Mapping of fiber orientation in human internal capsule by means of polarized light and confocal scanning laser microscopy

Hubertus Axer *, Diedrich Graf v. Keyserlingk

Institut für Anatomie I, Universitätsklinikum der RWTH Aachen, Pauwelsstraße 30, 52057 Aachen, Germany

Received 8 December 1998; received in revised form 13 August 1999; accepted 1 September 1999

Abstract

The nervous fibers in the human internal capsule were mapped according to their three-dimensional orientation. Four human cadaver brains were cut into comparable and standardized sections parallel to the ACPC-plane, stained with DiI, and analyzed using a combination of confocal and polarized light microscopy at the same time. This combination provides information about the structure and orientation of the fibers in great detail with confocal microscopy, and information about the localization and orientation of long myelinated fiber tracts with polarization microscopy. The internal capsule was parcellated in the areas CI 1 to CI 4 containing fibers of distinct orientation and structure, which enriches the macroscopically definable parcellation in the anterior and posterior limb. Fibers of the anterior thalamic peduncle intermingle with frontopontine tract fibers. Single fibers connect the caudate and the lentiform nucleus. The pyramidal tract is located in the anterior half of the posterior limb intermingled with fibers of the superior thalamic peduncle. Parietooccipitopontine fibers are located in the posterior part of the posterior limb. The slopes of the different systems of fibers change continuously in the anterior–posterior direction of the internal capsule. Using the 3D orientation of fibers as a criterion for parcellation, as well as the description of bundles as a collection of fibers belonging to particular tracts leads to a more function-related description of the anatomy of the internal capsule. The method can be used for interindividual, sex- or age-related comparisons of particular systems of fibers. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Fiber mapping; Internal capsule; Confocal; DiI; Polarization; Corticopontine tract; Pyramidal tract; Thalamic radiation

1. Introduction

The fiber architecture especially of the white matter in the human brain is presently the focus of interest. Many neurologic diseases affect the cerebral white matter, and modern imaging procedures such as magnetic resonance imaging (MRI) provide detailed visual information about the white matter. Anatomical knowledge about nervous fibers was achieved earlier by fiber preparations (Ebeling and Reulen, 1992), myelogenic studies (Kretschmann, 1988) and by observations after brain injury (Fries et al., 1993).

We were able to demonstrate the orientation and texture of the nervous fibers to influence the value of impedance measurements, and so an online verification of the needle’s position in a stereotactic procedure can be performed (Axer et al., 1998). In addition, the orientation of nervous fibers can be visualized by modern MRI procedures (Curnes et al., 1988; Douek et al., 1991; Peled et al., 1998). Nevertheless, a detailed anatomical mapping of fiber orientations in the adult human brain is difficult to assess. Our aim was to perform a mapping of the orientation of the fibers in the internal capsule. Thus we wanted to find out how anatomically defined fiber tracts are oriented in the white matter. The method should give information about fiber texture, fiber orientation and identification of specific fiber tracts.

Therefore we used the combination of confocal laser microscopy and polarized light microscopy. The confocal laser microscope allows information to be collected from well-defined optical sections. This is done by a sequential illumination which is focused on one volume element of the specimen at a time (Wright et al., 1993).
This way stacks of optical sections can be produced, which allow three-dimensional reconstruction of the fiber texture and provide good information about the orientation of the fibers at high resolution.

Polarized light microscopy can selectively visualize anisotropic structures. In polarization microscopy, optically polarized light passes through a sample of tissue and into a second polarizer (analyzer), which polarizes light in a perpendicular plane with respect to the first polarizer. Birefringence is able to twist some of the light so that it can pass through the analyzer and be imaged. The birefringence of the nervous tissue has been well known for a long time (Schmidt, 1923, 1924; Schmitt and Bear, 1936; Kretschmann, 1967; Wolman, 1975). As the presence of anisotropy indicates polarity and order, polarization microscopy can be used to visualize long fiber tracts in the brain (Fraher and MacConnaill, 1970; Miklossy and Van der Loos, 1991). The orientation of the fibers influences the transmission of plane-polarized light at different velocities at different azimuths.

The combination of both techniques allowed us to obtain detailed information of the fiber structure and orientation with confocal microscopy and to collect information about the localization and orientation of long myelinated fiber tracts with polarized light microscopy.

The internal capsule in the adult human brain represents a collection of different systems of fibers closely located in a small space. It is a structure of high clinical importance, since the fibers of the pyramidal tract are located here. Nevertheless the exact location of the pyramidal tract in the internal capsule was a matter of dispute for decades (Maurach and Strian, 1981).

2. Materials and methods

2.1. Macroscopic preparation

Four human cadaver brains without macroscopically definable pathology and without a history of neurologic or psychiatric disease were fixed in 4% aqueous formalin solution and macroscopically prepared. The meninges were removed and the brains cut in the median-sagittal plane. Important landmarks were identified on the median surface of the hemispheres: the anterior and posterior commissure, the interventricular foramen of Monro and the internal cerebral vein (or the tela chooroidea ventriculi tertii). The next two sections were cut parallel to the line between anterior and posterior commissure (ACPC). The first horizontal section was made at the level of the ACPC-line. The sections were orientated parallel to the ACPC-line. The sections were serially collected. Every second section was coverslipped without staining procedure. The other sections were kept in Tris buffer (0.1 M, pH 7.4) containing 0.1% Triton™ X-100 (Merck, Darmstadt, Germany) for 24 h. Afterwards these sections were placed on microscope slides and 3–4 drops of a DiI-solution (1 mg DiI [1,1'-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, FAST DiI™ oil, Molecular Probes Europe, Leiden, Netherlands] in 1100 μl dimethyl sulfoxide [DMSO]) were put on these slides, and then the slides were kept in a moist chamber at 37°C. DiI is highly lipophilic and dissolves into lipid membranes. This way the myelin sheaths of the nerve fibers in particular are labeled. After 7 days, the sections were mounted with the aqueous mounting medium Aquatex™ (Merck, Darmstadt, Germany) and coverslipped.

2.2. Histologic preparation

The slabs were first placed for 24 h in 10% sucrose solution and then in 30% sucrose solution until they sank to the bottom of the container. Afterwards the tissue was sectioned on a freezing microtome at 60 μm in the horizontal plane. This way all histologic sections were orientated parallel to the ACPC-line. The sections were serially collected. Every second section was coverslipped without staining procedure. The other sections were kept in Tris buffer (0.1 M, pH 7.4) containing 0.1% Triton™ X-100 (Merck, Darmstadt, Germany) for 24 h. Afterwards these sections were placed on microscope slides and 3–4 drops of a DiI-solution (1 mg DiI [1,1'-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, FAST DiI™ oil, Molecular Probes Europe, Leiden, Netherlands] in 1100 μl dimethyl sulfoxide [DMSO]) were put on these slides, and then the slides were kept in a moist chamber at 37°C. DiI is highly lipophilic and dissolves into lipid membranes. This way the myelin sheaths of the nerve fibers in particular are labeled. After 7 days, the sections were mounted with the aqueous mounting medium Aquatex™ (Merck, Darmstadt, Germany) and coverslipped.

2.3. Conventional polarized light microscopy

The unstained sections were analyzed by conventional polarized light microscopy. We used the Stemi SV 11 (Zeiss, Germany) with rotatable crossed polars for low magnification. This way we obtained a map of the entire sample. A Leica DM IRBE (Leica Microsystems, Heidelberg, Germany) microscope equipped with a rotatable stage and fixed crossed polars was used for higher magnification. The same microscope was used for confocal laser microscopy.

2.4. Confocal laser scanning microscopy

The DiI stained sections were analyzed with the Leica TCS NT confocal laser scanning microscope (Leica Microsystems, Heidelberg, Germany). Since DiI (maximum absorption: 549 nm, maximum emission: 564 nm) has fluorescent features similar to rhodamine we used the standard rhodamine filter settings of the confocal microscope.

2.5. Polarization with transmitted laser light

Polarized light microscopic pictures were produced on a second channel of the confocal microscope. Therefore the transmission mode of the microscope was used. In transmission mode the laser light passes through the sample and is detected in a photomultiplier at the other side of the sample. Since laser light is polarized we had to insert one polarization filter (analyzer) only into the
Fig. 1. Combination of confocal and polarization microscopy.

path of the laser beam after the light has passed through the sample. This way it was possible to analyze a single section with both confocal fluorescence and polarization methods (Fig. 1) at the same time.

3. Results

In polarization microscopy, the detectable signal depends on the order and the inclination of the fibers. In particular, parallel, horizontally cut fibers give a bright signal at a special azimuth whereas the signal decreases after rotation of 45°. The steeper these tracts of fibers are, the less bright the signal is. Thus different fiber tracts of different orientation and order produce areas of different brightness. In low magnification, the polarization picture provides information about the localization of different bundles of fibers (Fig. 2).

At higher magnification single bundles of fibers can be detected. In polarization mode, the area resembling a bundle of fibers is a summation over the whole thickness of the section (60 μm), as the light must pass through the entire sample. At the same time confocal imaging allows the identification of single fibers and their texture (Figs. 3–6). The image produced with the confocal microscope is a small optical section in the sample. The angle of the fibers was defined by 3D-reconstructions from confocal z-series (Fig. 7).

3.1. A revised parcellation of the internal capsule

Fig. 8 shows the parcellation of the internal capsule based on the distribution of differently oriented fibers. These areas are different from the borders of the macroscopically defined parcellation of the internal capsule (anterior limb, genu, posterior limb). The internal capsule was divided into four areas CI 1 to CI 4 for a better orientation.

Three distinct fiber systems can be distinguished in the anterior limb. Many fibers originate from the thalamus and run in the direction of the anterior limb. In our sections these fibers are cut almost horizontally (Fig. 3) and are located in the whole area of the

Fig. 2. Anterior limb of the internal capsule as revealed by conventional polarization microscopy. Systems of fibers of different orientation appear in characteristic scales of grey under this azimuth. The rectangle in the map in the right upper corner shows the orientation of the sample. Abbreviations are: PL, posterior limb; G, genu; AL, anterior limb; Tha, thalamus; Put, putamen; NC, caudate nucleus.
located at the lateral border of the first part of the posterior limb. A third fiber system is not organized in bundles. Single fibers run from the caudate nucleus to putamen and globus pallidus, thus these fibers traverse the anterior limb (Fig. 3). Isles of grey substance (the ponticuli grisei) can be found in between the fibers of the anterior limb.

Fibers directed very steeply (almost 80–90° to the ACPC-plane) run in the posterior part of the genu and in the anterior part of the posterior limb (area CI 3).

anterior limb (area CI 1 and CI 2 in Fig. 8). The course of a second fiber system is relatively steep. The fibers do not run into the thalamus, but pass the thalamus, and their slope is 60–80° in relation to the ACPC-plane. They are collected in bundles, which are organized in bands (Fig. 2). These steep bundles are intermingled with the horizontal fiber bundles mentioned before (Fig. 4) and the angle between these bundles varies from 60 to 70°. The area of these two intermingled fiber systems (area CI 2 in Fig. 8) is located in the posterior two-thirds of the anterior limb. Its medial border corresponds to the tip of the genu and its posterior border is

Fig. 3. Lateral part of the anterior limb (size 158.7 × 158.7 µm, DiI). The confocal image (a) and the same section in polarization mode (b) show parallel, horizontally running fibers which belong to the anterior thalamic peduncle. These fibers are traversed by single fibers which belong to the caudatopallidal system of fibers (a).

Fig. 4. Medial part of the anterior limb (size 158.7 × 158.7 µm, DiI), (a) confocal and (b) polarization. Two distinct systems of fibers can be distinguished. The bundle of fibers in the middle represents fibers of the frontopontine tract. The horizontally cut fibers belong to the anterior thalamic peduncle. Because (b) is a summation effect over the whole thickness of the sample (60 µm) the border between both fiber systems is not as clear-cut as in (a).
The anterior border of this fiber system is the middle part of the genu on the medial side. On the lateral side this fiber system begins at the edge of the putamen or the globus pallidus more posteriorly and so this border extends into the beginning of the posterior limb. These steep fibers are intermingled with bundles of fibers which run horizontally and arise from the thalamus (Fig. 5). The number of these horizontal bundles decreases continuously in the anterior–posterior direction.

In the posterior part of the posterior limb the course of the fibers is also steep, but they are not as homogeneously ordered as in the anterior part. Steep bundles of fibers are oriented at slightly different angles and are twisted. This produces an image of mosaic-like areas of different shades of grey in the polarization pictures (Fig. 6). In addition, fibers of large diameters like in area CI 3 were not observed in such a high number (Tomasch, 1969). Bundles of horizontal fibers running from the thalamus are sparse in this area CI 4. The border between area CI 3 and area CI 4 is located in the posterior middle of the posterior limb. But this borderline cannot be drawn very precisely as there is a continuous transition between these fiber systems.
3.2. Interindividual differences

In all brains the parcellation of these fiber structures was almost similar, but all fibers, especially in CI 3 (Fig. 8), were more densely packed in smaller brains than in larger ones. A statistical analysis will follow. In addition, the border of this steep fiber system (area CI 3) differed also slightly depending on the size of the brain. In smaller specimen the anterior border of the steep fibers was located more anteriorly in the genu.

Fig. 7. 3D-reconstruction of a confocal z-series. The picture illustrates the steep, parallel fibers of the pyramidal tract.

Fig. 6. Posterior part of the posterior limb (size 158.7 × 158.7 μm, DiI). The fibers are twisted, so fibers with slightly different slopes can be seen in the confocal image (a) and these appear as areas of different scales of grey in the polarization mode (b).

Fig. 8. Revised parcellation of the internal capsule in the three sections (S I, internal cerebral vein; S III, foramen interventriculare Monroi; S II, between I and III). We distinguish four major areas which differ in their fiber texture. These characteristic textures are described in the text.

The posterior border differed most in all specimens, but this border could not be defined clearly, because there was a continuous transition of these systems of fibers. The ill-defined borders are marked as a dotted band in Fig. 8.
4. Discussion

4.1. Methodological considerations

Traditionally the internal capsule is divided into the anterior limb, the genu and the posterior limb (Dejerine, 1901). The areas occupied by the distinct bundles of fibers differ from the borders of the macroscopically defined parcellation of the internal capsule. In his classical description, Vogt (1902) subdivided the posterior limb into three parts according to the intensity of the Weigert staining. This subdivision, however, does not take care of the orientation of the fibers.

The central tracts of nervous fibers are important, for instance, in stereotactic interventions focused on particular tracts of fibers (e.g. in anterior capsulotomy). Many neurologic diseases affect the white matter of the brain (e.g. multiple sclerosis, HIV) and result in neuropsychological impairments. Thus in many clinical settings more attention is presently paid to changes in the white matter. The orientation of myelinated fibers can be visualized by modern MRI procedures (diffusion tensor maps) (Douek et al., 1991; Peled et al., 1998). The technique presented here can be used to evaluate such results from an anatomical point of view.

Bundles of fibers are interesting if functional entities are investigated in the case of tracts of fibers. Changes in special tract systems can be found e.g. in amyotrophic lateral sclerosis. In addition, right–left differences may be detectable in some systems of fibers as well as sex-dependent or age-related differences.

The new technical approach presented here can be used for mapping the 3D structure of central nervous fibers in the human brain. The method provides information not only about single nervous fibers but also about bundles of fibers. Polarization microscopy is able to differentiate distinct bundles of fibers and is a good tool to describe the shape and size of these bundles.

Nevertheless, the slope of the fibers should provide information about the origin and the target of the fibers. In ontogenesis, central nerve fibers will take the shortest way to reach their target (Maurach and Strian, 1981). This shortest way could only be disturbed by anatomical structures which have to be avoided or by other systems of fibers. In the internal capsule the fibers originating in the frontal lobe have a different slope from fibers originating in the occipital lobe. Fibers from central cortical regions proceed very steeply. On the other hand fibers travelling to the thalamus have to turn more horizontally to reach their thalamic targets, while fibers travelling to the pons follow their steep path. Fig. 9 shows the different slopes of distinct fiber systems in the internal capsule. Difficulties in the differentiation of different tracts arise when fiber systems show only slightly different slopes as in the posterior limb (between area CI 3 and area CI 4), or when distinct fiber systems may be mainly intermingled as in the corona radiata.

The usefulness of confocal microscopy is rather obvious. Single nerve fibers can clearly be visualized at high resolution, and confocal z-series provide three-dimensional information about the orientation of the nerve fibers. DiI is a fluorescence marker which is often used to investigate connectivity of nervous fibers (Honig and Hume, 1986; Baker and Reese, 1993). The fact that the dye requires a relatively long time (3–5 months) for diffusion is a disadvantage. We used this dye to label all myelinated fibers in our sections and developed a procedure which yields many samples quickly by staining the probes after sectioning. This way the method provides the possibility to investigate a larger number of specimens in a shorter time.

The marker DiI labels myelin sheaths in particular. This way small unmyelinated fibers may not be imaged. They also do not contribute to the polarization images, since the anisotropy of the nervous tissue is the result of radially orientated lipids in the myelin sheath. In the central nervous system, unmyelinated fibers were visualized accurately with electron microscopy (Keyserlingk and Schramm, 1984; Leenen et al., 1985; Aboitiz et al., 1992), showing for instance, some sex-dependent differences in the ratio of myelinated to unmyelinated fibers to exist in the rat brain (Mack et al., 1995). Taking unmyelinated fibers into consideration may be very interesting for description of the organization of the various fiber tracts of our study, as there may exist differences in the distribution of the amount of myelination in the different tracts of fibers. This, however, was not possible to investigate here, but could form an interesting field for future research.

The combination of confocal and polarized light techniques allows us to obtain detailed information of the fiber structure and orientation with confocal microscopy and information about the localization and orientation of long myelinated fiber tracts with polarized light microscopy. Jouk et al. (1995) used both...
techniques for mapping the orientation of myocardial cells, but the use of the confocal microscope to produce both confocal and polarized pictures of the same section at the same time is new.

We successfully demonstrated the architecture of the nervous fibers to influence the value of the impedance measurement at this point (Axer et al., 1999). Thus online impedance measurements in a stereotactic intervention can be compared with the three-dimensional structure of nervous fibers in an atlas in order to perform a verification of the needle’s position.

Thus the method presented was developed to obtain detailed information about the structural organization of the myelinated fibers. However, the final identification of the various fiber tracts can only be done with knowledge of the localization of the different tract systems. This knowledge is recorded in the literature and will be discussed in detail. Each location in the internal capsule contains a few fiber tracts and these can be differentiated according to their slope.

4.2. Corticopontine tracts

One of the major systems of fibers located in the internal capsule is the corticopontine system of fibers projecting to the pontine nuclei which in turn project into the cerebellum. Many investigations of this system were performed on monkeys. Most fibers of this system were demonstrated to arise from pericentral cortices (Dhanarajan et al., 1977; Brodal, 1978a,b, 1982; Wiesendanger et al., 1979; Hartmann-von Monakow et al., 1981). But also prefrontal (Leichnetz and Astruc, 1976; Schahmann and Pandya, 1997), parieto-occipital (Schahmann and Pandya, 1992), and parahippocampal cortices (Schahmann and Pandya, 1993) project on the pontine nuclei.

In human beings, such results are relatively scarce (Tredici et al., 1990). The human corticopontine system of fibers however comprises 20 times more fibers than the pyramidal tract system (Tomasch, 1969). In addition, the association cortices in man are more developed than in monkeys and may contribute to cerebrocerebellar circuits which also influence higher behavioural functions (Leiner et al., 1991; Schahmann, 1991).

The relatively steep bundles of fibers in the anterior limb (area CI 2), which pass the thalamus must be frontopontine fibers. The frontopontine tract (Arnold’s bundle) is a well known fiber tract in man (Meyers, 1950). In post-mortem studies on degenerations in human brains after prefrontal leucotomy, frontopontine as well as frontothalamic fibers were localized in the anterior limb of the internal capsule (Meyer, 1949; Beck, 1950). The frontopontine tract of fibers can also be visualized by the preparation method of Klingler (Ludwig and Klingler, 1956 [Tabula 45, 47]). In studies of monkey brains the frontopontine tract was also localized in the anterior limb (Leichnetz and Astruc, 1976).

We demonstrated the frontopontine tract not to be a separate bundle in the anterior limb, as shown in classic anatomic textbooks (Kappers et al., 1967). Instead, it intermingles with the fiber bundles which come from the thalamus, and its border reaches into the lateral part of the posterior limb. Earlier anatomical studies using Weigert myelin stains did not describe such fiber bundles (Vogt, 1902).

In addition, the fibers in the posterior part of the posterior limb (area CI 4) should be the parietooccipitopontine projections because their course is not as steep as for the fibers from the pericentral cortex. This system of fibers was localized in the posterior part of the posterior limb in the course of studies on degeneration (Dejerine, 1901), and by macroscopical dissection (Ludwig and Klingler, 1956 [Tabula 38]). Temporopontine fibers (Tuerck’s bundle) were not detected in the course of our sections because these fibers are located in more caudal planes (Dejerine, 1901).

In monkeys the parietooccipitopontine fibers are also collected in the posterior limb (Schahmann and Pandya, 1992). The corticopontine fibers were distinguishable as largely separate, but partially overlapping bundles (Schahmann and Pandya, 1992). This finding is very similar to the picture we found in the posterior limb when using the polarization method (Fig. 6).

4.3. Pyramidal tract

Over decades the localization of the pyramidal tract in the internal capsule was a matter of dispute (Davidoff, 1990). The traditional concept of localization of the pyramidal tract in the anterior half of the posterior limb is based on the degeneration studies of Dejerine (1901). Analysis of lesions restricted to the internal capsule using computer tomography (CT) demonstrated the localization of the pyramidal tract in the anterior half of the internal capsule and its topological organization (Tredici et al. 1982; Bogousslavsky and Regli, 1990; Fries et al., 1993). In addition, it was possible to reproduce this concept in defined experimental investigations in monkeys (Dawney and Glees, 1986; Fries et al., 1993). Nevertheless, several other work groups located the pyramidal tract in the posterior half of the posterior limb in post-mortem case studies (Englander et al., 1975; Hanaway and Young, 1977), but also in a large number of exploratory stimulations of the internal capsule during stereotactic interventions (Hardy et al., 1979) and during MRI-studies on amyotrophic lateral sclerosis (Yagishita et al., 1994).

This ambiguity was settled by Ross (1980) using macroscopical dissection techniques. The pyramidal
tract is at first located in the first half of the posterior limb but shifts posterior in more caudal sections, which was also demonstrated in myelogenic studies (Kretschmann, 1988). A detailed description of the subcortical topography of the pyramidal tract was provided by Ebeling and Reulen (1992).

In summary, the exact location of the pyramidal tract depends on the level of section investigated, because the pyramidal tract features a change in its three-dimensional orientation (Maurach and Strian, 1981). In addition, the orientation of the section is also crucial. Horizontal sections may vary up to 25°, and these differences may change the relative location of the genu of the internal capsule. Our sections were cut parallel to the ACPC line and this standardization to reference landmarks allows all levels to be compared with each other. Hardy et al. (1979) also used this reference system and analyzed a level 2 mm above the ACPC line. In contrast, our sections are located higher (4–8 mm). Here, the pyramidal tract is located in the first half of the posterior limb (Ross, 1980; Kretschmann, 1988). The posterior shift of these fibers must be expected at lower planes we did not actually investigate.

In our investigations we localized the pyramidal tract in area CI 3. The slope of the fibers located here is 80–90°, and this has to be considered for the fibers running from pericentral cortices to the brain stem. We also found steep fibers located in the genu which apparently belong to corticobulbar fibers (Bogousslavsky and Regli, 1990). This localization is however not always found in all individuals. In smaller brains these fibers are located more anteriorly than in larger ones.

4.4. Thalamic radiation

Thalamocortical and corticothalamic fibers are often neglected in the description of the internal capsule or depicted as separate bundles (Kappers et al., 1967). This is apparently due to the complex connectivity of the various thalamic nuclei (Axer and Niemann, 1994). Nevertheless, thalamic fibers intermingle diffusely with the aforementioned fiber systems (Fries et al., 1993), but they can be differentiated, when they have to swing into the thalamus. The frontothalamic path consists of numerous bundles connecting the cortex of the frontal lobe to the medial and to a lesser extent to anterior thalamic nuclei (Hassler, 1982). This bundle of fibers converges in the anterior limb of the internal capsule (Kandel, 1989) and the great number of horizontally cut fibers in the anterior limb in our study (area CI 1 and CI 2) belong to the anterior thalamic peduncle. The localization of these fibers can also be shown by means of the preparation method of Klingler (Ludwig and Klingler, 1956 [Tabula 54, 55]; Kandel, 1989) and was likewise confirmed by earlier degeneration studies in the human brain (Dejerine, 1901; Beck, 1950).

The projection of the lateral thalamic nuclei is mainly to the pre- and postcentral gyri (Hassler, 1982). These fibers are located in the posterior limb of the internal capsule (Kandel, 1989). We located the superior thalamic peduncle in area CI 3 and CI 4 as also demonstrated by macroscopical preparation (Ludwig and Klingler, 1956 [Tabula 34, 35]). The thalamic bundles of fibers decrease numerically in the anterior–posterior direction of the posterior limb and they intermingle diffusely with corticobulbar and corticospinal fibers (Kretschmann, 1988).

4.5. Caudatopallidal fibers

At least single fibers were found traversing the anterior limb and extending from the nucleus caudatus to the nucleus lentiformis. In the monkey, anterogradely labeled fibers arising from the caudate nucleus were found, obliquely traversing the internal capsule and penetrating the dorsal portion of the pallidum (Hazzrati and Parent, 1992). These fibers traversing the anterior limb of the internal capsule have also been described in the human brain (Hassler, 1974; Carpenter, 1982) and could not be detected with conventional staining methods (Dejerine, 1901; Vogt, 1902). In addition, these fibers are also not detectable with polarization microscopy.

4.6. The organization of the internal capsule

In summary, the organization of the internal capsule as analyzed here differs from the classical models of anatomic textbooks in some respects. The different tracts of fibers are not individual, separate tracts of fibers. Bundles of fibers intermingle with other bundles, but all have their characteristic orientation on their own. The slope of the different systems of fibers changes continuously in the anterior–posterior direction of the internal capsule (Fig. 9). Thus different bundles of fibers are distinguishable and so it is possible to describe them separately. Using the 3D orientation of fibers as a criterion for parcellation as well as the description of bundles as a collection of fibers belonging to particular tracts results in a more function-related description of the anatomy of central nervous fibers.

Acknowledgements

We would like to thank Petra Ibold and Anita Agbedor for their technical assistance.