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Combining Fiber Dissection, Plastination, and Tractography for Neuroanatomy Education: Revealing the Cerebellar Nuclei and Their White Matter Connections

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In recent years, there has been a growing interest in white matter anatomy of the human brain. With advances in brain imaging techniques, the significance of white matter integrity for brain function has been demonstrated in various neurological and psychiatric disorders. As the demand for interpretation of clinical and imaging data on white matter increases, the needs for white matter anatomy education are changing. Because cross-sectional images and formalin-fixed brain specimens are often insufficient in visualizing the complexity of three-dimensional (3D) white matter anatomy, obtaining a comprehensible conception of fiber tract morphology can be difficult. Fiber dissection is a technique that allows isolation of whole fiber pathways, revealing 3D structural and functional relationships of white matter in the human brain. In this study, we describe the use of fiber dissection in combination with plastination to obtain durable and easy to use 3D white matter specimens that do not require special care or conditions. The specimens can be used as a tool in teaching white matter anatomy and structural connectivity. We included four human brains and show a series of white matter specimens of both cerebrum and cerebellum focusing on the cerebellar nuclei and associated white matter tracts, as these are especially difficult to visualize in two-dimensional specimens and demonstrate preservation of detailed human anatomy. Finally, we describe how the integration of white matter specimens with radiological information of new brain imaging techniques such as diffusion tensor imaging tractography can be used in teaching modern neuroanatomy with emphasis on structural connectivity. Anat Sci Educ 00: 000–000. © 2013 American Association of Anatomists.

Key words: neuroscience education; medical education; brain dissection; white matter; fiber dissection; plastination; diffusion tensor imaging (DTI) tractography; neuroanatomy; cerebellum

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INTRODUCTION

White matter anatomy has gained particular interest due to recent developments in neuroimaging and computer visualization methods. Emerging techniques, such as diffusion tensor imaging (DTI) and tractography, are able to characterize the architecture of the various axonal fiber bundles comprising the white matter of the human brain. Diffusion-based tractography is a MRI technique in which measurement of directional movement of water molecules in the human brain is

used to reconstruct virtual three-dimensional (3D) representations of white matter tracts (Le Bihan, 2003; Ciccarelli et al., 2008; Mori et al., 2009). These new techniques have resulted in important information on the structural connectivity of the brain and therefore contributed to the understanding of brain function (Johansen-Berg and Rushworth, 2009; Le Bihan and Johansen-Berg, 2012). As white matter can be visualized in the living brain, its importance is increasingly recognized in both research as well as medical practice. Recently, white matter abnormalities have been demonstrated in a wide variety of neurological and psychiatric diseases, such as stroke, Alzheimer's disease, and schizophrenia (Ciccarelli et al., 2008; Schmahmann et al., 2008). In neurosurgery and neuroradiology, fiber tracking is already becoming part of clinical protocols (Dimou et al., 2013), being used for perioperative grading, monitoring and the evaluation of brain tumors.

Hence, understanding the white matter anatomy of the brain is essential for medical students and researchers in the field of neuroscience (Familiari et al., 2013). Moreover, it can be consequential for medical specialists, as knowledge of brain connectivity will assist in the interpretation and critical appraisal of clinical and radiological information (Filley, 1998, 2005). With the rapid advance in medical imaging, interpretation of 3D information has become very important (Marks, 2000; Estevez et al., 2010). Consequently, the demand for insight in the 3D white matter anatomy is growing and the needs for anatomical education are changing. The traditional approach of classical medical neuroscience curricula often falls short in representing the complex 3D relationships of various brain structures (Estevez et al., 2010). The classical approach for studying neuroanatomy is based on cadaver dissections and orthogonal two-dimensional (2D) projections (Nolte and Angevine, 2007; Moon et al., 2010). It can be difficult to reveal the complex 3D architecture of white matter by gross anatomy or sectional anatomy as white matter appears as a homogeneously mass in which it is nearly impossible to identify the individual fasciculi in their full extent from origin to termination. This is certainly not trivial, as this is an important aspect in structural connectivity and therefore important for the functional and clinical implications of white matter anatomy (Filley, 1998, 2005). Classical fiber dissection is a technique that is used to isolate complete fiber pathways by removing parts of the cortex and underlying white matter of formalin-fixed brains. It is a well-defined preservation and dissection technique that was greatly improved by Professor Josef Klingler (1888–1963) at the University of Basel in the 1930s (Klingler, 1935; Ludwig and Klingler, 1956; Klingler and Goor, 1960; Agrawal et al., 2011). It is an effective method to isolate a limited number of white matter fascicles and create a 3D white matter specimen. In recent years, there has been a growing interest in fiber dissection to study the white matter anatomy of the human brain. Postmortem fiber dissections are often performed by neurosurgeons and anatomists (Ture et al., 2000; Peuskens et al., 2004; Fernandez-Miranda et al., 2008) and can provide new insight in surgical approaches. In addition, 3D specimens that are made by fiber dissection can serve as tools in teaching white matter anatomy. In this study, we give a detailed account of the materials and methods we used to obtain white matter specimens. As these specimens can easily be damaged by even the slightest touch, we combined classical fiber dissection with tissue plastination to produce detailed white matter specimens that are durable, easy to handle, and do not require special care or conditions. Plastination is a well-established preservation technique and consists of a process of fixation, dehydration, forced impregnation, and hardening of biological tissues (von Hagens, 1979, von Hagens et al., 1987; Weiglein, 1997; Pashaei, 2010). Plastination has become an important tool for the educational display of human anatomy (Jones, 2002; Reidenberg and Laitman, 2002). Hence, plastinated brain tissue has also become an important instrument for teaching human neuroanatomy (Weiglein, 1997). Several articles have been published on plastination of the human brain, the majority of which report on plastination of brain slices (Ulfig and Wuttke, 1990; Suriyaprapadilok and Withyachumnarnkul, 1997; Weiglein, 1997; Sora et al., 1999; Baeres and Møller, 2001; Wadood et al., 2001; Steinke et al., 2002; Riederer, 2013). In this study, we show the application of the plastination technique to fiber dissection specimens of both cerebrum and cerebellum. While we also show cerebral fiber tracts, we focus our present dissection on the cerebellum as few dissection studies of the cerebellum are performed (Gluhbegovic and Williams, 1980). Furthermore, our technique is well illustrated by the cerebellar nuclei and associated white matter tracts, as these are especially difficult to visualize in 2D specimens. Finally, we describe the integration of white matter specimens with radiological information of DTI tractography for teaching modern neuroanatomy with emphasis on structural connectivity.

METHODS

Preservation Technique

We used a specific preservation method in order to facilitate white matter dissection. This method was originally described by Professor Josef Klingler (Klingler, 1935; Ludwig and Klingler, 1956; Klingler and Goor, 1960). Two male and two female brains were obtained from the body donor program of the Anatomical Institute of the Radboud University Nijmegen Medical Centre. Donors were above 60 years of age and without history of neuropathological diseases in their clinical records. The brains were carefully removed from formalin-perfused human cadavers. After removal of the dura mater, brains were fixed in a 10% formalin solution at room temperature for at least two months. To prevent deformation of the anatomy, the brain was suspended from the basilar artery in a bucket of formalin with a small cord. After the fixation period, brains were washed for one night and the remaining arachnoid membrane, pia mater and vasculature were removed. The brains were placed in fresh 10% formalin solution and stored in a refrigerator at −10 to −15 degrees Celsius for 8–10 days. Afterward, the brains were thawed in running water for one day and stored in the refrigerator once more for 8–10 days, as this protocol facilitates dissection (De Castro et al., 2005). Finally, the brains were thawed in running water for one day before dissection was started. Our brain specimens were stored in fresh 10% formalin in between dissection procedures. However, it is recommended that, if the dissection takes longer than one month, the specimens are kept frozen for at least twelve hours and thawed before the dissection is recommenced (Ture et al., 2000).

Dissection Technique

Before dissection, the specimen-specific surface anatomy of the sulci and gyri of the cerebrum and the surface anatomy of the cerebellum was studied in detail as this directs the initial steps of the dissection. Dissection of both the cerebrum and

Figure 1.

Dissection tools.

the cerebellum was performed using wooden spatulas, fine curved metal spatulas, fine forceps, and microdissectors (Fig. 1). The major pathways of the cerebrum could be exposed by peeling the fibers along the axonal direction from one end of the tract to the other. In this study, the cerebellum was approached from both its superior and inferior aspect, in different specimens. First, while dissecting the superior aspect of the cerebellum, numerous small blood vessels were removed between the white matter blades of the arbor vitae. The superficial cortical mantle of the cerebellar hemispheres was removed with a small wooden spatula and a small scissor. Hereafter, lobules of the cerebellar hemispheres and their white matter blades were removed by carefully tearing them toward the pons from the fissures bounding them inferiorly. The lobules were removed one by one from superior to inferior as far as the postero-inferior lobule. Once the contour of the dentate nucleus (DN) was observed, the overlying white matter was removed by using a dissection microscope (6×–40× magnification). When the DN was isolated, the other cerebellar nuclei were exposed by carefully peeling the remainder of the white matter from lateral to medial. The vermis was removed, taking care to spare the inferior peduncles.

The dissection of the inferior aspect of the cerebellum was performed using a similar approach. The cerebellar tonsils were partially dissected and the lobules of the inferior parts of the cerebellum were removed as far as the postero-inferior lobule. As the cerebellar nuclei could be identified from their distinctive colors, the dissection microscope was used to isolate these small structures.

Plastination Technique

Plastination is the technique developed by Gunther von Hagens in 1979 in which tissue fluids are replaced by curable polymers (von Hagens, 1979). It consists of four basic steps: specimen preparation, dehydration, impregnation, and curing. The followed procedure described below is based on the Dow™/Corcoran/room-temperature technique (Raoof et al., 2007) and the cold-temperature technique (DeJong and Henry, 2007) using the curable polymers from the room-temperature technique (PR10, Ct32, and Cr20; Dow Corning Corporation, Midland, MI).

Dehydration. To decrease shrinkage of the brain tissue due to degreasing of white matter, all dehydration steps were carried out in cold acetone (−20 degrees Celsius deep freezer; custom made by DKC Totaaltechniek, Wijchen, The Netherlands). During the dehydration procedure, which lasted approximately three weeks, acetone was stirred twice a day on average (excluding weekends).

After rinsing the dissected brains several days in running tap water, they were placed in specimen baskets and precooled for four hours in tap water in the fridge (four degrees Celsius). Subsequently, the baskets with the brains were submerged in baths containing 85%, 96%, and 100% acetone for one week in each bath. After these three weeks, the purity of the acetone was measured with an acetonometer (VisDocta® Research, Tignale, Italy) and confirmed to be >99%: dehydration was completed.

Impregnation. After dehydration, the baskets containing the brains were placed in a vacuum chamber (custom made by DeMaCo, Noord-Scharwoude, The Netherlands) containing a silicon polymer mixture of PR10 and Cr20 (100:5) at room temperature. Tissue was allowed to accommodate over the weekend. In contrast to the room-temperature technique (Raoof et al., 2007), we used the slow impregnation regimen as described by DeJong and Henry (2007): daily decreases of 1/3 in pressure until the acetone starts to vaporize. Pressure was measured with an analog manometer and a digital vacuum sensor (Baratron® manometer type 722A; MKS Instruments, Woerden, The Netherlands) with a gauge controller (Terranova Model 908A; Duniway Stockroom Corp., Mountain View, CA) placed in the fume hood for the fine tuning when pressure was >200 mbar.

Active bubble formation was apparent at 70 mbar, after which we decreased pressure in steps of 2–10 mbar 2–4 times a day. During nights and weekends, the vacuum chamber was kept at the current pressure by closing the system, while the vacuum pump (Scrollpump Dry Run I; ILMVAC GmbH, Ilmenau, Germany) was turned off. At day 11 of impregnation, active bubble formation had ceased at a pressure of 23 mbar: impregnation was completed. Valves were opened and the pressure was slowly returned to atmospheric pressure.

Curing. Tissue was allowed to accommodate over the weekend. Specimen baskets were raised from the reaction mixture and excess silicone-mix was allowed to drain into the vacuum chamber. Subsequently, the brains were removed from the baskets and were drained in a fume hood for 8–15 days in a container. During this period, brains were placed on absorbent paper. The brains were turned 1–2 times a day and the absorbent paper was changed every day (except weekends). When the paper remained (almost) dry, we started the curing procedure in the fume hood. Extra care was taken for the olfactory bulbs and the pituitary in the dissected brains. They were placed in the anatomical position and the pituitary was fixed to the brain with an iron pin until curing was completed.

A paint brush was used to apply a small amount of mixture of catalyst Ct32 and acetone (1:5) on the surface of the specimen. In contrast to the procedure described by Raoof et al. (2007), no foil was used to seal the specimen. Because (1) foil can leave an imprint of cured silicone on the surface of the specimen, and (2) our experience indicated that specimen also cure without sealing the specimen in an air-tight environment. Again, the brains were placed on absorbent paper and were turned 1–2 times a day. Excess silicone-mix was wiped from the surface and the absorbent paper was changed every day (except weekends). Applying the Ct32-acetone mixture once was enough to complete the curing within three weeks.

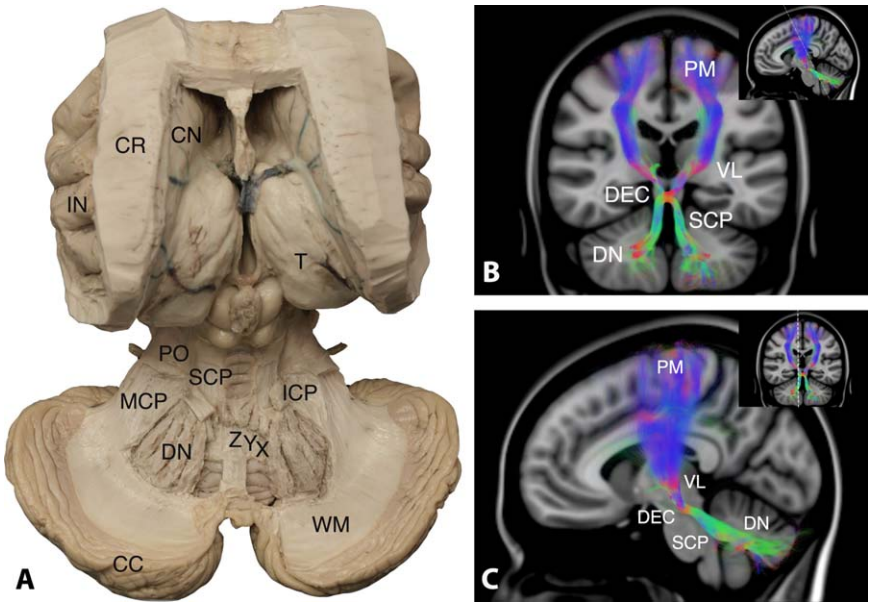

Figure 2.

A, Superior aspect of the cerebellum and its cerebellar nuclei and white matter tracts; CN = caudate nucleus; CC = cerebellar cortex; CR = corona radiata; DN = dentate nucleus; ICP = inferior cerebellar peduncle; IN = gyri of the insula; MCP = middle cerebellar peduncle; SCP = superior cerebellar peduncle; T = thalamus; WM = white matter; X = emboliform nucleus; Y = globose nucleus; Z = fastigial nucleus; **B** and **C**, T_1-weighted template image overlaid with tractography results of the dentatorubrothalamic tract with extensions from thalamus to cerebral cortex and from dentate nucleus to cerebellar cortex (modification of van Baarsen et al., 2013). DEC = decussation of the SCPs; DN = dentate nucleus; PM = premotor cortex; SCP = superior cerebellar peduncle; VL = ventrolateral nucleus of the thalamus.

Imaging Technique

In addition to our dissection and plastination, we used a high quality diffusion weighted imaging dataset from a healthy volunteer to obtain "virtual dissections" of many of the same tracts as uncovered in our specimens. The volunteer was scanned on a 3T Tim Trio MR system (Siemens, Erlangen, Germany) with a 32-channel head-coil using a twice-refocused spin-echo sequence with echo-planar readout (EPI) and TR = 13 sec and TE = 101 msec. A matrix size of 110×110 was sampled covering a field of view of 220×220 mm (GRAPPA AF = 2). Seventy slices of 2 mm were acquired resulting in a whole-brain dataset with an isotropic resolution of 2 mm. Diffusion weighted images were acquired in 256 directions equally distributed on the sphere at a *b*-value of 1500 sec mm^{-1}, interleaved with 24 volumes without diffusion weighting. The data were preprocessed with software developed in our institute and consisted of motion and cardiac pulsation artifact correction (Zwiers, 2010) and unwrapping of the distorted EPI images (Visser, 2012). Voxel-wise modeling and tractography were performed in Mrtrix v 0.2.9 (Tournier et al., 2012). Constrained spherical deconvolution with a maximum harmonic order of 8 was performed for every brain voxel to reconstruct the fiber configurations, estimating the response function from voxels with a fractional anisotropy higher than 0.7. Tractography was performed using the streamlining method, but in other cases using a probabilistic approach whenever simple streamlining did not reconstruct all elements of the anatomy of the fiber bundle. For each tract presented in this article, seed regions (as well as include regions and exclude regions) were segmented manually from the color-coded fractional anisotropy map following common anatomical knowledge. A thousand fibers were generated in each tract, using a radius-of-curvature constraint of 1 mm. The threshold for the amplitude of the fiber orientation distribution was set to 0.1. Unidirectional tracking with a stop criterion in the include region was used where the length of the tract exceeded the region of interest.

RESULTS

The dissection of our white matter specimens of both cerebrum and cerebellum was completed in two weeks. As the dissection was carried out by expert hands, trained on dissection, it took approximately seven to eight hours to produce one true and instructive brain specimen. With the use of the dissection microscope, it was relatively easy to dissect the small cerebellar nuclei and white matter of the cerebellum from both the superior and inferior aspect. The white matter tracts that were dissected in postmortem human brains could be successfully identified and compared with fiber tracts derived from in vivo DTI tractography.

Description of Specimens

The cerebellar nuclei and white matter tracts of the cerebellum: Superior view. In the first specimen, the superior parts of the vermis and cerebellar hemispheres were removed in order to isolate the cerebellar nuclei and their associated fiber tracts (Fig. 2A). The DN is located deep

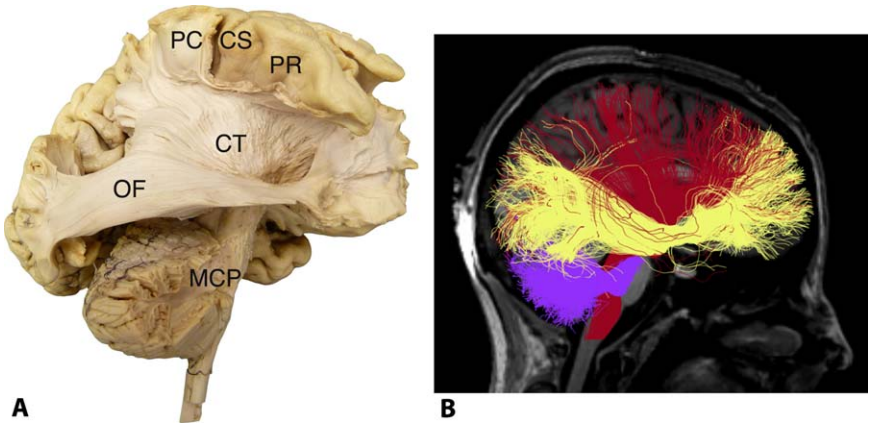

Figure 3.

A, Fiber dissection specimen of the long association and projection fiber tracts; CT = corticospinal tract; CS = central sulcus; MCP = middle cerebellar peduncle; OF = occipitofrontal fasciculus; PC = postcentral gyrus; PR = precental gyrus; **B**, T_1-weighted template image overlaid with tractography. Purple = white matter of the cerebellum and middle cerebellar peduncle; Red = corticospinal tract; Yellow = occipitofrontal fasciculus.

within the white matter of the cerebellum. The DN is characterized by crenated lamellae of gray matter and has an open anterior-median hilus to connect with fibers of the superior cerebellar peduncle (SCP). Fibers of the SCP are projected on the ventral side of the DN and can be followed upward as far as the inferior colliculus, under which they disappear. The inferior cerebellar peduncle (ICP) appears as a thick rope-like strand that crosses over the SCP. The fastigial nucleus (Z) lies near the midline, close to the fourth ventricle, from which it is separated by a thin section of the dorsal parts of the vermis and underlying white matter. The oval-shaped emboliform nucleus (X) is situated medially to the DN and is partially covered in its hilum. The globose nucleus (Y) has a close relationship with the emboliform nucleus (together they are often referred to as the interposed nucleus). The middle cerebellar peduncles (MCP) can be seen lateral to the superior and ICPs and fan out into blades of white matter that run to the cerebellar cortex.

The cerebral hemispheres have been dissected away in order to reveal the dorsal surface of the mesencephalon and diencephalon. In the median cleft of the specimen, the slit-like third ventricle can be observed which communicates with the lateral ventricles rostrally, and the fourth ventricle caudally. The posterior part of the pineal body is seen in the midline rostral to the two superior colliculi of the mesencephalon. The thalamostriate vein can be seen overlying the thalamus (T). The caudate nucleus is also visible. The thin, triangular, vertical membrane of the septum pellucidum separates the anterior horns of the left and the right lateral ventricles. The insular portion of the cerebral cortex is left intact and the gyri of the insula can be perceived on both sides of the specimen.

Finally, the specimen was combined with tractography results of the dentatorubrothalamic tract with extensions from thalamus to cerebral cortex and from DN to cerebellar cortex (Figs. 2B and 2C).

The cerebellar nuclei and white matter tracts of the cerebellum: Inferior view. The cerebellum was approached from its inferior aspect. The caudal parts of the vermis and the cerebellar tonsils were removed and the cerebellar nuclei were isolated and exposed against surrounding cerebellar white matter. The DN appeared as an irregularly dented mass of gray matter. The emboliform nucleus could be recognized and the spherical fastigial nucleus formed part of the roof of the fourth ventricle. The globose nucleus could be observed on the right side of the cerebellum but remained obscure in the left, as it appeared hard to isolate due to its small size. The white matter blades flowing from the MCP, connecting the pons with the cerebellum, were visualized. Finally, the specimen was combined with tractography results of cerebellar white matter and surrounding structures such as the medulla oblongata and vestibulocochlear and facial nerves.

Long association and projection fiber tracts of the cerebrum. The specimen shows several white matter structures, mainly long association fibers and projection fibers of the cerebrum (Fig. 3A). The right hemisphere is dissected, whereas the left hemisphere is left intact in order to use the gyral and sulcal pattern of the left hemisphere as a reference. The precentral gyrus (PRC) is intact, but the inferior part of the postcentral gyrus has been removed. In this way, the depth and orientation of the central sulcus can be studied. The corticospinal tract, containing projection fibers originating from the PRC, can be seen caudally and medially to the PRC running to the spinal cord. Another prominent white matter bundle, one of the major long association fiber tracts, is the occipitofrontal fasciculus. It connects the occipital with the frontal lobe. The posterior part of this fascicle has been exposed by manual tearing, without using a tool, and therefore exposing its most natural course. Note that this is possible only by virtue of the stack-of-sheets organization of the white matter in this region, i.e., without extensive fiber crossing. Furthermore, the pons and the right MCP have been partly dissected. The left temporal pole can be seen, indicating the amount of tissue taken away on the right side of the brain.

Finally, the specimen was combined with tractography results of both white matter tracts of the cerebellum and cerebrum (Fig. 3B).

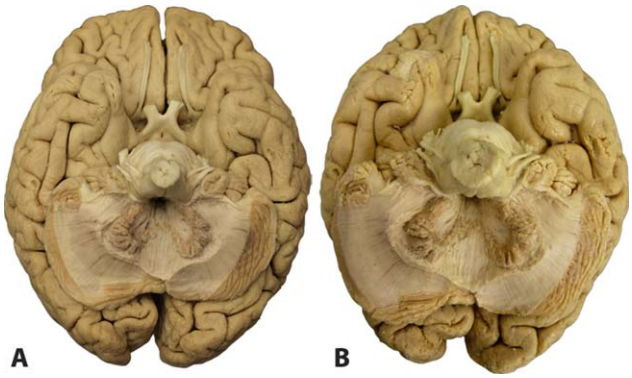

Figure 4.

A, Embalmed, nonplastinated specimen; **B**, Plastinated specimen. Fiber dissection specimen showing the inferior aspect of the cerebellum and its cerebellar nuclei and white matter tracts. The amount of shrinkage can be seen when comparing both frontal and temporal lobes of A or B.

DISCUSSION

In this study, we have described the use of fiber dissection and plastination to obtain durable and easy to use 3D white matter specimens that do not require special care or conditions for the purpose of using them as a tool in teaching white matter anatomy. White matter constitutes a complex 3D architecture in the human brain. Because in 2D sections of regular specimens of formalin-fixed brains the white matter appears mostly homogeneously white, it is impossible to show its individual fasciculi and their full extent from origin to termination. By using fiber dissection, it is possible to reveal this hidden 3D white matter structure. Professor Klingler's preservation technique is an essential step in the process of making fiber dissection specimens, as it prepares brain tissue and facilitates dissection. The freezing and thawing process of Klingler's technique is thought to allow ice crystals to form between the nerve fibers and loosen up the compact structure of the white matter of the brain (Ture et al., 2000; Agrawal et al., 2011). Formalin solution poorly penetrates myelinated nerve fibers and remains between the fibers. When the formalin solution expands as it freezes, it separates the nerve fibers and facilitates the dissection of white matter bundles in the human brain. While fiber dissection allows visualization of the 3D features of human brains, in practice, it has some limitations. First, if the main direction of the fiber bundles is not closely followed in the dissection, fibers break easily, giving the specimen's surface an artifactual appearance and the added value above dissection with a scalpel is wasted. Another important note concerns the unavoidable and ubiquitous fiber crossings that form the major difficulty in the fiber dissection. As fiber crossings are an integral feature of white matter organization, we believe that the best strategy to deal with these is to show them as clearly as possible. Perhaps the visually most pleasing results are obtained by manually splitting and tearing large sections of a tract along its anatomical course. Note, however, that this technique can be used for only the largest tracts without macroanatomical fiber crossings (e.g., the tracts in the sagittal stratum) and for the fine white matter blades of the cerebellum. Furthermore, little control can be exerted once a split along a surface is initiated, i.e., there is a chance of doing irreversible damage to the specimen. Therefore, it is important that the dissection is done by someone with practiced hands, who is already experienced in fiber dissections and has proper knowledge of human neuroanatomy (Agrawal et al., 2011).

Wet prosections of white matter can be extremely vulnerable to stress as the thin white matter blades are easily damaged by even the slightest touch. To allow use in a teaching environment, it is therefore necessary to plastinate the material in order to preserve its structure and allow tactile manipulation. Plastinated specimens are durable as they do not lose their appeal and beauty despite being heavily used in practical teaching (Riederer, 2013). Plastination of whole brains and brain dissections is usually performed using silicone polymers as this results in natural appearing specimens (Weiglein, 1997). While several silicone polymers, additives, and processing techniques have been developed since the introduction of the plastination technique (Henry et al., 2001), the S10 silicone plastination process has become the gold standard for preservation of biological tissues (DeJong and Henry, 2007). In this study, we based our plastination technique on the S10-cold-temperature technique (DeJong and Henry, 2007), while using the polymers from the room-temperature technique (Raoof et al., 2007). As these polymers remain a stable impregnation-mixture when kept at room-temperature, less cooling equipment is needed at the institution. Furthermore, both cold- and room-temperature techniques are able to produce high-quality and detailed plastinated specimens that are dry, durable, and free of offensive odors. Both cold- and room-temperature specimens have already been used for teaching as well as research (Henry, 2007). Brain slices, plastinated by the S10 technique, often require macroscopic staining to differentiate gray and white matter (Suriyaprapadilok and Withyachumnarnkul, 1997). For fiber dissection specimen, staining is not necessary as the characteristic contrast between the fiber tracts and neuronal components is retained during the plastination process which is demonstrated in our specimens. One disadvantage of plastination is shrinking of brain tissue. Shrinkage can be reduced by performing the plastination process at freezing temperature (Sora et al., 1999). By dehydrating the specimens at freezing temperature, we were able to preserve the detailed human neuroanatomy in our specimens (Fig. 4).

While plastination is a relatively new technique, it is an effective way of tissue preservation and has become widely accepted for use in educating gross anatomy (Reidenberg and Laitman, 2002; Sugand et al., 2010). The use of plastinated specimens prevents excessive exposure of staff and students to the toxic and possibly carcinogenic substances used in many embalming fluids, such as formalin (NTP, 2010; Hammer et al., 2012). It has also been recorded that the majority of students appreciate the availability of plastinated prosections as a resource for their anatomical learning (Murillo et al., 2006; Latorre et al., 2007; Fruhstorfer et al., 2011). However, plastinated specimens are perceived to be compromised because of limitations in terms of tactile and emotional experience (Fruhstorfer et al., 2011). Therefore, plastinated material does not remove the need for using traditional resources such as regular 2D specimens and textbook visualizations. Rather, they should be used as a tool in conjunction with traditional teaching methods and material. It has been shown that prosections allow the presentation of detailed human anatomy and can be an effective way of learning

human gross anatomy (Nnodim, 1990; Nnodim et al., 1996; Drake et al., 2009; Cornwall, 2011). However, a review of modern methods in teaching human anatomy reported a slight advantage for traditional dissection over prosection (Winkelmann, 2010). Cadaveric dissection is, therefore, considered as the gold standard for learning anatomical spatial relationships because of its engagement of multiple senses, 3D interaction and tactile manipulation of tissues (Sugand et al., 2010; DeHoff et al., 2011). Although dissection might remain the best tool to acquire anatomical knowledge, the fact remains that due to financial and time constraints placed on the modern medical curriculum it is often impractical to teach through hands-on dissection (Collins, 2008). In addition, white matter dissection can be time consuming. Preparing a true and instructive white matter specimen is preferably executed by someone trained on dissection with preexisting knowledge of the gross white matter anatomy of the brain. Besides, it is time-consuming and expensive to supply a large group of students with pretreated human brains. Such a white matter dissection course would only be suitable for postgraduate trainees, such as neurosurgical, anatomical, and radiological professionals that require a higher level of detail of anatomical understanding. While in recent years, there has been growth in the use of new learning resources, such as 3D computer models (Jastrow and Vollrath, 2003; Nicholson et al., 2006; Sergovich et al., 2010; Venail et al., 2010; Adams and Wilson, 2011; Codd and Choudhury, 2011; Nguyen et al., 2012) and physical models (Waters et al., 2005; Motoike et al., 2009; Oh et al., 2009; Estevez et al., 2010), to our present knowledge, there are no new methods that focus specifically on the anatomy of the white matter.

In this study, we focused our dissection on the cerebellum. In the last decade, the cerebellum and its afferent and efferent white matter tracts have become more relevant as they may have a role in cognition, behavior, and psychiatric illness (Schmahmann et al., 2008). By using fiber dissection, it is possible to reveal the anatomy of the cerebello-cerebral system, a complex but relevant subject in neuroanatomy. Moreover, we chose to focus our present dissection on the cerebellum as the small structures are not among the easiest to dissect and plastinate, providing an excellent demonstration of the efficacy of our approach. We showed that it is possible to successfully obtain plastinated prosections of nuclei and small white matter tracts of the human cerebellum. It can, therefore, be presumed that this combination of dissection and plastination is suitable for many white matter tracts in the brain.

In addition to our prosections that are described in this article, we acquired radiological images of the dissected white matter tracts using DTI tractography. Fiber tractography resembles the classical fiber dissection technique and has, therefore, been named virtual dissection (Catani et al., 2002). The DTI data in this study was acquired at our institute, but high-quality tractography images for use in neuroanatomical education are available in books and from several sources on the internet (Wakana et al., 2004; Mori et al., 2005; Catani and Thiebaut de Schotten, 2008). White matter prosections and tractography provide complementary aspects for learning white matter anatomy. For example, Skadorwa et al. (2009) already showed that the implementation of neuroimaging in neuroanatomical training can facilitate fiber dissection and understanding of white matter structures in the human brain. While prosections can only show the outline of a major tract, tractography can show the full distribution of endpoints of a tract. Moreover, tractography can reconstruct all major fiber tracts in the same brain, while only a limited number of tracts can be dissected in any prosection. Hence, tractography allows the study of the spatial relations between fiber tracts, especially if visualized in 3D. Furthermore, tractography can be used to show the interindividual anatomical variability of a tract in a group of subjects, which can be difficult with prosections (Heiervang et al., 2006; Clatworthy et al., 2010). The value of the use of tractography images in medical education has already been demonstrated by Familiari et al. (2013) who showed that an integrated neuroanatomy model consisting of clinical cases and neurosurgical images from DTI tractography can significantly improve academic performance of medical students.

While computer-based learning modules can have a positive impact on learning outcomes, they appear to have significant disadvantages compared with traditional specimens in learning anatomy (Khot et al., 2013). Hence, it is still important that computer-based resources are used in conjunction with physical models or specimens. Therefore, we propose that the combination of plastinated fiber dissection specimens—which allow students to hold, feel, and rotate specimens close to their natural form—with tractography—allowing visualization the full set of tracts, the anatomical variability and deconstructing and reconstructing the white matter in the constituent fiber tracts—could provide a learning experience that leads to deep understanding of white matter anatomy.

CONCLUSIONS

In summary, combining fiber dissection with plastination is an elegant method to obtain durable and easy to use 3D white matter specimens that do not require special care or conditions and can be used as a tool in teaching white matter anatomy and structural connectivity in conjunction with traditional educational material. Combining white matter specimens with 2D or 3D radiological information of modern brain imaging techniques provides a unique opportunity to visualize complex 3D spatial relationships of white matter in the human brain. Our first experience using a combination of plastinated specimens with fiber tractography images in an educational course is positive, but further studies in a controlled setting have to reveal its effects and value in learning and teaching human neuroanatomy.

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LITERATURE CITED

Adams CM, Wilson TD. 2011. Virtual cerebral ventricular system: An MR-based three-dimensional computer model. Anat Sci Educ 4:340–347.

Agrawal A, Kapfhammer JP, Kress A, Wichers H, Deep A, Feindel W, Sonntag VK, Spetzler RF, Preul MC. 2011. Josef Klingler's models of white matter tracts: Influences on neuroanatomy, neurosurgery, and neuroimaging. Neurosurgery 69:238–252.

Baeres FM, Møller M. 2001. Plastination of dissected brain specimens and Mulligan-stained sections of the human brain. Eur J Morphol 39:307–311.

Catani M, Howard RJ, Pajevic S, Jones DK. 2002. Virtual in vivo interactive dissection of white matter fasciculi in the human brain. Neuroimage 17:77–94.

Catani M, Thiebaut de Schotten M. 2008. A diffusion tensor imaging tractography atlas for virtual in vivo dissections. Cortex 44:1105–1132.

Ciccarelli O, Catani M, Johansen-Berg H, Clark C, Thompson A. 2008. Diffusion-based tractography in neurological disorders: Concepts, applications, and future developments. Lancet Neurol 7:715–727.

Clatworthy PL, Williams GB, Acosta-Cabronero J, Jones SP, Harding SG, Johansen-Berg H, Baron JC. 2010. Probabilistic tractography of the optic radiations—An automated method and anatomical validation. Neuroimage 49: 2001–2012.

Codd AM, Choudhury B. 2011. Virtual reality anatomy: Is it comparable with traditional methods in the teaching of human forearm musculoskeletal anatomy? Anat Sci Educ 4:119–125.

Collins JP. 2008. Modern approaches to teaching and learning anatomy. BMJ 337:665–667.

Cornwall J. 2011. The diverse utility of wet prosections and plastinated specimens in teaching gross anatomy in New Zealand. Anat Sci Educ 4:269–274.

De Castro I, Christoph Dde H, dos Santos DP, Landeiro JA. 2005. Internal structure of the cerebral hemispheres: An introduction of fiber dissection technique. Arq Neuropsiquiatr 63:252–258.

DeHoff ME, Clark KL, Meganathan K. 2011. Learning outcomes and student-perceived value of clay modeling and cat dissection in undergraduate human anatomy and physiology. Adv Physiol Educ 35:68–65.

DeJong K, Henry RW. 2007. Silicone plastination of biological tissue: Cold-temperature technique Biodur S10/S15 technique and products. J Int Soc Plast 22:2–14.

Dimou S, Battisti RA, Hermens DF, Lagopoulos J. 2013. A systematic review of functional magnetic resonance imaging and diffusion tensor imaging modalities used in presurgical planning of brain tumour resection. Neurosurg Rev 36:205–214.

Drake RL, McBride JM, Lachman N, Pawlina W. 2009. Medical education in the anatomical sciences: The winds of change continue to blow. Anat Sci Educ 2:253–259.

Estevez ME, Lindgren KA, Bergethon PR. 2010. A novel three-dimensional tool for teaching human neuroanatomy. Anat Sci Educ 3:309–317.

Familiari G, Relucenti M, Heyn R, Baldini R, D'Andrea G, Familiari P, Bozzao A, Raco A. 2013. The value of neurosurgical and intraoperative magnetic resonance imaging and diffusion tensor imaging tractography in clinically integrated neuroanatomy modules: A cross-sectional study. Anat Sci Educ (in press; doi: 10.1002/ase.1349).

Fernandez-Miranda JC, Rhoton AL Jr, Kakizawa Y, Choi C, Alvarez-Linera J. 2008. The claustrum and its projection system in the human brain: A microsurgical and tractographic anatomical study. J Neurosurg 108:764–774.

Filley CM. 1998. The behavioral neurology of cerebral white matter. Neurology 50:1535–1540.

Filley CM. 2005. White matter and behavioral neurology. Ann N Y Acad Sci 1064:162–183.

Fruhstorfer BH, Palmer J, Brydges S, Abrahams PH. 2011. The use of plastinated prosections for teaching anatomy—The view of medical students on the value of this learning resource. Clin Anat 24:246–252.

Gluhbegovic N, Williams TH. 1980. The Human Brain: A Photographic Guide. 1st Ed. Hagerstown, MD: Harper & Row, Inc. 176 p.

Hammer N, Löffler S, Feja C, Sandrock M, Schmidt W, Bechmann I, Steinke H. 2012. Ethanol-glycerin fixation with thymol conservation: A potential alternative to formaldehyde and phenol embalming. Anat Sci Educ 5:225–233.

Heiervang E, Behrens TEJ, Mackay CE, Robson MD, Johansen-Berg H. 2006. Between session reproducibility and between subject variability of diffusion MR and tractography measures. Neuroimage 33:867–877.

Henry RW. 2007. Silicone plastination of biological tissue: Cold-temperature technique North Carolina technique and products. J Int Soc Plast 22:15–19.

Henry RW, Reed RB, Henry CL. 2001. "Classic" silicone processed specimens vs "new formula" silicone plastinated specimens: A two year study. J Int Soc Plast 16:33.

Jastrow H, Vollrath L. 2003. Teaching and learning gross anatomy using modern electronic media based on the visible human project. Clin Anat 16:44–54.

Johansen-Berg H, Rushworth MF. 2009. Using diffusion imaging to study human connectional anatomy. Annu Rev Neurosci 32:75–94.

Jones DG. 2002. Re-inventing anatomy: The impact of plastination on how we see the human body. Clin Anat 15:436–440.

Khot Z, Quinlan K, Norman GR, Wainman B. 2013. The relative effectiveness of computer-based and traditional resources for education in anatomy. Anat Sci Educ 6:211–215.

Klingler J. 1935. Erleichterung der makroskopischen Praeparation des Gehirns durch den Gefrierprozess. Schweiz Arch Neurol Psychiatr 36:247–256.

Klingler J, Gloor P. 1960. The connections of the amygdala and of the anterior temporal cortex in the human brain. J Comp Neurol 115:333–369.

Latorre RM, García-Sanz MP, Moreno M, Hernández F, Gil F, López O, Ayala MD, Ramírez G, Vázquez JM, Arencibia A, Henry RW. 2007. How useful is plastination in learning anatomy? J Vet Med Educ 34:172–176.

Le Bihan D. 2003. Looking into the functional architecture of the brain with diffusion MRI. Nat Rev Neurosci 4:469–480.

Le Bihan D, Johansen-Berg H. 2012. Diffusion MRI at 25: Exploring brain tissue structure and function. Neuroimage 61:324–341.

Ludwig E, Klingler J. 1956. Atlas Cerebri Humani: Der innere Bau des Gehirns dargestellt auf Grund mackroskopischer Praeparate. Basel, Switzerland: S. Karger. 136 p.

Marks SC Jr. 2000. The role of three-dimensional information in health care and medical education: The implications for anatomy and dissection. Clin Anat 13:448–452.

Moon K, Filis AK, Cohen AR. 2010. The birth and evolution of neuroscience through cadaveric dissection. Neurosurgery 67:799–809.

Mori S, Wakana S, Nagae-Poetscher LM, van Zijl PCM. 2005. MRI Atlas of Human White Matter. 1st Ed. Amsterdam, The Netherlands: Elsevier, B.V. 239 p.

Mori S, Oishi K, Faria AV. 2009. White matter atlases based on diffusion tensor imaging. Curr Opin Neurol 22:362–269.

Motoike HK, O'Kane RL, Lenchner E, Haspel C. 2009. Clay modeling as a method to learn human muscles: A community college study. Anat Sci Educ 2: 19–23.

Murillo OR, López-Soler M, Roda-Murillo A, Ramírez-Ortiz I. 2006. Plastination in the teaching of neuroanatomy. Eur J Anat 10:85–89.

Nguyen N, Nelson AJ, Wilson TD. 2012. Computer visualizations: Factors that influence spatial anatomy comprehension. Anat Sci Educ 5:98–108.

Nicholson DT, Chalk C, Funnell WR, Daniel SJ. 2006. Can virtual reality improve anatomy education? A randomised controlled study of a computer-generated three-dimensional anatomical ear model. Med Educ 40:1081–1087.

Nnodim JO. 1990. Learning human anatomy: By dissection or from prosections? Med Educ 24:389–395.

Nnodim JO, Ohanaka EC, Osuji CU. 1996. A follow-up comparative study of two modes of learning human anatomy: By dissection and from prosections. Clin Anat 9:258–262.

Nolte J, Angevine JB Jr. 2007. The Human Brain in Photographs and Diagrams. 3rd Ed. St Louis. MO: C.V. Mosby Inc. 272 p.

NTP. 2010. National Toxicology Program. Final Report on Carcinogens Background Document for Formaldehyde. January 22, 2010. Research triangle Park, NC: U.S. Department of Health and Human Services, National Toxicology Program. 552 p. URL: http://ntp.niehs.nih.gov/ntp/roc/twelfth/2009/november/formaldehyde_bd_final.pdf [accessed 13 March 2013].

Oh CS, Kim JY, Choe YH. 2009. Learning of cross-sectional anatomy using clay models. Anat Sci Educ 2:156–159.

Pashaei S. 2010. A brief review on the history, methods and applications of plastination. Int J Morphol 28:1075–1079.

Peuskens D, van Loon J, Van Calenbergh F, van den Bergh R, Goffin J, Plets C. 2004. Anatomy of the anterior temporal lobe and the frontotemporal region demonstrated by fiber dissection. Neurosurgery 55:1174–1184.

Raoof A, Henry RW, Reed RB. 2007. Silicone plastination of biological tissue: Room-temperature technique Dow/Corcoran technique and products. J Int Soc Plast 22:21–25.

Reidenberg JS, Laitman JT. 2002. The new face of gross anatomy. Anat Rec 269:81–88.

Riederer BM. 2013. Plastination and its importance in teaching anatomy. Critical points for long-term preservation of human tissue. J Anat (in press; doi: 10.1111/joa.12056).

Schmahmann JD, Smith EE, Eichler FS, Filley CM. 2008. Cerebral white matter: Neuroanatomy, clinical neurology, and neurobehavioral correlates. Ann N Y Acad Sci 1142:266–309.

Sergovich A, Johnson M, Wilson TD. 2010. Explorable three-dimensional digital model of the female pelvis, pelvic contents, and perineum for anatomical education. Anat Sci Educ 3:127–133.

Skadorwa T, Kunicki J, Nauman P, Ciszek B. 2009. Image-guided dissection of human white matter tracts as a new method of modern neuroanatomical training. Folia Morphol (Warsz) 68:135–139.

Sora M-C, Brugger P, Traxler H. 1999. P40 plastination of human brain slices: Comparison between different immersion and impregnation conditions. J Int Soc Plast 14:22–24.

Steinke H, Pfeiffer S, Spanel-Borowski K. 2002. A new plastination technique for head slices containing brain. Ann Anat 184:353–358.

Sugand K, Abrahams P, Khurana A. 2010. The anatomy of anatomy: a review for its modernization. Anat Sci Educ 3:83–93.

Suriyaprapadilok L, Withyachumnarnkul B. 1997. Plastination of stained sections of the human brain: Comparison between different staining methods. J Int Soc Plast 12:27–32.

Tournier JD, Calamante F, Connelly A. 2012. MRtrix: Diffusion tractography in crossing fiber regions. Int J Imaging Syst Technol 22:53–66.

Ture U, Yasargil MG, Friedman AH, Al-Mefty O. 2000. Fiber dissection technique: Lateral aspect of the brain. Neurosurgery 47:417–426.

Ulfig N, Wuttke M. 1990. Plastination of stained sections of the human brain. Anat Anz 170:309–312.

van Baarsen K, Kleinnijenhuis M, Konert T, van Cappellen van Walsum AM, Grotenhuis A. 2013. Tractography demonstrates dentate-rubro-thalamic tract disruption in an adult with cerebellar mutism. Cerebellum (in press; doi: 10.1007/s12311-013-0473-z).

Venail F, Deveze A, Lallemant B, Guevara N, Mondain M. 2010. Enhancement of temporal bone anatomy learning with computer 3D rendered imaging software. Med Teach 32:e282–e288.

Visser E, Poser BA, Barth M, Zwiers MP. 2012. Reference-free unwarping of EPI data using dynamic off-resonance correction with multiecho acquisition (DOCMA). Magn Reson Med 68:1247–1254.

von Hagens G. 1979. Impregnation of soft biological specimens with thermosetting resins and elastomers. Anat Rec 194:247–255.

von Hagens G, Tiedemann K, Kriz W. 1987. The current potential of plastination. Anat Embryol (Berl) 175:411–421.

Wadood AA, Jabbar A, Das N. 2001. Plastination of whole brain specimen and brain slices. J Ayub Med Coll Abbottabad 13:11–13.

Wakana S, Jiang H, Nagae-Poetscher LM, van Zijl PC, Mori S. 2004. Fiber tract-based atlas of human white matter anatomy. Radiology 230:77–87.

Waters JR, Van Meter P, Perrotti W, Drogo S, Cyr RJ. 2005. Cat dissection vs. sculpting human structures in clay: An analysis of two approaches to undergraduate human anatomy laboratory education. Adv Physiol Educ 29: 27–34.

Weiglein AH. 1997. Plastination in the neurosciences. Keynote lecture. Acta Anat (Basel) 158:6–9.

Winkelmann A. 2007. Anatomical dissection as a teaching method in medical school: A review of the evidence. Med Educ 41:15–22.

Zwiers MP. 2010. Patching cardiac and head motion artefacts in diffusion-weighted images. Neuroimage 53:565–575.