Clinical Research

3D anatomy of cerebellar peduncles based on fibre microdissection and a demonstration with tractography

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ABSTRACT

Objective: To perform an anatomical and radiological study, using fibre microdissection and diffusion tensor tractography (DTT), to demonstrate the three-dimensionality of the superior, middle and inferior cerebellar peduncles.

Material and methods: A total of 15 brain-stem, 15 cerebellar hemispheres, and 5 brain hemispheres were dissected in the laboratory under the operating microscope with microsurgical instruments between July 2014 and July 2015. Brain magnetic resonance imaging was obtained from 15 healthy subjects between July and December of 2015, using diffusion-weighted images, in order to reproduce the cerebellar peduncles on DTT.

Results: The main bundles of the cerebellar peduncles were demonstrated and delineated along most of their trajectory in the cerebellum and brain-stem, noticing their overall anatomical relationship to one another and with other white matter tracts and the grey matter nuclei the surround them, with their corresponding representations on DTT.

Conclusions: The arrangement, architecture, and general topography of the cerebellar peduncles were able to be distinguished using the fibre microdissection technique. This knowledge has given a unique and profound anatomical perspective, supporting the correct representation and interpretation of DTT images. This information should be incorporated in the clinical scenario in order to assist surgeons in the detailed and critical analysis of lesions that may be located near these main bundles in the cerebellum and/or brain-stem, and
Anatomía de los pedúnculos cerebelosos en 3D basada en microdisección de fibras y demostración a través de tractografía

RESUMEN

Objetivo: Realizar un estudio anatómico de microdisección de fibras y radiológico mediante tractografía basada en tensor de difusión (DTT) para demostrar tridimensionalmente los pedúnculos cerebelosos superiores, medios e inferiores.

Material y métodos: Bajo visión microscópica y con el uso de instrumental microquirúrgico en el laboratorio, se disecaron 15 troncoencéfalos, 15 hemisferios cerebelosos y 5 hemisferios cerebrales humanos, entre julio de 2014 y julio de 2015. Se obtuvieron imágenes de resonancia magnética cerebrales realizadas a 15 sujetos sanos entre julio y diciembre de 2015, empleando secuencias potenciadas en difusión para el trazado de los pedúnculos cerebelosos y su reproducción mediante DTT.

Resultados: Se demostraron y describieron anatómicamente las principales fibras de los pedúnculos cerebelosos a lo largo de gran parte de su trayectoria en el cerebelo y troncoencéfalo, identificando las relaciones entre sí y con otros haces de sustancia blanca y núcleos de sustancia gris que los rodean, con la correspondiente representación mediante DTT.

Conclusiones: Mediante la técnica de microdisección se apreció la disposición, arquitectura y organización topográfica general de los pedúnculos cerebelosos. Este conocimiento ha aportado una perspectiva anatómica única y profunda que ha favorecido la representación y correcta interpretación de las imágenes de DTT. Esta información debe ser trasladada a la práctica clínica para favorecer el análisis crítico y exhaustivo por parte del cirujano ante la presencia de lesiones que puedan localizarse cercanas a este grupo de haces en el cerebelo y/o troncoencéfalo, y, en consecuencia, mejorar la planificación quirúrgica y alcanzar una técnica microquirúrgica más segura y precisa.

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All this has motivated the conduct of an essentially anatomical laboratory study using the nerve fibre microdissection technique with the main objective of demonstrating the topography and relationships of the main projection fibre systems of the human brain: the superior, medial and inferior cerebellar peduncles. This study offers a microsurgical perspective of the configuration of the cerebellum which is supplemented with the demonstration of these systems by means of MRI DTT performed in healthy subjects.

Material and methods

The anatomical study was performed in the neuroanatomy laboratory at Hospital Universitario de la Ribera (Alzira, Spain) from July 2014 to July 2015. There, multiple human brain specimens—15 brainstems, 15 cerebellar hemispheres and 5 cerebral hemispheres—were examined and dissected using the fibre dissection technique, widely reported in the literature.10,12–14,38 To do this, the specimens were removed from the cranial cavity, stripped of dura mater and placed in a 10% formaldehyde solution for at least 2 months. After that, they were carefully stripped of pia mater, arachnoid mater and blood vessels. Next, the specimens were frozen at −10°C to −15°C for 7–10 days. Thereafter, they were submerged in water until they thawed (2–3 h). At that point, they were ready for anatomical dissection. They were preserved during the dissection process in a 5% formaldehyde solution.

In order to progressively follow anatomical planes and detail and identify even the thinnest fibres, bundles of white matter were systematically microdissected using microscopic vision (6–40×) and the following instruments: scalpel with 15 and 11 mm blades, microsurgery scissors, microdissection forceps of different sizes, fine aspirators and, on occasion, fine-pointed wooden spatulas (<1 mm thick and 3 mm wide). Dissection started on the superior aspect and lateral border of the cerebellum, from the most superficial structures to the deepest structures, accompanied by dissection of the anterolateral surface of the midbrain and pons, and finally culminated in dissection of the inferior aspect of the cerebellum. During the different steps, each specimen was photographed using a Nikon D3000 camera (with an AF-S VR Micro-Nikkor 105 mm f/2.8G IF-ED lens), Nikon Corp, Sendai, Japan, and 2 free wireless flashes. A tripod with a built-in pan and tilt head was also used to perform 2 captures of the same image from 2 different perspectives and thus prepare 3-dimensional images. The images were fused in an anaglyph to create three-dimensional photographs using the software program Adobe Photoshop CS6 version 13.0 × 64 for Macintosh.

The second part consisted of a radiological study with DTT images obtained from brain MRIs performed with a 1.5-Tesla Philips Achieva device on 15 healthy subjects from July to December 2015. The fascicles chosen in tractography were traced with enhanced diffusion sequences using DTVII SR toolbox software (Hospital Universitario de la Ribera Radiology Department). This allowed tractographies with 30 directions (b = 1,000; voxel = 2 × 2 × 2 mm) to be prepared. The technique based on selection of regions of interest (ROIs) reported by Catani et al.21 was applied. This was essentially guided by classic anatomy books1,2,39–41 and the knowledge obtained in the anatomical phase. Thus, three-dimensional volumes of the white matter projection fascicles selected—the superior, medial and inferior cerebellar peduncles—were created.

Results

Anatomy and dissection of the superior surface and lateral border of the cerebellar hemisphere and brainstem

The superior surface of the cerebellum is also known as the tentorial surface due to its relationship to the tentorium cerebelli. The anteromedial portion of this surface constitutes its apex, formed by the anterior part of the vermis, the culmen, the highest point of the cerebellum. The part of the cerebellar hemisphere corresponding to this surface includes the quadrangular, simple and superior semilunar lobules, while the vermis includes the culmen, declive and folium. The tentorial or primary fissure, between the quadrangular and simple lobules in the hemisphere and the culmen and declive in the vermis, divides this surface into an anterior lobe and a larger posterior lobe. The larger and deeper horizontal fissure separates the superior and inferior semilunar lobules and is identified on the anterior surface and lateral border of the cerebellar hemisphere, extending up to the foramen of Luschka in the cerebellopontine cistern (Fig. 1).

Initial dissection of the superior surface and lateral border of the cerebellum consists of removing the cerebellar cortex which covered the anterior lobe (the lingula, the central lobule together with the wing of the central lobule, and the culmen together with the quadrangular lobule) and part of the posterior lobe (the declive together with the simple lobule, and the folium together with the superior semilunar lobule), thus exposing the narrow sheets of white matter which constitute the cerebellar folia as projections from the deep white matter of the cerebellum. After dissecting the white matter of the cerebellar folia, we identified superficial radiations mainly from the middle cerebellar peduncle, which course and project in a posterior direction both laterally and medially, to show terminations in most of the lobules of the cerebellum, minus the nodule and the flocculus. The middle cerebellar peduncle, which contains more fibres, originates from transverse pontine fibres largely from the contralateral pontine nuclei, which travel obliquely on the lateral surface within the pons, crossed by the fibres of the trigeminal nerve, to form part of the floor of the cerebellopontine angle before entering the cerebellum, thus positioning itself lateral to the superior and inferior cerebellar peduncles, with no direct relationship to the cavity of the fourth ventricle.

After removing deeper fibres of the middle cerebellar peduncle, mainly in the depth of the quadrangular lobule, the fibres of the inferior cerebellar peduncle, whose bundles have a characteristic arrangement and pathway, are observed. The white matter of the centre of the cerebellum crosses from lateral to medial to continue dorsomedially around the hilum of the dentate nucleus and proceed mainly towards the vermis. The rostral border of the inferior cerebellar peduncle is then seen anterior to the level of the union of the superior cerebellar peduncle with the dentate nucleus. Similarly, dissection of the deep fibres of the middle cerebellar peduncle exposed...
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Fig. 1 – Tentorial or superior surface (A), suboccipital or inferior surface (B) and petrosal or anterior surface (C) of the cerebellum. Abbreviations with white letters refer to fissures. bil: biventer lobule; ca: cerebellar amygdala; cel: central lobule; cu: culmen; de: declive; flc: flocculus; isl: inferior semilunar lobule; mo: medulla oblongata; pf: primary fissure; pon: pons; py: medullary pyramid; quil: quadrangular lobule; sl: simple lobule; ssl: superior semilunar lobule; uv: uvula.

A

B

C

a thin capsule of fibres covering the superior surface of the dentate nucleus (Fig. 2A).

On the lateral surface of the midbrain tegmentum, a thin and superficial layer of fibres forming the tectospinal tract is identified behind the lateral midbrain sulcus and anterior to the fibres of the superior cerebellar peduncle. The tectospinal tract is an efferent bundle from the superior colliculus that descends and continues towards the pontine tegmentum. When this tract is removed, a group of fibres is exposed that ascends obliquely, superficially and ventrally to the fibres of the superior cerebellar peduncle, some of which reach the ipsilateral inferior colliculus while others continue below the brachium of the inferior colliculus. These fibres, from posterior and medial to anterior and lateral, correspond to the lateral lemniscus, the spinothalamic tract and some fibres from the dorsolateral portion of the medial lemniscus, which constitutes the most superficial portion of their trajectories through the brainstem, occupying the area known as the lemniscal trigone.3,47 The lateral lemniscus terminates in the ipsilateral inferior colliculus, while the spinothalamic tract and the medial lemniscus turn dorsally and ascend in the depth of the brachium of the inferior colliculus towards their final destinations in the thalamus. From the pontomesencephalic sulcus, dissection continued on the anterior surface of the pons. This enabled determination of how the corticospinal tract divides longitudinally into several bundles which interdigitate with the transverse pontine fibres connecting the pontine nuclei to the middle cerebellar peduncle (Fig. 2A).

Dissecting part of the fibres of the inferior cerebellar peduncle and all other fibres comprising the capsule of the dentate nucleus allows the superior surface of this nucleus along with the fibres of the superior cerebellar peduncle to be distinguished. The dentate nucleus consists of well-defined islets of grey matter forming nearly parallel bars, separated by superficial sulci which contain white matter. The fibres which depart from this nucleus come together in its hilum and combine with those originating in the globular and emboliform nuclei to form the superior cerebellar peduncle, located in the depth of the culmen and central lobule of the vermis as well as part of the quadrangular lobule and the wing of the central lobule of the cerebellar hemisphere. The superior cerebellar peduncle is arranged medial to the medial and inferior cerebellar peduncles and continues on a pathway that ascends in an anterior and superior direction, initially as part of the lateral wall of the fourth ventricle, to later contribute, along with the superior medullary velum and its contralateral counterpart, to forming the ceiling of the fourth ventricle. It ascends towards the interior of the midbrain tegmentum under the fibres of the lateral lemniscus and inferior colliculus (Fig. 2B–D, G–I). The inferior cerebellar peduncle ascends dorsolaterally in the medulla oblongata, dorsal to the olive, lateral to the gracile and cuneate tubercles, and deep to the medullary striae and dorsal cochlear nucleus in the lateral recess of the fourth ventricle, laterally covered by the flocculus, to form part of the lateral wall of the fourth ventricle. On its ascent, the inferior cerebellar peduncle is intimately related to intrapontine fibres of the facial and trigeminal nerves in its ventromedial portion. It also intersects with fibres from the middle cerebellar peduncle, where it changes direction to proceed backwards obliquely and enter the cerebellum, mainly between the superior and middle cerebellar peduncles (Fig. 2E and F).

Anatomy and dissection of the inferior surface of the cerebellar hemisphere and posterior surface of the brainstem

The main hemispheric structures on the inferior or suboccipital surface of the cerebellum are the biventer lobules and the cerebellar amygdalae, as well as the uvula on the midline. The fissures that surround the cerebellar amygdalae and delimit its free borders—the cerebellomedullary fissure, the amygdalar–biventral fissure and the fissure that separates it from the uvula—are recognised (Fig. 1B and Fig. 3A). The right
The amygdala is dissected by separating the fibres from its superolateral portion. Through these fibres, the peduncle of the cerebellar amygdala, it inserts into and connects to the rest of the cerebellar hemisphere. This exposes the inferior medullary velum, the tela choroidea with the choroid plexus and the telovelar junction, forming the inferior part of the ceiling of the fourth ventricle. The structures of the cerebellar vermis (uvula and nodule in depth, from which the inferior medullary velum arises) may be clearly visualised. Next, the left cerebellar amygdala and both telae choroideae were dissected, largely exposing the cavity of the fourth ventricle and both lateral recesses, as well as the posterolateral surface of the brainstem, mainly the medulla oblongata. The inferior medullary velum extends as a sheet of white matter to each side of the nodule; its convex border continues with the white matter of the cerebellum, specifically through the so-called peduncle of the flocculus, at the level of the outer margin of the lateral recess. The inferior cerebellar peduncle ascends from the posterolateral surface of the medulla oblongata to later form part of the lateral border of the IV ventricle, as well as the anterior and superior margins of the lateral recess, where it will maintain contact with fibres of the medial and superior cerebellar peduncles while proceeding posteromedially towards the cerebellar hemisphere on the

Fig. 2 – Progressive dissection on the superior surface and lateral border of the cerebellar hemisphere and brainstem. Abbreviations with white letters refer to sulci and fissures. (A) Dissection on the superior surface and lateral border of the cerebellum exposes the superior cerebellar peduncles (scp), middle cerebellar peduncles (mcp) and inferior cerebellar peduncles (icp). (B–D) Advanced dissection, enabling observation of the relationships between the cerebellar peduncles and the dentate nucleus (dn) (lateral, posterolateral and posterior view, respectively). Fibres belonging to the medial and lateral lemnisci (lle) and in the basilar portion of the pons and the corticospinal tract (cst) unfolded in several bundles are identified on the lateral surface of the midbrain tegmentum. (E and F) Part of the ascent of the inferior cerebellar peduncle (icp) is seen on the anterolateral surface of the brainstem. G–I correspond to images B–D in 3D, respectively (anaglyph glasses in red and cyan must be used to visualise them properly). bico: brachium of the inferior colliculus; cp: cerebral peduncle; cst: corticospinal tract; cu: culmen; de: declive; dn: dentate nucleus; flc: flocculus; ico: inferior colliculus; icp: inferior cerebellar peduncle; lle: lateral lemniscus; lms: lateral midbrain sulcus; mcp: middle cerebellar peduncle; ol: olive of the medulla oblongata; pf: primary fissure; pg: pineal gland; pon: pons; py: medullary pyramid; qul: quadrangular lobule; sco: superior colliculus; scp: superior cerebellar peduncle; sl: simple lobule; smv: superior medullary velum; ssl: superior semilunar lobule; tl: temporal lobule; tp: thalamic pulvinar; ver: vermis of the cerebellum; III: oculomotor nerve; IV: trochlear nerve; V: trigeminal nerve; VII–VIII: facial–vestibulocochlear nerves.
same side. The superior cerebellar peduncles are observed constituting the lateral walls of the superior portion of the fourth ventricle. The dentate tubercle, a prominence of the dentate nucleus, is located in the region of the lateral recess, close to the lateral border of the inferior medullary velum, which is superior and lateral to the vestibular area and lateral to the inferior cerebellar peduncle, as well as medial to the peduncle of the cerebellar amygdala (Fig. 3C and G).

On the superior surface of the cerebellar hemisphere we observed the fibres of the middle cerebellar peduncle following a pathway towards the midline, where finally the majority of them would cross to the contralateral hemisphere. However, when we removed the folia from the right biventer and inferior semilunar lobules, we revealed a set of fibres belonging to the middle cerebellar peduncle which advances curvilinearly below the dentate nucleus in a posterior direction towards the periphery, without crossing the midline. The flocculus, together with its connection to the inferior medullary velum through the peduncle of the flocculus, as well as the site of insertion of the amygdala into the cerebellar hemisphere, may easily be seen (Fig. 3D and H). Finally, dissecting the white matter of the right cerebellar hemisphere in greater depth exhibits the grey matter of the inferior surface of the dentate nucleus, thereby showing its relationships to the cerebellar peduncles and the IV ventricle (Fig. 3E and I).

**DTT of the cerebellar peduncles**

A better and clear perception of the three-dimensional arrangement of the fibres of the 3 pairs of cerebellar peduncles, acquired through the microdissections performed, was the fundamental pillar for identifying them properly when studying brain MRI DTI axial sequences, especially in those zones that are the most constant and have the greatest anatomical distinction. This enabled more rigorous selection of the corresponding ROIs during tracking on the colour DTI map. Thus reproduction and subsequent triplanar demonstration through tractography images of the superior, medial and inferior cerebellar peduncles were achieved, thereby providing additional qualitative and descriptive information.
Superior cerebellar peduncle

The area of the dentate nucleus, corresponding to the origin and therefore to the initial portion of the superior cerebellar peduncle recognised in the axial slices of the colour DTI map, was selected as the first ROI. The highest portion was identified approximately 1 mm below the ipsilateral inferior colliculus, just after entering the midbrain tegmentum, which constituted the second ROI. Along its ascent on the ceiling of the fourth ventricle (cerebellopontine level), approximately 7 mm equidistant from the first 2 ROIs and 5 mm from the midline, the third ROI, corresponding to the midpoint of its path, was selected. Tractography of the superior cerebellar peduncle illustrated the known pathway from the cerebellar grey nuclei to their destination in the thalamus. However, it was not possible to demonstrate its decussation in the midbrain tegmentum. In this case, the fibres of both peduncles ascended to approach the midline without coming into contact with those on the contralateral side at the height of the red nucleus. Therefore, the tractographical phenomenon known as kissing was not shown (Fig. 4). Identifying an ROI at the site of the decussation of the superior cerebellar peduncles reproduced erratic fibres, potentially related to the central tegmental tract, very close in a posterolateral position to the red nucleus, or also from the ventral tegmental decussation, which includes descending rubrospinal fibres deriving from the superior cerebellar peduncle.2,43,44

Middle cerebellar peduncle

To represent the cerebellopontine fibres, a 3 mm axial slice inferior to the emergence of the trigeminal nerve was used and 2 ROIs were selected corresponding to the area of the middle cerebellar peduncle on each side, from its start just lateral to the emergence of the trigeminal nerve in the pons to its entry into the cerebellum (occupying an area of approximately 15 mm on its anteroposterior axis). As occurred in the microdissections, accurate demonstration of cerebellopontine fibres from contralateral pontine nuclei was not possible in the radiological phase; in its place both middle cerebellar peduncles were simultaneously demonstrated and the continuity and connection of their fibres through the midline, suggestive of the contralateral origin of many of them, were observed (Fig. 5).

Inferior cerebellar peduncle

The search for the inferior cerebellar peduncle started in the dorsolateral and inferior portion of the medulla oblongata. Its identification was clearer starting from the height of the olive of the medulla oblongata, ascending to constitute the corpus restiforme on the superior part of the medulla oblongata, where the first ROI was selected (around 8 mm superior to the obex, 2 mm under the lateral recess and occupying the area from 5 to 8 mm lateral to the posterior medial sulcus). We followed it towards the cerebellum and located the confluence of the large peduncular mass on the lateral walls of the fourth ventricle, to later advance and continue towards the inside of the cerebellum, where the second ROI was selected (approximately 1–2 mm superior and anterior to the ipsilateral dentate nucleus). Thus the tractography sequences show the main connection between the medulla oblongata and the cerebellum through the inferior cerebellar peduncle, continuing in front of and above the dentate nucleus to spread out mainly in the vermal and paravermal region of the anterior lobe of the cerebellum (Fig. 6A and B). Finally, the set of fibres of the 3 cerebellar peduncles was represented through tractography, which exhibited the relationships, arrangement and organisation of these bundles of white matter on the axial, coronal and sagittal brain MRI planes (Fig. 6C and D).

The limitations of the study include the characteristic technical limitations related to the equipment and software used in this study, such as the lack of quantitative information with respect to size, fibre volume, number of fibres in a tract, fraction of anisotropy and apparent diffusion coefficient, as well as the impossibility of accurately selecting the same ROIs in different subjects. All this had an impact on the analysis of the tractography results and motivated focusing the objective on achieving radiological reproduction of the fibre bundles studied during the laboratory phase, for demonstration purposes only.
Fig. 5 – Demonstration of the middle cerebellar peduncles through DTT images on different brain MRI planes.

Discussion

Historical–anatomical account of the study of the cerebellar peduncles based on fibre dissection

The first representations of the superior and middle cerebellar peduncles, among other central nervous system structures, emerged in 1543 with the publication of De Humani Corporis Fabrica by Andrea Vesalius (1514–1564). In his atlas Neurographia universalis (1685), the French anatomist Raymond Vieussens (1641–1715) described and represented, although imprecisely and with low-quality images, the cerebellar peduncles, especially the medial and inferior peduncles, as well as their connections to the brainstem. Franz Joseph Gall (1758–1828), in collaboration with his student Johann C. Spurzheim (1776–1832), strengthened the study of the projection fibres, and his anatomical studies, published in 1810, identify good illustrations of the middle cerebellar peduncles.

The studies of Herbert Mayo (1796–1852) stand out, for their era, due to the better dissections and illustrations of the superior, medial and inferior cerebellar peduncles, where the main relationships between them and to other neighbouring tracts are distinguished (Fig. 7). Subsequently, other anatomists, such as Friedrich Arnold (1803–1890), Achille L. Foville (1799–1878) and JB Luys (1828–1895) contributed information on the white matter of the cerebellum and brainstem with great detail and spectacular images. Later on, in the middle of the 20th century, Joseph Klingler (1888–1963) and his teacher, Ludwig, published their masterpiece, Atlas Cerebri Humani, which displayed a wide variety of detailed dissections, including dissections of the cerebellum and brainstem. Recently, with the development of the microscope, the technique has been rescued and interest has been awakened in the study of fibre dissection, including the region of the cerebellum, oriented more towards microsurgical anatomy.

Anatomy of the surface of the cerebellum and its relationship to the dissection of the cerebellar peduncles

On the superior surface of the cerebellum are the culmen, declive and folium, which form part of the vermis, and the quadrangular, simple and superior semilunar lobules, which form part of the cerebellar hemispheres, respectively. These dissections confirmed the close relationship of the 3 cerebellar peduncles to this surface. Thus, fibres of the middle cerebellar peduncle, in an anteroposterior and lateromedial direction, are found in the depth of the quadrangular and simple lobule. Many of these follow a curve that projects towards most of the cerebellar lobules, thereby constituting part of the final destination of the cortico-ponto-cerebellar afferences. In an anterior and medial direction, the fibres of the inferior cerebellar peduncle which pass in front of the dentate nucleus towards the vermis and paravermis were identified. A group of fibres from the inferior cerebellar peduncle crosses the midline while others continue ipsilaterally; however, during microdissections, the intimate relationship between some of these fibres and those of the middle cerebellar peduncle makes them very difficult to differentiate. In the most anterior portion of the depth of the superior surface (quadrangular and simple lobules), approximately 5 mm from the midline on each side, the superior cerebellar peduncles are arranged obliquely from inferior and posterior to superior and anterior, as part of the ceiling of the fourth ventricle, along with the superior medullary velum, proceeding towards the posterior aspect of the midbrain and continuing medial to the fibres of the lateral lemniscus, covered by the inferior colliculi (Figs. 1A and 2). Akakin et al. separated the fibres of the middle cerebellar peduncle which run in the depth of the superior surface of the cerebellum in 2 groups: the fibres in the first group are oriented parallel to the midline and are called corticocerebellar fibres, and the fibres in the second group project parallel to the dentate nucleus towards the superior and inferior semilunar lobules and are called cerebellopontine fibres. However, in this microdissection study, it was not possible to discern a clear separation between the two groups of fibres, and so application of this nomenclature, which can even create a certain amount of topographical and anatomical confusion, was avoided.
The anterior surface of the cerebellum is related on its superior part to the cerebellopontine angle and foramen of Luschka through the cerebellopontine fissure, which contains a superior brachium and an inferior brachium which come into direct contact with the middle cerebellar peduncle on its path towards the cerebellum to later be covered by the apex of the fissure, where the two brachia meet. The lateral recess and the foramen of Luschka open towards the medial portion of the inferior brachium of the cerebellopontine fissure, where the flocculus, the choroid plexus and the cranial nerve pairs—facial, vestibulocochlear, glossopharyngeal and vagus—are identified. Thus, during its ascending course the inferior cerebellar peduncle is closely related to the anterior and superior border of the lateral recess, being partially covered by the flocculus (which projects towards the cistern of the cerebellopontine angle) and by the most anterior border of the biventer lobule, both forming part of the posterior, superior and lateral border of this recess. As we descended on the anterior surface, we found the complex cerebellomedullary fissure as a continuation of the cerebellopontine fissure, close to the inferior portion of the floor of the fourth ventricle. On the anterior wall of this fissure we mainly observed the fibres of the inferior cerebellar peduncle and, behind them, the biventer lobule and the ipsilateral cerebellar amygdala, leaving the inferior medullary velum and the tela choroidea in a more medial location on the ceiling of the fourth ventricle (Figs. 1C and 2E,F). On the inferior surface of the cerebellum we identified the folium, the tuber, the
pyramid and the uvula as structures of the vermis, the superi- 
or and inferior semilunar and biventer lobules, and the cerebellar amygdala as structures of the cerebellar hemi- 
sphere, respectively (Figs. 1B and 3A). The lateral surface of the amygdala is in contact with the biventer lobule and separated from it through the amygdalar–biventral or retroamygdalar 
fissure; in the depth we identified fibres that proceed mainly from the middle cerebellar peduncle, which run inferior to the dentate nucleus, in close relationship to the so-called pedun- 
cle of the amygdala, which connects it from its superior and 
lateral border to the rest of the cerebellar hemisphere.

Therefore, the fibres of the middle cerebellar peduncle were 
demonstrated wrapping the dentate nucleus in a superior and 
inferior direction: those that pass the superior surface of the cerebellar hemisphere form a curve, emitting radia-
tions towards multiple hemispheric regions, including some that are oriented towards the midline, part of which will 
potentially cross towards the contralateral hemisphere; those 
that continue on the inferior surface do so predominantly in 
an anteroposterior oblique direction on the cerebellar amygd-
dalae while remaining in the same hemisphere, similar to that 
reported by other authors58 (Fig. 3). However, microdissection 
of peduncular fibres in the area close to the lateral recess did 
not enable appropriate separation of the limits between those 
that ascend as part of the inferior cerebellar peduncle from 
those corresponding to the inferior radiations of the middle 
cerebellar peduncle. Unlike Perrini et al.,50 when we followed 
the fibres of the inferior cerebellar peduncle, we did not clearly 
identify radiations that continue under the ipsilateral dentate 
nucleus. This is consistent with data from classic literature 
that indicate their major ascending trajectory above and in 
front of this nucleus.1–3,52

Cerebellar peduncles and surgical implications

When surgically approaching the cerebellum, together with 
the cavity of the fourth ventricle, their relationships to the grey-matter structures (dentate nucleus) and white-matter 
structures (cerebellar peduncles) found inside must be taken 
into account. As in the cerebrum, the normal cortex and cerebellar white matter, mainly peduncular fibres, should be 
preserved to the extent possible when resecting lesions inside 
or neighbouring them. The morbidity inherent to violating 
them (due to inappropriate dissection or retraction, or even 
partial retraction) requires implementation of strategies that 
attempt to protect their integrity during surgery.

The middle cerebellar peduncle is most vulnerable to being 
affected during approaches to the cerebellomedullar fissure 
(retosigmoid or suboccipital lateral craniotomy towards 
the region of the cerebellomedullar angle), in both their suprafla-
cular and infralocular variants.53–56 Lesion of this peduncle 
causes ataxia and dysmetria during voluntary movements of 
isilateral limbs and hypotonia similar to those that 
appear when the lateral part of the cerebellar hemisphere is 
affected.57,58 When accessing the inside of the middle cere-
bellar peduncle and the lateral portion of the pons, incision 
and dissection must be done preferably in a horizontal direc-
tion, lateral to the emergence of the trigeminal nerve and 
following the parallel anatomical trajectory of their cerebel-
lopontine fibres,59,60 in an attempt to preserve their integrity.

As the fibres of the middle cerebellar peduncle go deeper, the 
corticospinal tract will be located in an anterior and medial 
direction in the basilar portion of the pons, while the fibres of the medial and lateral lemnisci will be located in a medial 
direction in the depth of the pontine tegmentum and the inferior cerebellar peduncle will be located in a medial and 
caudal direction (mainly its spinocerebellar fibres). Extending 
dissection through the inferior portion of the cerebellomedullar 
fissure and its continuation with the superolateral border of the cerebellomedullary fissure exposes the dorsolateral sur-
face of the medulla oblongata, low cranial nerve pairs and 
lateral border of the inferior cerebellar peduncle, recently 
reported as a route of access to resect lesions affecting this 
peduncle.61

On the inferior surface of the cerebellar hemisphere, the 
relationships between the cerebellar amygdala and the biven-
ter lobule are significant for the supra-amygdalar approach 
through the amygdalar–biventral fissure, proposed by Lawton 
to resect arteriovenous malformations in the inferior cere-
bellar peduncle.62 This approach requires retraction of the 
amygdala and may affect the fibres that connect it to the cere-
bellar hemisphere. However, it is recognised as a favourable 
route to access both the medial fibres of the inferior cerebel-
lar peduncle as they pass through the ceiling of the lateral 
recess of the fourth ventricle and those of the middle cere-
bellar peduncle which cross the inferior border of the dentate 
nucleus. This nucleus, which is found just above and there-
fore has a close relationship to the peduncle of the amygdala, 
may be lesioned during dissection and cause mainly balance 
abnormalities and intention tremor in limbs.53

Studies by Matsushima et al.58 emphasised the micro-
surgical anatomy of the fourth ventricle and its approach 
through the tela choroidea and the inferior medullary velum. 
Yaşargil1 reported entering the fourth ventricle through the 
sulcus between the amygdala and the uvula, along the medial 
division of the posteroinferior cerebellar artery. Subsequently, 
the concept of the “transcerebellomedullary” approach was 
modified by detailing its benefits and limitations.63–66

Although the 3 cerebellar peduncles converge on the lateral 
walls and the ceiling of the fourth ventricle, the direct prox-
imity of the superior and inferior cerebellar peduncles to the 
inside of the cavity of the fourth ventricle confers upon them 
a higher risk of being lesioned during surgical approaches to 
this region. Thus, in the approach to the cerebellomedullary 
fissure, initial dissection of the space between the amygdala 
and the posterolateral surface of the medulla oblongata, as 
well as lateral dissection through the opening of the tela 
choroidea to reach and expose the lateral recess, render the 
inferior cerebellar peduncles more vulnerable. Opening the 
inferior medullary velum to gain access to higher areas of 
the ventricular ceiling exposes the superior cerebellar pedun-
cles, especially above the height of the lateral recesses and 
dentate tubercle, thereby making them more prone to being 
lesioned during dissection of lesions in this area. Damage to 
the fibres of the superior cerebellar peduncle causes ipsilateral 
tension tremor, dysmetria and decomposition of movement, 
while damage to the inferior cerebellar peduncle causes bal-
ance abnormalities similar to those caused by impairment of 
the flocculonodular lobe, with truncal ataxia, unstable gait 
and a tendency to fall towards the same side of the lesion.58
Finally, the superior cerebellar peduncles also have a higher risk of being affected during approaches to the cistern of the cerebellar–mesencephalic fissure. Special mention is made of the paramedian infratentorial supracerebellar approach, reported by Yaşargil and widely used for aneurysm disease of the superior cerebellar artery, arteriovenous malformations and tumour lesions involving the superior surface of the cerebellum, the dorsolateral region of the midbrain and the pineal and parapineal region, as well as more lateral variations of this approach reported by other authors. Dissection along the superior surface of the cerebellum, 2 or 3 cm from the midline, enables opening of the cerebellar–mesencephalic cistern and subsequent exposure of the superior cerebellar peduncle just after retracting the quadrangular lobule, a place in which it may easily be lesioned. Transtentorial interhemispheric posterior (transtentorial occipital) approaches allow access to the ipsilateral half of the cerebellar–mesencephalic fissure with exposure of the pineal and parapineal region, the dorsolateral region of the midbrain and part of the ceiling of the fourth ventricle, including the superior cerebellar peduncle.

**Tractography and microdissection of the cerebellar peduncles**

The development of diffusion tensor-based DTI and tractography techniques more than a decade ago represented an extraordinary achievement. The information obtained through DTI and thus from tractography images possesses considerable value, providing visualisation and qualitative and quantitative characterisation of the major routes of white matter. Its incorporation as a tool in the preoperative study of patients with brain lesions is becoming more and more common, with the corresponding responsibility that it represents for neurosurgeons to tackle an appropriate and critical interpretation of its results.

As the fibre microdissection technique was adopted, the laboratory study enabled observation of the fundamental macroscopic and microscopic arrangement of the 3 pairs of peduncles that connect the cerebellum to the rest of the hindbrain and forebrain, which are intimately related to the grey matter nuclei and other surrounding tracts. It should be remembered that dissection of one system of fibres generally results in destruction of another, and that clearly demarcating small fibre bundles as well as identifying their origin or termination may become an arduous task, despite the availability of microsurgical instruments and high magnification, as this study showed. However, it was possible to show them and follow their main path in the cerebellum and brainstem as well as conceive of their course and main spatial relationships to one another and to other parenchymatous structures. This knowledge is difficult to acquire solely through study of historical illustrations, which are common and widely reported in the literature.

This three-dimensional anatomical knowledge unique to white matter is particularly beneficial for neurosurgeons involved in the study and treatment of patients with intrinsic lesions of the central nervous system, including the cerebellum and the brainstem, the main subject of this study, as it increases precision at the time of reconstruction in vivo through tractography of these bundles. Therefore, the two techniques complement and enrich one another, despite their shared limitations: difficulty distinguishing areas where fibres intersect, difficulty determining the cortical and subcortical origins and terminations of tracts, and lack of anatomical accuracy when attempting to delimit contiguous tracts with a similar trajectory.

Finally, it should be noted that the study of “normality” (both anatomical and tractographical) in relation to the arrangement of the main projection fibres, including the cerebellar peduncular fibres, may be translated to the clinical practice setting, being helpful when performing clinical and radiological analysis of a patient with an intrinsic lesion of the posterior fossa (highlighting tumours and cavernomas) and facilitating understanding of the relationships between the lesion and the surrounding healthy tissue, including potential changes in the spatial configuration of these bundles—all with the objective of a better indication, planning, strategy and microsurgical technique to achieve maximum resection of the lesion while avoiding damage to these functional structures and minimising morbidity.

**Conclusions**

The microdissection technique enabled observation of the general topographical arrangement, architecture and organisation of the superior, medial and inferior cerebellar peduncles, crossing and connecting the cerebellum to the brainstem, and determination of their relationships to one another, to intrinsic neighbouring neural structures and to the surface of the cerebellum and brainstem. This knowledge contributed a unique, in-depth anatomical perspective that promoted the representation and proper interpretation of DTI images. This information should be translated to clinical practice to promote comprehensive critical analysis by the surgeon when there are lesions that may be located close to this group of bundles in the cerebellum and/or brainstem and thus improve surgical planning and achieve a safer and more precise surgical technique.

**Conflicts of interest**

The authors declare that they have no conflicts of interest.

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