness were evaluated. Using integrative multi-modal imaging the analysis was protected against an unwanted impact of callosal fibers. The obtained results provided evidence of substantial variation between hemispheres and gender in terms of both the volume and architecture of the MCB.

As in prior studies, the statistical analysis of the micro-structural MCB architecture points to differences between hemispheres. The finding of a higher anisotropy in the left as compared to the right MCB agrees with previous observations. This asymmetry in anisotropy of the left and right MCB has meanwhile been observed using different methodological approaches such as fiber tracking [Kubicki et al., 2003], scale-invariant parametrization [Gong et al., 2005a,b], assessment via manually drawn regions of interest [Wang et al., 2004], as well as the integration of multi-modal images. The concordance across studies suggests this finding to be valid. Whether the effect is caused by a higher degree of directional similarity, denser axonal packing or stronger myelination needs further investigation. Interestingly, the left MCB also seems to be larger than the homologue right fiber tract. This result replicates the work of Allen et al. [2003] who found this asymmetry by manually tracing the regional white matter. Recapitulating, together with the cortical and gross morphological asymmetries observed in this region [gray matter volume, fissurization etc.; Huster et al., 2007; Paus et al., 1996a,b; Vogt et al., 1995; Yücel et al., 2001, 2002], the pattern of hemi-

spheric differences in local neuroanatomy suggests a lateralization of anterior cingulate functioning.

Regarding gender-associated diversity, differences were again found concerning both the volume and microstructure of the MCB. Males showed a higher anisotropy as determined via DTI with respect to midcingulum fibers. To the authors' knowledge the study of Gong et al. [2005a] was the only previous survey that assessed gender differences in anisotropy of the cingulate bundles. While finding a trend similar in direction to the one observed in our analysis, their results did not reach statistical significance. However, the effect size of this difference between sexes cannot be classified as large and Gong et al. worked with a rather small-sized sample. This might have resulted in low statistical power and failure to achieve significance. Future studies will have to replicate and extend this outcome. A gender difference with higher regional white matter volume in males was also found by Allen et al. [2003]. Several studies on schizophrenic patients and controls draw analogous conclusions. Again, although the pattern of morphologic differences suggests functional relevance, the exact functional consequences of these variations between women and men require further clarification.

Controlling for the diversity of individual brain volumes in the statistical analyses differences between the left and the right MCBs disappeared. This holds true with respect to both volume as well as microstructural characteristics. Such results are in agreement with theories on the development of hemispheric specialization. Ringo et al. [1994] argue that larger brain size is inevitable associated with an increase in interregional transfer time which finally leads to impaired interhemispheric neuronal computation. Favoring proximal over distal connectivity is a way to circumvent such problems, thereby causing intraregional functional specialization and hemispheric asymmetry. This line of reasoning is also in accordance with studies on callosal characteristics showing a positive association between corpus callosum size and brain volume [Bermudez et al., 2001] as well as forebrain size [Jancke et al., 1997]. Furthermore, in our analyses gender differences at the microstructural level persist with strongly diminished effect sizes only, when controlling for the influence of global brain volume. Volumetric differences between male and female subjects seem to be associated to the covariate in even larger parts, as they do not achieve statistical relevance after the covariate is added. This could again be indicative of adaptive effects concerning the overall organization of the MCB to cope with the already cited obstacles associated with larger male brains.

In one of their studies, Gong et al. [2005a] reported an effect of handedness with higher anisotropy in right-handers ($\omega^2 = 0.06$). Using a voxel-based morphometric approach to FA values, Büchel et al. did not find such a difference in the MCB, nor did the present investigation. What could explain such contradictory outcomes? Varying definitions of handedness could have influenced the observed statistics. Gong et al. compared a group of

extreme righthanders to a subsample of lefthanders who were to span a wide range on the handedness continuum. In our study, the degree of manual lateralization was more variable in both handedness subgroups. This increase in representativeness of the subsamples could at the same time explain the lower effect sizes obtained here. In addition, given the estimated size of the effect in the study of Gong et al., larger sample sizes are recommended in order to have a fair chance to reliably detect such moderate or medium-sized differences. Besides, the factor sex was not analyzed very thoroughly in the study of Gong et al. because of the small sample size. The somewhat unequal distribution of gender within Gong's handedness subgroups (i.e., higher percentage of males among righthanders) might have additionally biased the results. Previous studies also failed to obtain handedness effects regarding volumetric differences of the MCB [e.g., see the classical study of Good et al., 2001]. Taken together, effects of handedness on white matter characteristics of the MCB do not seem to be very pronounced, although further studies would be welcome. Given that the MCC contains three cingulate motor areas with distinct arm representations and that handedness seems to moderate gender effects concerning the degree of regional fissurization [Huster et al., 2007; Picard and Strick, 2001], this becomes an interesting issue. The underlying notion is, whether a primary motoric phenomenon is reflected in modules subserving the integration of higher cognitive functions and motoric information regarding both regional gray and white matter characteristics [e.g., Beaton, 2003].

Another finding, although not relevant from a statistical point of view, seems noteworthy. Comparisons indicate a substantially higher volume of the MCB in the left as compared to the right hemisphere. This indeed holds true for most of the subgroups, except for the right-handed males, who show an inverted trend. Interestingly, it is this group exhibiting the most pronounced fissurizational asymmetry with a higher degree of regional midcingulate folding in the left hemisphere [Huster et al., 2007]. It was also shown that the occurrence of a gyral doubling in this region is associated with smaller intrasulcal gray matter volume in the cingulate sulcus [Paus et al., 1996a]. This raises the question whether the amount of midcingulate gray and white matter may be associated. A less pronounced cingulate gyrus, due to the common gyral doubling, is more often found in the left as compared to the right hemisphere. This phenomenon might go hand in hand with smaller MCB volumes in the left midcingulate region. However, several points advise caution. First, the ROI applied to extract the MCB was adjusted to also include white matter falling into the paracingulate region. Therefore, one would in any case have expected a more pronounced MCB in the left hemisphere in all subgroups as a second or superior section of the midcingulate bundle, underlying the paracingulate gyrus, was included in the ROI. Second, it cannot be ruled out that this effect is, at least partially, introduced by the relatively low image resolution in the axial plane. In cases of strong fissurizational asymmetry (the occur-

rence of a paracingulate sulcus in the left hemisphere) the MCB might have more reliably been extracted from the right hemisphere. However, a manual inspection of the datasets does not support this assumption. Third, analyses of the three-way interaction (hemisphere $\times$ gender $\times$ handedness) as well as for a hemispheric comparison in right-handed males reveal nonrelevant effect sizes (0.02 and 0.00, respectively). Beyond this, directly addressing possible relationships of midcingulate folding patterns (for a thorough description please refer to Huster et al., 2007) and white matter characteristics, none of the correlations reached statistical significance (max. $r = 0.25$). Further investigations of this volumetric inversion in right-handed males seem to be recommended, but the current data do not strongly support the relevance of this phenomenon.

White matter fiber tracts form the basis for functional interactions between brain regions. The MCB is strongly tied to the MCC and therefore seems to be a crucial component of a system devoted to attentional control or performance monitoring [Devinsky et al., 1995]. As stated earlier, there is ample evidence that the organization of the MCC is asymmetric [e.g., Huster et al., 2007; Paus et al., 1996a,b; Yücel et al., 2001] and the MCB seems to mirror this cortical lateralization. It is interesting, therefore, that deviations from this leftward asymmetry have been found in psychiatric disorders such as schizophrenia concerning both the gray and the white matter [e.g., Kubicki et al., 2003; Park et al., 2004; Wang et al., 2004; Yücel et al., 2002]. However, whether the effects observed in this study only globally follow cortical development and for this reason show regional hemispheric as well as gender differences as to guarantee an effective connectivity needs further investigation. Alternatively, one might think of indices of connectivity, as represented by macro- and microstructural white matter characteristics, as another and partly independent factor contributing to a brain-system's functioning. Current studies do not help to unambiguously clarify this issue as they addressed structure-function associations for the midcingulate region concerning predominantly either the cortex or the cingulum bundle [e.g., Fornito et al., 2004; O'Sullivan et al., 2005; Pujol et al., 2002].

In this study an approach of integrative multi-modal imaging was applied to derive estimates of MCB volumes of both hemispheres as well as their microstructural characteristics. Care has to be taken not to over-interpret the data especially regarding the volumetric measures. It is recommended to construe these estimates as relative rather than true or absolute values. This approach for sure is valid concerning the inference of differences between groups of interest, but likely is not suited to draw strong conclusions concerning single subjects. Large sample sizes also foster group comparisons as to increased statistical power and representativeness of samples. Although methods such as fiber tracking may promise to derive more specific estimates of a tracts neuroanatomy, from a general scientific point of view converging results obtained from differing methods are more trustable as they do not leave

us blind to shortcomings of a single and perhaps exclusively applied strategy of analysis. However, the technical settings utilized for this study (diffusion process measured in only 6 directions and $b \approx 600 \text{ s/mm}^2$) could be improved in future surveys. More recent studies should utilize measurements with higher b values and in more directions to minimize sampling direction biases and to increase the signal to noise ratio. Nevertheless, a systematic effect associated with these settings and hampering our conclusions certainly is not to be expected.

Summarizing, evidence was found indicating macro- and microstructural differences between the left and right MCB. In light of studies focusing on other neuroanatomical aspects, a lateralization of midcingulate functioning can be assumed. The obtained results also suggest that male and female subjects may vary with respect to functional aspects, given the marked differences in volumetric and microstructural characteristics of the MCB as measured with DTI methods. However, these gender-related effects also seem to be substantially associated with differences in brain volumes. Overall, the pattern of observations may reflect a higher functional connectivity of the left MCB and in males as compared to females.

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