Callosal dysgenesis (CD) is observed in many neurodevelopmental conditions, but its subjacent mechanisms are unknown, despite extensive research on animals. Here we employ magnetic resonance diffusion tensor imaging and tractography in human CD to reveal the aberrant circuitry of these brains. We searched particularly for evidence of plasticity. Four main findings are described—1) in the presence of a callosal remnant or a hypoplastic corpus callosum (CC), fibers therein largely connect the expected neocortical regions; 2) callosal remnants and hypoplastic CCs display a fiber topography similar to normal; 3) at least 2 long abnormal tracts are formed in patients with defective CC: the well-known Probst bundle (PB) and a so far unknown sigmoid, asymmetrical aberrant bundle connecting the frontal lobe with the contralateral occipitoparietal cortex; and 4) whereas the PB is topographically organized and has an ipsilateral U-connectivity, the sigmoid bundle is a long, heterotopic commissural tract. These observations suggest that when the developing human brain is confronted with factors that hamper CC fibers to cross the midline, some properties of the miswired fibers are maintained (such as side-by-side topography), whereas others are dramatically changed, leading to the formation of grossly abnormal white matter tracts.

Keywords: callosal agenesis, callosal development, corpus callosum, cortex development, cortical commissures, DTI

Introduction

Plasticity has been recognized as a fundamental and universal property of the nervous tissue, capable of providing changes to its structure and function in response to environmental challenges, from invertebrates to mammals including humans. Plastic changes can vary widely, from subtle modulations of synaptic transmission (Blitz and others 2004; Nordeen KW and Nordeen EJ 2004) to gross displacements of functional areas in the brain (Elbert and others 2002), as much as the environmental changes that provoke them, which can vary from subtle sensory events to large, destructive lesions of the nervous system. In general, it is believed that younger mammals are more susceptible to structural reorganization of a compensatory nature (Kennard 1942), such as neuronal proliferation, axonal (re)growth, and circuit reconstruction (Clowry and others 2004; Maffei and others 2004; Feller and Scanziani 2005), whereas older animals have their plastic possibilities restricted to the synaptic level (Froemke and others 2005), although there are documented exceptions to this “Kennard principle” (Schneider 1979; Arnold and others 2005; Talby and others 2005).

Clinicopathological observations suggest that the immature human brain is capable of major structural and functional reorganization, as exemplified by the excellent recovery of children subjected to extensive removal of one hemisphere as the last therapeutic resource to control epilepsy that is otherwise intractable (Bernasconi and others 2000; Villablanca and Hovda 2000). Some children born without a corpus callosum (CC) may represent another example of this remarkable plastic property of the brain as they lack the interhemispheric disconnection syndrome that is typical of split-brain adults (Sperry 1970).

Because the CC is the major commissural fiber bundle in the human brain, investigating the reorganization of white matter in different forms of isolated callosal dysgenesis (CD) provides a unique window to understand human neuroplasticity. CD can appear as 1) a partial defect of the CC (e.g., lack of the body and splenium, with the presence of a rostral remnant), 2) hypoplasia (homogeneous reduction of the callosal size), or 3) a complete lack of the commissure (agenesis). The first description of CD was made by Reil (1812), and the first evidence for plastic reorganization of callosal fibers was provided by Probst (1901), who recognized the aberrant longitudinal bundle of fibers that was named after him (Probst bundle [PB]). CD can present as an isolated abnormality, or may come associated with other lesions or malformations. The result is a wide spectrum of clinical features (Lassonde and others 2003), from a complete lack of recognizable symptoms to severe mental retardation, language deficits, and motor impairment. CD has been described in association with more than 50 different metabolic and genetic disorders of the central nervous system (Richards and others 2004), although its molecular and cellular causes remain largely unknown.

Whereas earlier cases were only identified postmortem, with the introduction of computerized tomography (CT) and magnetic resonance imaging (MRI), most cases are now diagnosed directly in vivo (CT: Meyer, Röricht, and Woiciekowsky 1998; MRI: Kuker and others 2003). In virtually all the cases documented by neuroimaging techniques, the PBs are the only morphological indication that plasticity of cortical circuits may have occurred during development, despite the fact that their functional role, either compensatory or maladaptive, has not been revealed so far. The trajectory of some of the Probst fibers was studied in animal models (Ozaki and others 1987, 1989; Ozaki and Shimada 1988), but the precise topography and function of these fibers in humans remain unknown.

Until very recently, noninvasive neuroimaging techniques (including conventional MRI) had very limited power to characterize white matter structures in humans. In the past decade, new magnetic resonance (MR) pulse sequences had a major and increasing impact on the assessment and management of many neurological diseases. More recently, the development of diffusion tensor imaging (DTI) allowed the estimation of vector fields describing the directional diffusivity of water molecules...
in the living human brain. DTI describes the anisotropic properties of water diffusion on a voxel basis, thus revealing important details of fiber tract orientation. Diffusion tensor can thus be used to reconstruct the trajectories of major fiber systems in 3-dimensional spaces (Shrager and Basser 1998; Basser and others 2000). Computer graphics-based renderings of these reconstructed “fiber tracts” have been termed MR “tractography.” The rapid progress of MR hardware technology (powerful and stable gradients) together with important advances in pulse sequence designs has pushed DTI into the clinical neuroscience arena. DTI scanning is now feasible in most clinical conditions, both in adults and in children (Albayram and others 2002).

A recent DTI/tractography short study of 4 cases of CD (Lee and others 2004) was able to show abnormalities in white matter structure in this condition. These preliminary findings encourage a more thorough and detailed use of DTI for the investigation of specific patterns of white matter developmental changes in CD. Here we employ DTI/tractography to characterize the white matter structural reorganization in patients with different types of CD in vivo, showing that developmental plasticity in humans may produce major reorganization of great tracts in the brain. We aimed at 1) comparing the topographic arrangement of PBs and their putative connectivity with those of the CC of normal subjects, 2) evaluating the trajectory and connectivity patterns of callosal fibers that cross through the remnant callosum in partial dysgenesis, and 3) searching for evidence of additional aberrant circuits.

Our results provide evidence that when the developing human brain is confronted with factors that hamper CC fibers to cross the midline, some properties of the miswired fibers are maintained (such as side-by-side topography), whereas others are dramatically changed, leading to the formation of grossly abnormal white matter tracts.

### Materials and Methods

#### Patients

Eleven patients aged from 1 to 33 years and with different types of CD were analyzed: 3 with callosal agenesis (complete lack of the CC), 3 with callosal hypoplasia, and 5 with partial CD. None had contraindications for MRI. An experienced neurologist performed clinical evaluations. Patients’ characteristics are summarized in Figure 1. Ten individuals with no evidence of neurological disease served as normal controls. Written informed consent was obtained from the patients or their parents. All procedures were approved by the Ethics Committee of our institution and performed according to international regulations (Declaration of Helsinki 2000).

#### Neuroimaging

Anatomical images were obtained with a 1.5-T Philips-Intera scanner, using the following pulse sequences: spin-echo $T_1$ weighted ($TR/TE/matrix/field of view [FOV] = 550 ms/20 ms/256 × 256 × 256/240 mm$), turbo spin-echo $T_2$ weighted ($TR/TE/matrix/FOV = 3500 ms/90 ms/256 × 256 × 256 mm$), inversion recovery $T_1$ weighted ($TR/TE/FOV = 3000 ms/30 ms/256 × 256 mm$), and fluid-attenuated inversion recovery ($TR/TE/TI/FOV = 9000 ms/100 ms/2300 ms/256 × 256 × 256 mm$), all with a slice thickness of 5 mm without gap.

### Table 1

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (years)</th>
<th>Psycho-\ mental abnormalities</th>
<th>Type of dysgenesis</th>
<th>Probst bundles</th>
<th>Cingulate gyrus</th>
<th>Lateral ventricles</th>
<th>Other brain abnormalities</th>
<th>Eroticranial abnormalities</th>
<th>Anatomical Images</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case1</td>
<td>M</td>
<td>25</td>
<td>Partial/genu remnant</td>
<td>Present</td>
<td>Inverted</td>
<td>Parallel Colpo-cephalus</td>
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<tr>
<td>Case2</td>
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<td>26</td>
<td>Partial/genu remnant</td>
<td>Present</td>
<td>Inverted</td>
<td>Parallel Colpo-cephalus</td>
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<td>None</td>
<td></td>
</tr>
<tr>
<td>Case3</td>
<td>M</td>
<td>33</td>
<td>Partial/genu remnant</td>
<td>Present</td>
<td>Inverted</td>
<td>Parallel Colpo-cephalus</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Case4</td>
<td>F</td>
<td>10</td>
<td>Mild mental retardation</td>
<td>Undiscussable from cingulate</td>
<td>Inverted</td>
<td>Parallel Colpo-cephalus</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Case5</td>
<td>M</td>
<td>8</td>
<td>Mild mental retardation</td>
<td>Undiscussable from cingulate</td>
<td>Inverted</td>
<td>Parallel Colpo-cephalus</td>
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<td>None</td>
<td></td>
</tr>
<tr>
<td>Case6</td>
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<td>2</td>
<td>Autism</td>
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<td></td>
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<tr>
<td>Case7</td>
<td>M</td>
<td>1</td>
<td>Moderate to severe fascic paraparesis</td>
<td>Present</td>
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<td>Parallel Colpo-cephalus</td>
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<tr>
<td>Case8</td>
<td>CFS</td>
<td>7</td>
<td>Psycho-motor retardation</td>
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<td>Not identified</td>
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<td>None</td>
<td></td>
</tr>
<tr>
<td>Case9</td>
<td>KCA</td>
<td>7</td>
<td>Severe mental retardation</td>
<td>Present</td>
<td>Not identified</td>
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<tr>
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<td>5</td>
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<td>None</td>
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</tr>
</tbody>
</table>

Figure 1. Clinical characteristics and MRI findings in studied patients.
Diffusion-weighted images were acquired in axial and sagittal planes with single-shot, spin-echo echo-planar sequences—1) axial: TR/TE = 4000/110 ms, FOV = 256 mm, matrix = 112 x 128, slice thickness = 5 mm without gap and 2) sagittal: TR/TE = 4491/121 ms, FOV = 256 mm, matrix = 112 x 128, slice thickness = 5 mm without gap. Diffusion sensitization gradients were applied in 6 noncollinear directions (x, y, z, xz, yz, xz), with a b factor of 800 s/mm². Diffusion tensor data were transferred and postprocessed using a software written in Interactive Data Language, Philips Research Integrated Development Environment software (IDDE research platform, FiberTrack 4.1). The diffusion tensor data set for each voxel was generated including eigenvalues and eigenvectors using multivariate fitting. Fractional anisotropy (FA) maps and color-coded FA maps for fiber direction were produced according to procedures described in detail by Pajevic and Pierpaoli (1999). Fiber tracking (tractography) was performed using a technique known as the Fiber Assignment by Continuous Tracking (Mori and others 1999; Xue and others 1999). For the purpose of the present paper, the white matter was identified in the anatomical images and then in the color-coded FA maps. Both the anatomical images and the FA color-coded maps were used to guide the proper placement of 2-dimensional regions-of-interest (ROIs) in order to reconstruct the 3-dimensional fiber bundles. Fiber tracking was accomplished by manually specifying ROIs over the trajectory of 1) the callosal remnant, 2) the PB, 3) the cingulate bundle, and 4) additional white matter locations emerging from this analysis. We further employed multiple ROIs in different locations both ipsi- and contralaterally in patients and controls to exclude "skipping-tract" artifacts (Pierpaoli and others 2001; Mori and van Zijl 2002; Lazar and Alexander 2003; Huang and others 2004). Equivalent ROIs were specified for control subjects. For precise localization, all the ROIs were placed on the color-coded FA maps and on the corresponding axial, coronal, and sagittal anatomical images. Automatic tracking of fibers was performed with a marching algorithm, restricting fiber tracking to voxels with a minimum FA of 0.30. Fiber deflection threshold was set to 0.85 (corresponding to 45° for the angle between 2 "eigenvectors" in contiguous voxels). These procedures were repeated independently by 2 investigators.

Placement of ROIs

To trace the fibers crossing at the CC, a polygonal ROI was drawn in the midsagittal plane of each control subject, encompassing the entire CC. In order to detect a rostrocaudal topography of the callosal fibers, 5 equally spaced ROIs with equivalent areas were placed along the CC in the midsagittal plane of each control subject (Fig. 3, Panel 1A). To investigate the existence of a dorsoventral callosal topography, 5 triplets of contiguous ROIs were defined dorsoventrally, as shown in Figure 3, Panel 1B. A similar strategy was used to uncover fiber topography in callosal hypoplasia. In order to determine the connectivity of the callosal remnant in each patient with partial CD, single irregular polygonal ROIs were drawn encompassing the entire remnant area at the midsagittal plane (Fig. 3, Panel 2A). PBs were identified in the anatomical images and in the color-coded FA maps of 5 patients. Directional color-coded FA images in a coronal plane 6 mm posterior to the anterior commissure were used as references to place the ROIs designed to trace both PBs of each patient. A similar procedure was used to trace the cingulate bundles in both patients and control groups. To confirm the asymmetry of the aberrant bundles that were found crossing through the callosal remnants, 2 ROIs were positioned in different places of their trajectory in the contralateral hemisphere (Fig. 5, Panel J), and the FA threshold was decreased as low as 0.05, along with a lower deflection threshold of 0.60. Using the same procedure in the control subjects (Fig. 5, Panel 2) and observing that no tracts could be reconstructed, it was confirmed that the presence of asymmetrical aberrant bundles could not be attributed to reconstruction artifacts.

To characterize the topographic organization of the PB, each bundle was divided into 4 quadrants (as shown in Fig. 4, Panel 2D) at the coronal plane in the color-coded FA maps. Each quadrant was treated as a separate ROI for tracing fibers in an equivalent number of seed points. In one patient (Case 5, Fig. 2D), PBs were identified but could not be discerned from the adjacent cingulate bundles based on anatomical or color-coded FA map. A single thickened bundle was observed in each hemisphere, possibly containing both cingulate and PB fibers. These thick bundles were then arbitrarily divided into lateral and medial regions at the coronal plane in the color-coded FA map. In each region, an equivalent number of seed points were placed to trace the fibers, transversally to the medial and lateral regions.

Because there is controversial evidence concerning the occurrence of plasticity in the anterior commissure of CD animals and humans, we tried to place ROIs in this structure at the sagittal plane to clarify this issue. However, technical limitations of the DTI acquisition (slice thickness and resolution) precluded reliable assessment of the anterior commissure in the present study.

Results

Conventional Imaging

Conventional MRI (i.e., anatomical images) showed the typical anatomical features of CD, including parallel, enlarged lateral ventricles, downward displacement of the cingulate gyrus, radial sulci at the median brain surface, and PBs. The main clinical characteristics and conventional MRI findings of all patients are listed in Figure 1, and the basic anatomical types are exemplified in Figure 2.

Fiber Organization of Normal CC

Anatomical and recent DTI fiber-tracking studies have shown a rostrocaudal topographic organization of callosal fibers in normal individuals (de Lacoste and others 1985; Xu and others 2002; Abe and others 2004). However, the fiber organization of callosal remnants in CD is largely unknown. Our findings in normal individuals confirmed the rostrocaudal topographic organization of normal CC fibers as described in the previous studies, with frontal fibers crossing through the genu, parietal fibers mostly through the body, and occipital fibers through the splenium (Fig. 3, Panel 1A,1C,1D). We also demonstrated an evident dorsoventral fiber distribution pattern within the normal CC: fibers derived from medial cortical sectors cross dorsally in the CC, whereas those originated in dorsolateral sectors of the cortex were positioned ventrally in the CC (Fig. 3, Panel 1B,1E,1G).

Topographic Organization in Callosal Remnants and Hypoplastic Tracts

Radiologists and pathologists usually assume that callosal remnants situated rostrally correspond to the genu, whereas caudal remnants correspond to the splenium. However, this assumption remains unproven, and remnants might simply represent other segments of the CC, mechanically displaced under abnormal conditions. Moreover, the connectivity pattern established by fibers that make up the CC remnants could be accounted for by at least 2 alternative possibilities: either they would contain "compressed" tracts with an expanded connectivity encompassing large extents of cortex or their fibers would keep their connection fields restricted to specific sectors of the cortex as occurs in the normal CC. We found that fibers of rostral remnants connect the rostral prefrontal cortex of both hemispheres (Fig. 3, Panel 2A-D), confirming that they indeed represent the genu in these patients, rather than fibers with enlarged projection fields. A "tail" of fibers projecting backward at the left side can be observed consistently in many of these cases (arrow in Fig. 3, Panel 2) and will be described below.

Another important issue concerns the possible preservation of topography within the remnant or within a hypoplastic callosum. Do the fibers therein conserve their overall topographic relations as if they were comprised within a normal callosum? We found a consistent rostrocaudal and dorsoventral distribution
of fibers in all patients with either partial CD or hypoplasia that agrees with the topography seen in the normal individuals (Fig. 4, Panel 1A–C). This finding indicates that the topographic rules orienting the rostrocaudal arrangement of the remaining callosal fibers are preserved despite the developmental defects that hindered commissuration of a greater number of fibers.

Developmental Plasticity of Cortical Fibers in CD
Aberrant fibers forming the PBs were traced in 5 subjects, 2 of them with CC agenesis and 3 with partial dysgenesis. We first posed the question whether the fibers within the PB displayed a pattern of connectivity in any way similar to the normal callosal fibers. Second, we wanted to know whether Probst fibers conserved a topographic organization consistent with the callosal fibers that formed it. We found that Probst fibers leave the bundle at successive rostrocaudal levels and deflect dorsally or ventrally toward the cortical gray matter (Fig. 4, Panel 1F–H, 2A–C, fibers in purple), creating a multiple U-system of longitudinal connections in the cortex, similar to the pattern described in acallosal mice by Wahlsten and his collaborators (Ozaki and others 1987, 1989; Ozaki and Shimada 1988). This multiple U-arrangement differed clearly from the long, rostrocaudal pattern typical of the cingulate bundle (shown in yellow in Fig. 4, Panel 1F–H, 2A–C).

In addition, by employing a set of small ROIs positioned within the PB (Fig. 4, Panel 2D), we were able to demonstrate that it presented a consistent, topographic, and spatial organization of fiber trajectories (Fig. 4, Panel 2E–F) that was maintained within the PB as well as in the cortical white matter after leaving the bundle. Thus, PB fibers positioned ventrally exhibited a longer, longitudinal trajectory and connected the parietooccipital regions with more anterior sectors of the frontal lobe, whereas those situated dorsally in the PB were more arched, U-shaped, and projecting to dorsal cortical sectors in the same hemisphere.

The PB could not be individualized in some of the patients with callosal hypoplasia (Case 5, Fig. 1). Instead, a single thick, longitudinal bundle was observed in each hemisphere in the conventional anatomical images and also in the FA and color-coded FA maps (Fig. 2B). We wondered whether this thick bundle contained both the cingulum and the PB and whether the corresponding fibers were segregated or mixed therein. These issues were addressed by arbitrarily dividing this thick bundle into lateral and medial sectors and placing an equivalent number of seed points in each sector (Fig. 4, Panel 1D). Fibers with trajectories matching those described above for the PB and the cingulum were identified, indicating that these 2 bundles were adjacent in these patient (Fig. 4, Panel 1F–H), although a morphological septum between them was not apparent, as in all other cases. We could note that the lateral fibers exhibited the U-arrangement of the PB as described above and that the medial fibers showed trajectories that are more longitudinal and ventral, thus compatible with the cingulate bundles.

A Hitherto Undescribed Aberrant Tract in CD
The neurological literature attributes a large variability of symptoms to patients with CD, from mental retardation to mild intelligence quotient subnormality and from aphasia to
hyperactivity (Lassonde and others 2003). This extensive variability suggests that a number of largely unknown neuroplastic events may take place in dyscallosal brains. We had observed a caudal tail of fibers when tracing the connections of the callosal remnant (see Fig. 3, Panel 2) and hypothesized that this could be an aberrant callosal tract that could be distinguished from PB. We tested this hypothesis 1) by placing of a single ROI at the genual callosal remnant, at the midsagittal plane, and 2) by using a combination of 2 strategically positioned ROIs, one at the callosal remnant and the other more laterally, in the coronal plane, approximately 2–4 cm posterior to the anterior commissure (Fig. 5, Panel 1BE–F). These analyses revealed a so far unknown asymmetric, abnormal tract in 4 CD patients (Fig. 3, Panel 2 and Fig. 5, Panel 1B–D). This tract followed a sigmoid trajectory beneath the PB, connecting the left parietooccipital region with the contralateral frontal pole, through the genu callosi and PB (patients 1 and 2) or through the hypoplastic body (patients 4 and 7). The “sigmoid aberrant bundle” was asymmetrical (Fig. 5, Panel 1F) because it consistently connected the right prefrontal cortex with the left occipital cortex but was absent (patients 1, 2, and 7) or very small in the mirror-symmetric configuration in one case with hypoplastic CC (patient 4). To exclude the possibility of an artifact (“kissing” or “skipping” fibers), we placed an equivalent ROI in the callosal genu of 10 normal controls (Fig. 5, Panel 2A–C). Only the regular, homotopic callosal connections were reconstructed (Fig. 5, Panel 2D), even when very low FA threshold (0.05) was adopted. Using the 2-ROI procedure, similar thresholds and even larger ROIs at the coronal plane, no tracts linking the callosal genu and parietooccipital regions were identified. We concluded that the sigmoid aberrant bundle is a real anatomical entity formed in CD subjects, suggesting that the brain of these subjects can undergo extensive rewiring, including the formation of long, massive, aberrant tracts connecting cortical regions located far apart.

Discussion
The CC is the major commissural fiber bundle in the human brain. In adults, its damage or surgical transection leads to the
classical interhemispheric disconnection syndrome (Sperry 1970), which is usually not observed when the CC is congenitally absent (Lassonde and others 1991). However, patients with CD even without other anatomical anomalies can show variable clinical presentations, ranging from no symptoms at all to severe cognitive impairment (Lassonde and others 2003). Remarkably, this clinical variability is poorly correlated with gross anatomical features, suggesting the existence of major changes at a finer level. Although CD was extensively explored in animals, most studies in humans have been limited to postmortem anatomical descriptions or conventional MRI. DTI/tractography now provides a powerful tool to characterize finer changes in white matter structure in vivo and, as observed in our cases, indicates the occurrence of plastic events leading to the formation of aberrant tracts in the brain.

We have reported 4 basic findings in patients with CD—1) when there is a rostral callosal remnant, fibers therein do not expand their target territory in the neocortex; instead, they connect the rostral regions of the frontal lobes as genual fibers normally would; a similar conclusion holds for cases with a hypoplastic CC; 2) callosal remnants and hypoplastic CC display a fiber topography reminiscent of normal CC; 3) at least 2 long abnormal tracts are formed in patients with defective CC: the long-time known PB and a sigmoid, asymmetrical aberrant bundle connecting the right frontal lobe with the contralateral occipitoparietal cortex; and 4) the PB maintains a topographic organization, albeit ipsilateral, as callosal fibers would normally do contralaterally; the sigmoid bundle, on the other hand, is a heterotopic commissural tract about which no topographic or functional information is available. These observations suggest that when the human brain is confronted with factors that hamper CC fibers to cross the midline normally during development, some properties of the miswired fibers are maintained (such as a side-by-side topographic organization), whereas others are dramatically changed, leading to the formation of grossly aberrant white matter tracts.

The Developmental Framework for Callosal Plasticity

During development, callosal axons grow over long distances to reach their final targets. Their first task after emerging from the somata is to grow toward the white matter, what they seem to do following polarity cues intrinsic to the neuron (Polleux and
(Serafini and others 1996; Bagnard and others 1998; Hu and others 2003; Uziel and others 2003). Close to the midline, the prospective callosal axons enter a tunnel of glial cells that secrete repulsive molecules (e.g., Slit-2), channeling them toward the opposite hemisphere (Shu and Richards 2001; Richards 2002; Richards and others 2004; Lent and others 2005). Actual pathfinding in search of the midline seems to be performed only by pioneering axons originating in the cingulate cortex (Koester and O’Leary others 1998), and extrinsic gradients of diffusible molecules (semaphorins, ephrins, netrin) that attract them toward the ventricular layer and then make them deflect or bifurcate subventricularly taking a medial direction (Shu and Richards 2001; Richards 2002; Richards and others 2004; Lent and others 2005).

Figure 5. Panel 1: The aberrant sigmoid bundle (SIG) in a CD case. (A) shows the transverse planes illustrated in (B–D) from ventral to dorsal. The aberrant SIG was seeded by the 2 ROIs in (B) and is shown to connect the right frontal pole with the left parietooccipital cortex by way of the anterior callosal remnant. The bundle seems to overlap the ventricle because it is slightly curved upward out of the background plane. The 2 effective ROIs in (B) are also shown over the FA sagittal (E) and coronal (F) maps. Larger ROIs positioned at mirror-symmetric locations with FA threshold as low as 0.05 (G, H) fail to reconstruct a contralateral bundle, demonstrating its asymmetric nature and discarding the possibility of a skipping-tract artifact. Panels (I) and (J) show that the SIG (in green) is segregated from the PB (in purple). (I) is a transverse plane, whereas (J) is a coronal plane slightly tilted around a vertical axis. Panel 2: Connectivity of genual fibers in a control case, showing no caudal fibers emerging from the CC. (A) shows a color-coded FA map in the sagittal plane, with an ROI placed at the genu. The inset shows the same map without the genu ROI. (B) shows the corresponding reconstruction of callosal fibers crossing the genu and connecting the frontal lobes of both hemispheres. (C) shows a lateral perspective of the 2 ROIs shown on a transverse FA map in (D), one ROI placed at the genu (represented sagittally in C, orthogonal to a transverse background plane) and another in the white matter caudally (coronal plane, slightly tilted around a vertical axis). Both ROIs were set to a low FA threshold of 0.05. However, as demonstrated in (B), callosal fibers cross the genu and connect the frontal lobes of both hemispheres as expected, but no fibers connecting these 2 ROIs were reconstructed in any of the normal brains.
1994; Rash and Richards 2001; but see Ozaki and Wahlsten 1998), whereas the bulk of axons are presumed to simply follow the pioneers. At the opposite hemisphere, little is known about the guidance cues and growing mechanisms of callosal axons, except that they overshoot the homotopic targets, leaving a branch that arborizes at the right point, and then eliminate somehow the overshooting fiber (Hedin-Pereira and others 1999). The establishment of a mature pattern of callosal projections involves the transient growth of supernumerary axons and arbor that are later selectively eliminated (for a recent review, see Innocenti and Price 2005).

These developmental mechanisms have only indirectly been demonstrated in humans, as suggested by the formation of midline cellular and extracellular matrix structures involved in axonal guidance (Lent and others 2005) and a perinatal reduction of cross-sectional callosal area that might indicate the massive axonal elimination shown to occur in animals (Clarke and others 1989). Recent MRI measures have added evidence to this latter suggestion, both in the callosal tract (Keshavan and others 2002) and in the cortical tissue (Thompson and others 2000). Classical descriptions have proposed a rostrocaudal gradient in formation of the callosal tract beginning at approximately 11 weeks postovulatory (Rakic and Yakovlev 1968). According to this view, a rostral locus of commissuration would form first at the prospective position of the genu, followed by the rostro-caudal addition of axons to form the body and splenium and an inverse sequence to form the callosal rostrum. However, other authors have suggested that the embryonic CC has a bicentric origin, with a rostral sector forming at the lamina rostralis and a more caudal region appearing simultaneously over the hippocampal commissure (Kier and Truwit 1996, 1997). The fact that genual fibers (i.e., fibers located rostrally in the CC and projecting to prefrontal cortical territories) are the most common callosal remnants in CD tends to favor the former hypothesis, although there are reported cases in which remnants are located more posteriorly (reviewed by Richards and others 2004). Thus, it can be concluded that the pathological disturbance in CD cases with genual remnants spares the rostral pioneer fibers and, therefore, presumably does not interfere with the guidance mechanisms provided to the pioneers by the midline cellular and matrix structures. Along the same lines, the subsequent rostral and caudal addition of callosal fibers is obstructed by the pathological insult.

An Interplay between Rigidity and Plasticity in CD

Despite indirect data, none of the above mechanisms have been unequivocally shown to be impaired in CD. However, the available evidence seems to indicate that callosal neurons survive in CD cases, projecting axons to normal and abnormal targets. This is substantiated by the existence of remnant and aberrant tracts that presumably contain callosal fibers. Therefore, the hodological information derived from DTI data can be used to draw important conclusions concerning the balance between plasticity and rigidity (or lack of plasticity) in CD. The fact that a hypoplasic CC exists in some cases, whereas a small remnant is placed rostrally in others, poses the question whether the fibers within these smaller tracts would conserve their specific connectivity (rigidity) or enlarge their territory (plasticity) to occupy the sites left vacant by impaired callosal fibers. We found the first hypothesis to be true as most fibers in the rostral remnants connect the frontal lobes in a very specific, focused manner (compare Fig. 3, Panel 2 with Fig. 5, Panel 2) and do not invade other territories, except in some particular situations (see below). Similar findings have been reported in mice with congenital deficiencies of the CC (Olavarria and others 1988).

A second important observation concerns the maintenance of a topographic arrangement by fibers within callosal remnants (Fig. 4, Panel 1B–C) and the PB (Fig. 4, Panel 2). In this case, if rules for topography were completely disrupted by the pathological processes, we would not find such a topographic order within these tracts. Topography in the normal CC has been shown in animals (Barbas and Pandya 1984; Cipolloni and Pandya 1985; Rockland and Pandya 1986; Olavarria and others 1988; Nakamura and Kanae 1989; Matsumami and others 1994) and in humans (Xu and others 2002; Abe and others 2004; see also de Lacoste and others 1985). In general (Fig. 3, Panel I), frontal regions become connected through the genu, parietal regions through the body, and occipital regions through the splenium. Also, dorsomedial cortical fibers cross dorsally within the CC, whereas more lateral axons occupy the ventral sectors of the callosal tract. A topographic order was also seen within the PB (Fig. 4, Panel 2), suggesting that fibers therein use cues to sort themselves that do not depend on the mechanisms impaired by the developmental disturbances of CD. Given the normal pattern of projections of rostral callosal remnants, the frontal poles of the hemispheres are appropriately interconnected, and it should be expected that at least the corresponding frontal functions are spared in these cases.

An example of plastic rewiring of callosal connections derives from the robust aberrant tracts that form in many CD cases: the well-known PB (Probst 1901) and the sigmoid bundle described here for the first time. The PB has been extensively studied in mutant mice (Ozaki and others 1987, 1989; Ozaki and Shimada 1988; Ozaki and Wahlsten 1993) and in surgically ablated hamsters (Lent 1982, 1983), employing tract-tracing techniques. In humans, PB is consistently observed in cases of CD (Lassonde and others 2003) and may even be found in individuals with a normal CC (Hori and Stan 2004). In rodents, Probst fibers usually display tortuous trajectories, leaving and returning to the bundle and eventually crossing the midline through the septum bordering the ventricular surface (Lent 1982, 1983). Many of these longitudinal fibers form U-shaped connections in the medial sectors of the ipsilateral cortex. In addition, a topographic organization was discerned within the PB, according to the cortical region of origin of the fibers (Ozaki and Shimada 1988). Most authors assume that PB is composed of callosal fibers whose way across the midline was blocked during development. The topographic similarities between normal callosal target regions and the ipsilateral connections established by Probst fibers in animals and, according to our data, in humans strongly support this assumption. The preservation of fiber order in both callosal remnants and the longitudinal aberrant bundle comes in line with the idea that topography is a presupposed property of some fiber systems, the CC among them. In this context, it has been shown that callosal axons are topographically organized from the outset (Innocenti and Clarke 1984). We, therefore, conclude that in CD, the pathological insult at the origin of this condition does not target the cues for fiber ordering in the callosum, even though their trajectories become entirely abnormal.

The sigmoid bundle, on the other hand, is hard to uncover using descriptive anatomical techniques, as it courses within
the normal-looking white matter of CD patients after crossing the remnant or hypoplastic callosum. Postmortem tract-tracing techniques have not been employed in these patients as they would require an improbable association of the congenital defect with acquired cortical lesions followed by short survival plus the availability of the brains for studies with silver impregnation of degenerating fibers. DTI/tractography, however, has proven to be capable of revealing this "hidden" tract. We are confident to exclude the possibility that this bundle be artifactual because 1) it consistently connects the right frontal lobe with the left occipital cortex and does not appear contrateraterally (except in one case, as described), despite our efforts to place mirror-symmetric ROIs with low FA thresholds to reveal a contralateral bundle (Fig. 5, Panel 1), and 2) it was never seen in the 10 control brains studied with similar ROIs as in the patients (Fig. 5, Panel 2).

As aberrant bundles in CD cases contain fibers that emerge from living neurons, they are presumed to be functional. Whether their function is compensatory or maladaptive, however, is a matter for speculation because a direct approach of their functional performance has not been available so far. One would imagine that the PB, being so consistent among patients, would serve a compensatory rather than a maladaptive role (Lassonde and others 1988, 1991; Sauerwein and Lassonde 1994; Lessard and others 2002), at least in acallosal individuals who lead a normal life without cognitive impairment (Meyer, Röricht, and Niehaus 1998). Therefore, it would not respond for the great variability of symptoms of CD patients. Clarification of this assumption, however, will have to await functional investigations using electrophysiological techniques, as well as clinicoradiological and neuropsychological investigations in larger samples of patients with CD.

The opposite hypothesis could hold in the case of the sigmoid aberrant tract found in 37% (4 of 11) of our cases. No similar structure such as this has been described so far either in animals or in humans, except for a brief mention by Lee and others (2004, 2005) of an occipital branch of fibers exiting from the callosal remnant in one of their cases. Remarkably, these 4 patients had the most severe impairments: 3 of them had moderate mental retardation and 1 had severe motor impairment. We, therefore, hypothesize that this highly asymmetric, aberrant circuit may somehow be related to the disabilities of these patients.

In conclusion, the search for aberrant projections in CD, as well as in other developmental disorders, by using imaging techniques can potentially be combined with electrophysiological methods in a way to address the functional relevance of neuroplastic changes and to better account for the variable and broad clinical presentations that are often observed. In addition, it might be possible to distinguish between plastic changes that cause functional disabilities and symptoms and those that are compensatory and beneficial to patients.

Notes

This work is part of the PhD thesis of FT-M submitted to the Programa de Ciências Morfológicas, Instituto de Ciências Biomédicas and was supported by the Conselho de Desenvolvimento Científico e Tecnológico (CNPq # 471625/2003-2) and the Centro de Educação Continuada em Medicina, Rede D’Or Hospitals. We thank Drs Leonardo C. Azevedo and Elaine L. Gerk for providing access to the patients of Hospital Fernandes Figueira, Fiocruz, Brazil, Prof. Linda Richards for comments on the text, and Dr Maria Beatriz A.M. Gonzaga for patients’ sedation support during image acquisition. We also thank all patients and health volunteers for their collaboration in the study. Conflict of Interest: None declared.

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References


Innocenti GM, Clarke S. 1984. The organization of immature callosal structure such as this has been described so far either in animals or in humans, except for a brief mention by Lee and others (2004, 2005) of an occipital branch of fibers exiting from the callosal remnant in one of their cases. Remarkably, these 4 patients had the most severe impairments: 3 of them had moderate mental retardation and 1 had severe motor impairment. We, therefore, hypothesize that this highly asymmetric, aberrant circuit may somehow be related to the disabilities of these patients.

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