Method for Preparing Permanent Brain Slices and Serial Slice Images for Education and MRI Correlation

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It is important to understand the anatomical structures of the human brain in horizontal planes. Serially sectioned brain slices can easily be made with a meat slicer. The objective of this research was to enhance the educational value of serial brain slices made with a meat slicer through various applications. Two brains were taken out of two cadavers and embedded with gelatin solution to make two brain blocks. The first brain block was serially sectioned at 5 mm thickness using a meat slicer to make 28 horizontal brain slices. Each brain slice was embedded with a synthetic resin mixture to make 28 permanent specimens. The second brain block was magnetic resonance-scanned at 1.4 mm thickness to make 130 horizontal magnetic resonance images, then serially sectioned at the same thickness using the meat slicer to make 130 horizontal brain slices. Each brain slice was scanned into a computer to make a series of slice images. Ten anatomical structures in the slice images were outlined to make segmented images. Corresponding magnetic resonance images, slice images, and segmented images were stacked and volume-reconstructed to make three-dimensional images, which were sectioned and rotated at free angles. We show that the serial brain slices made with a meat slicer can be permanently preserved and used in a variety of educational settings. Anat Rec (Part B: New Anat) 289B:64–71, 2006. © 2006 Wiley-Liss, Inc.

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INTRODUCTION

It is important for medical students to understand the anatomical structures of the human brain in horizontal planes. Especially in recent decades, the popularization of magnetic resonance images (MRIs) has made such knowledge an essential part of medical education. Color atlases and plastic models of the brain, which are not as realistic or accurate as the human brain specimens, have widely been used in previous decades. The improved method on this was to section the brain serially using a knife to make brain slices (Martinez and Astruc, 1975; Opeskin and Anderson, 1994). Such manual serial sectioning, however, rarely yielded thin brain slices with even surfaces that are parallel to each other, showing the anatomical structures in their proper location. Therefore, serial sectioning methods using either a polycut after celloidin embedding (Giles and Taylor, 1983; Qui, 1990) or a cryomacrotome after freezing (Toga et al., 1994) had been developed. However, such serial sectioning requires expensive equipment and an extraordinary amount of time. In order to solve these problems, the brain could be serially sectioned at 3 mm minimal thickness with a meat slicer after gelatin embedding (Roberts and Hanaway, 1969; Barnett et al., 1980; Heller and Stoddard, 1986). By using a meat slicer, the brain slices could easily be made with satisfactory quality. However, the brain slices made by this method also had some problems: they could be easily damaged, they might not show anatomical structures smaller than 3 mm, and they could not be observed together with corresponding MRIs.

The objective of this research is to enhance the educational value of the serially sectioned brain slices made with a meat slicer through the following applications: making permanent specimens of the brain slices, cutting thinner brain slices, matching the MRIs with their corresponding brain slices, and utilizing the computer images of brain slices in new ways.

In order to achieve this objective,
the following trials were performed. Two brains were extracted from two cadavers and embedded with gelatin solution to make two brain blocks. The first brain block was serially sectioned at 5 mm thickness using a meat slicer to make 28 horizontal brain slices. Each brain slice was embedded with a synthetic resin mixture to make a permanent specimen. The second brain block was MR-scanned at 1.4 mm thickness to make 130 horizontal MRIs, then serially sectioned at the same thickness using the meat slicer to make 130 horizontal brain slices.

Each brain slice was scanned into a computer to create a series of slice images. Ten anatomical structures in all slice images were outlined to make segmented images. Corresponding MRIs, slice images, and segmented images were stacked and volume-reconstructed to make three-dimensional (3D) images of the brain, which were sectioned and rotated at free angles.

MATERIALS AND METHODS

Extraction and Embedding of Two Brains to Make Two Brain Blocks

Two Korean male cadavers who did not die of brain diseases were selected for brain extraction. Formalin solution (100% formalin solution, 140 ml; 95% ethanol, 455 ml; distilled water, 1,000 ml) was injected into the cadavers through the femoral artery. The scalp of the cadaver was stripped from the calvaria and occipital bone. The outer table of the calvaria was cut with an electrical saw; the inner table was cut using a chisel and hammer. Likewise, the posterior part of the occipital bone was cut off to the level of the foramen magnum. After removing the dura mater, the cranial nerves and internal carotid arteries were cut at the internal surface of cranial base; the border of the brain and spinal cord as well as the vertebral arteries were cut at the foramen magnum or at a lower level. At this point, we tried to save as many anatomical structures of the brain as possible. The brain, including the arachnoid mater, was detached from the internal surface of cranial base (Felle et al., 1995). After extraction from the cadavers, each brain was immersed in 10% formalin solution for a week. The height of the first brain (height from the parietal lobes to the brain stem) was 140 mm; the height of the second brain was 182 mm because the second brain was larger than the first brain, and the second brain additionally involved the first and second cervical segments of the spinal cord.

An embedding box for each brain was made by the following methods. A transparent acrylic plate (width, 300 mm; length, 300 mm; height, 5 mm) was prepared as the bottom plate for the embedding box. A transparent acrylic cylinder (outer diameter, 250 mm; inner diameter, 240 mm; height, 350 mm) was prepared and vertically divided into two even side parts in order to take out the brain block easily from the embedding box. The bottom plate and two side parts were pasted with silicone (Fig. 1). Two vertical threads were attached on opposite sides of the cylinder; a horizontal thread was attached around the circumference of the cylinder.

Each brain was embedded with gelatin solution to make a brain block. Gelatin solution, a mixture of 200 g gelatin and 1,000 ml distilled water, was poured into the embedding box to the height of 70 mm and solidified in room temperature (20°C) to make the gelatin base. A shallow round pit (diameter, 30 mm; depth, 5 mm) was dug in the center of the surface of the gelatin base using a scalpel. The first brain was placed on the gelatin base upside down; as a result, the bilateral parietal lobes of the brain were placed on the shallow round pit of the gelatin base. The gelatin solution was poured again up to half the height of the brain and solidified a little. The brain direction was adjusted so the longitudinal cerebral fissure and cerebellar fissure fitted with the two vertical threads in order to prevent the brain from tilting left or right. Also, the brain direction was adjusted so the olfactory tracts and optic nerves were parallel to the horizontal thread in order to prevent the brain from tilting back and forth. After making the adjustments, the embedding box was filled with gelatin solution, which was solidified to make a brain block. The silicone was cut from the embedding box using a scalpel, and the bottom plate and side parts were separated to extract the brain block, including the gelatin base (Fig. 1). The brain block was immersed in 10% formalin solution for 3 days to make the gelatin as solid as the brain in the brain block. The second brain block was prepared in the same way.
Serial Sectioning of First Brain Block Using a Meat Slicer to Make Brain Slices of 5 mm Thickness

A rotary meat slicer was prepared. The rotary meat slicer (HFS-330L; Fuzee) is designed to section serially a moving subject with a rotating blade. The moving speed of the subject near the blade is 870 mm/sec; the diameter and rotating speed of the blade is 363 mm and 52 rpm, respectively. In this experiment, serial sectioning was performed not continuously but one by one, holding onto the button to move the subject back and forth. The blade height, measured by a ruler, was adjusted to be 5 mm, which corresponds to the thickness of serial sectioning (Fig. 2). The first brain block was placed on the meat slicer and firmly fixed. On the meat slicer, the gelatin base of the brain block faced downward; as a result, the gelatin base was serially sectioned first, then the brain was serially sectioned from the parietal lobes to the brain stem orderly. The brain block was firmly fixed on the meat slicer by pressing the fixing plate with spines on the upper surface of the brain block and by pressing two other fixing plates on two other sides of the brain block in order to prevent the brain block from shaking on the meat slicer during serial sectioning (Fig. 2).

The first brain block, in which brain height was 140 mm, was serially sectioned at 5 mm thickness to make 28 horizontal brain slices. After serial sectioning, each brain slice was placed on a vinyl resin mat (200 mm × 200 mm). It was verified whether the slice surfaces had any scratches or the anatomical structures had any pathological findings. As a result, the brain slices had neither scratches nor pathological findings. The gelatin outside the brain of the brain slices was cut off by using the scissors (Fig. 3). However, the gelatin, which was linking several brain parts (i.e., bilateral cerebral hemispheres, several cerebral gyri, cerebral arteries, cranial nerves, and arachnoid mater), was left intact. When the linking gelatin was torn, the gelatin and several brain parts were returned to their proper location according to the gelatin’s tearing shape.

Embedding of First Brain Slices to Make Permanent Specimens

The first brain slices were immersed in 50%, 75%, and three times in 100% glycerin solutions sequentially for dehydration. After immersing the brain slices in the final 100% glycerin solution, the solution was wiped off the brain slices with paper towels.

Permanent specimen frames for the first brain slices were made by the following procedures. A transparent glass plate (width, 190 mm; length, 170 mm; height, 5 mm) was prepared as a bottom plate for the permanent specimen frame. Two pairs of transparent acrylic plates (width, 170 mm; length, 25 mm; height, 5 mm) and two other transparent acrylic plates (width, 180 mm; length, 25 mm; height, 5 mm) were prepared as four side plates of the permanent specimen frame. The bottom plate and four side plates were pasted with silicone to make a permanent specimen frame (Fig. 4). Wax was painted inside the permanent specimen frame in order to extract the permanent specimen easily out of the frame. To conserve time of making permanent specimens, four more frames were prepared in a likewise manner.

Each slice of the first brain was embedded with the synthetic resin mixture to make a permanent specimen. Synthetic resin (Polycoat-141; 1,000 ml) was mixed with an accelerator (A-51; 8 ml) and then with a catalyzer (55% MEKP; 10 ml) to make the synthetic resin mixture, which is naturally solidified in room temperature in 1 hr. The synthetic resin mixture was poured into the permanent specimen frame to a height of 5 mm and solidified to make the synthetic resin base. A brain slice was then immersed into the synthetic resin mixture, prepared in another container; at this point, bubbles around the brain slice (especially, in the brain sulci) were eliminated. The brain slice was then taken out of the mixture and placed on the synthetic resin base; at this time, bubbles between the brain slice and synthetic resin base were also eliminated. The synthetic resin mixture was poured into the permanent specimen frame to a height of 5 mm above the brain slice and solidified to make a permanent specimen of 15 mm thickness. After cutting the silicone out of the permanent specimen frame, the bottom and side plates were separated to extract the permanent specimen (Fig. 4). Permanent specimen that had scratches on its surface was ground with an electric sandpaper machine and an electric polishing machine until the scratches disappeared and the synthetic resin became thin. The other 27 permanent specimens were prepared in the same way with the five permanent specimen frames (Fig. 3).

Determination of Thickness (1.4 mm) of MRIs and Brain Slices of Second Brain

According to the handbook of the meat slicer, the thickness of serial sectioning ranges from 0 to 25 mm. In the preliminary experiments using the
meat slicer, the brains were serially sectioned at 1 mm thickness, which sometimes resulted in torn brain slices. After several trials of serial sectioning at various thicknesses, we recognized that 1.4 mm was the minimal thickness required to make the brain slices with constantly good quality. In order to display as many anatomical structures in the sectioned surfaces as possible, we decided to section serially the second brain at 1.4 mm thickness. In addition, we decided to MR-scan the second brain at the same thickness to match the MRIs and corresponding brain slices.

**MR Scanning of Second Brain Block to Make MRIs**

The second brain block was placed on an MRI machine (GE Signa Horizon 1.5 Tesla MRIs System; GE, Milwaukee, WI). On the MRI machine, the brain block was stabilized to prevent movement during MR scanning. The brain block was MR-scanned by the following procedures. Slice thickness and interslice gap were adjusted to be 1.4 and 0 mm, respectively, so the number of MRIs from the second brain (height, 182 mm) equaled 130. Head coil was used so the field of view was 310 mm × 310 mm. Resolution was adjusted to 512 × 512. Proton density was weighted, interleave method was used, and number of excitation was adjusted to two. After MR scanning, horizontal direction of the MRIs was verified by examining symmetry of the bilateral anatomical structures in the MRIs. If the MRI was not horizontal, the brain block was appropriately trimmed and MR-scanned again. One hundred thirty MRIs of the brain images were transferred to a personal computer using picture archiving and communication system (PACS). The MRIs were converted from digital imaging and communication in medicine (DICOM) files to tag image file format (TIFF) files (color depth, 8 bits gray) on PiView software (Infinitt). Excessive margins of the MRIs were cut off, so the resolution (512 × 512) was reduced to 300 × 360 (Fig. 5, Table 1).

**Serial Sectioning of Second Brain Block Using a Meat Slicer to Make Brain Slices of 1.4 mm Thickness**

The serial sectioning method of the second brain block using the meat slicer was almost the same as that of the first brain block. However, in order to make the slices of the second brain corresponding to the MRIs, two procedures were altered. First, when the brain block was placed on the meat slicer, extra precautions were taken to make sure there was no tilting whatsoever. Second, the blade height of the meat slicer was adjusted to be 1.4 mm to make 130 brain slices of 1.4 mm thickness.

**Scanning of Second Brain Slices to Computer to Make Slice Images, in Which the Anatomical Structures Were Segmented**

The inferior surfaces of the second brain slices were scanned one by one...
using a scanner (ScanJet 4c; Hewlett Packard) to make 130 slice images of TIFF files (resolution, 512 × 512; color depth, 24 bits color; Table 1). In the slice images, the gelatin linking several brain parts was deleted.

The slice images were aligned and resized in reference to the MRIs, which were already aligned, by the following procedures. On each slice image, the corresponding MRI was superimposed and displayed semi-transparently on Adobe Photoshop software (version 7.0; Adobe). The slice image was enlarged, reduced, moved, and rotated until it matched the superimposed MRI. The margins of the slice image were cut off until the resolution (512 × 512) of the slice image fit 300 × 360, which was equal to the MRI (Table 1). After finishing previous procedure, the superimposed MRI was deleted (Fig. 5), and the procedures were repeated for all slice images.

Ten important anatomical structures of the brain (cerebrum, cerebellum, brain stem, lentiform nucleus, caudate nucleus, thalamus, fornix, optic nerve, cerebral artery, and ventricle) were selected for segmentation. Outlines of 10 anatomical structures in all slice images were manually drawn using CorelDRAW software (version 10; Corel) to make 130 segmented images of TIFF files (resolution, 300 × 360; color depth, 8 bits color; Fig. 5, Table 1).

Creating, Sectioning, and Rotating 3D Images of Second Brain

Three-dimensional images of the second brain was created, sectioned, and rotated by using Visual C++ programming language (version 6.0) as follows. All 130 MRIs were stacked in sequence and subsequently volume-reconstructed to make a 3D image. Likewise, two more 3D images of the slice images and segmented images were made. The 3D images were coronally sectioned to display the coronal planes of MRIs, slice images, and segmented images, all of which were correspondingly matched (Fig. 5). In the same manner, the 3D images were sagittally and obliquely sectioned to display their sectional planes. 3D images either of the whole brain or of the selected anatomical structures were displayed and rotated at free angles (Fig. 6).

RESULTS

The brain slices and permanent specimens were easily prepared as described. It took 1 week to embed a brain and to section a brain block serially; 3 weeks to dehydrate 28 brain slices; 1 week to embed 28 brain slices; and 1 week to trim 28 permanent specimens’ surfaces (Figs. 1 and 4).

Twenty-eight slices were prepared with 5 mm thickness of the first brain and 130 slices with 1.4 mm thickness of the second brain. Constant thickness (1.4 mm) of the slices of the second brain was confirmed by matching them to the corresponding MRIs with...
permanent specimens, which were taken
of the first brain slices were prepared.

1.4 mm thickness (Fig. 5). Furthermore, it was confirmed by the result that the 3D image made from the slices of the second brain was similar in appearance to the actual second brain.

Brain slices with even, parallel, and horizontal surfaces were prepared. The even surfaces were parallel to each other, which is a prerequisite for horizontal slice surfaces. The even and parallel surfaces of the second brain slices were confirmed by the result that the 3D image of the second brain slices was not distorted (Fig. 6). The horizontal direction of the slice surfaces was also confirmed by symmetry of the bilateral anatomical structures (Fig. 5).

Brain slices, in which the anatomical structures were marked, were prepared. The slice surfaces with no scratches showed the border between the gray matter (i.e., cerebral cortex, caudate nucleus, lentiform nucleus, and thalamus) and white matter even though the brain slices were not stained. Apparent slice surfaces of the first brain could be observed in the permanent specimens through the transparent synthetic resin without bubbles or scratches (Fig. 3). Slice surfaces of the second brain also appeared in the slice images with equal quality, making it easy to segment the anatomical structures (Fig. 5).

In the brain slices, several brain parts kept their proper locations thanks to the gelatin linking several brain parts. The proper locations were permanently preserved in the permanent specimens. In the specimens, some gelatin remained; however, it was negligible because the gelatin, which is almost transparent, did not cover the slice surfaces (Fig. 3).

Twenty-eight permanent specimens of the first brain slices were prepared. The original thickness (15 mm) of permanent specimens, which were taken out of the frame, was reduced as the surfaces of the permanent specimens were ground. As a result, the permanent specimens were convenient to manage. In the permanent specimens, the colors of the brain slices were permanently preserved (Fig. 3).

Corresponding MRIs and slice images of the second brain were prepared. The MRIs and slice images had the same direction (horizontal), thickness (1.4 mm), and number (130; Table 1); in other words, both images were corresponding. Therefore, the slice images could be aligned with reference to the corresponding MRIs. The alignment of the slice images was confirmed by the result that the coronal plane, made of slice images, was not distorted. The proton density-weighted MRIs showed apparent borders between the gray and white matters (Fig. 5).

The segmented images of 10 important anatomical structures were prepared so that 3D images of the selected anatomical structures among the 10 segmented anatomical structures could be displayed. Correct segmentation was confirmed by the result that the contours of the anatomical structures were smooth either in sectional planes of the 3D images or in the rotated 3D images (Figs. 5 and 6).

**DISCUSSION**

Horizontal brain slices used for medical education can easily be prepared by using a meat slicer. Desirable brain slices have the following qualities: the thickness is constant and thin, the slice surfaces are even and parallel to each other, and the brain slices show the apparent anatomical structures. If only the quality of the brain slices is considered, the brain may be serially sectioned either by using a polycut after embedding with celloidin (Giles and Taylor, 1983; Qui, 1990) or by using the cryomacrotome after freezing (Toga et al., 1994). However, the polycut and cryomacrotome are difficult and expensive techniques to use for classroom purposes, and embedding the brain with celloidin takes a lot of time. For our educational purposes, it is not necessary to obtain brain slices with the quality obtained by polycut or cryomacrotome; it is more important to obtain brain slices in a quick and easy manner from many brains. In this research, two brains were embedded with gelatin and serially sectioned using the meat slicer. With this technique, the price is inexpensive, difficult maneuvers are unnecessary, and it requires only 1 week for embedding and serially sectioning the brain.

The quality of the brain slices can be determined in preliminary experiments by checking the sharpness of the blade and stability of the brain block placed on the meat slicer. In the main experiment, the blade was sharpened sufficiently, the brain block was made as heavily as possible by using large amounts of gelatin, and the brain block was firmly fixed on the meat slicer (Fig. 2). As a result, the quality of the brain slices was standardized to the following conditions. First, the minimal thickness of 1.4 mm allows observation of anatomical structures greater than 1.4 mm in the brain slices. Second, the thickness is generally constant. Third, the sectioned surfaces are generally even and parallel to each other in horizontal direction. Last of all, the sectioned surfaces without scratches showed the apparent anatomical structures in their proper locations. The quality of the brain slices can be said to be good enough for education and for making various educational tools (Fig. 3).

Permanent brain slices can be easily prepared by embedding brain slices with the synthetic resin mixture. We need permanent brain specimens since untreated brain slices are easily damaged. If quality is the only consideration in making permanent brain slices, plastination method may be used. For plastination, water of brain slices should be dehydrated at low temperatures, fat should be eliminated at room temperature, and plastic should be penetrated using a vac-

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**TABLE 1. One hundred thirty sets of the MRIs, slice images, and segmented images of a human brain**

<table>
<thead>
<tr>
<th>Images</th>
<th>Color depth</th>
<th>One file size</th>
<th>Total file size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRIs</td>
<td>8 bits gray</td>
<td>105 Kbytes</td>
<td>13.3 Mbytes</td>
</tr>
<tr>
<td>Slice Images</td>
<td>24 bits color</td>
<td>316 Kbytes</td>
<td>40.0 Mbytes</td>
</tr>
<tr>
<td>Segmented images</td>
<td>8 bits color</td>
<td>105 Kbytes</td>
<td>13.3 Mbytes</td>
</tr>
</tbody>
</table>

Thickness: 1.4 mm, Resolution: 300 × 360.
The anatomical structures are helpful in recognizing the shape of anatomical structures in the MRIs and slice images (Fig. 6). Third, the corresponding MRIs and slice images are helpful in confirming the correct segmented images prepared in this research will be presented worldwide free of charge (Table 1).

Based on the results of the present study, and recognizing their value to the educational setting, future avenues of research on these methods include the following. One, a brain taken out of a fresh cadaver, into which fixatives are not injected, is serially sectioned to make brain slices, which will have colors similar to a living person. Two, a brain with pathological findings is serially sectioned in order to present an educational tool for pathology. Three, a whole head, including the brain, skull, and other anatomical parts, is serially sectioned by using a bone slicer, which will contribute to the comprehensive understanding of locational relationship of the brain and the neighboring structures such as the skull (Tiede et al., 1993). Four, a brain is serially sectioned in the coronal and sagittal directions. Five, the brain slices are stained to make the gray and white matter more distinguishable (Roberts and Hanaway, 1969; Barnett et al., 1980; Sheehan and Hrapchak, 1980). Six, the brain slices are scanned into a computer with higher resolution. Seven, a brain, into which MRI contrast medium is injected through the cerebral arteries, is MR-scanned. Eight, more anatomical structures of brain in the MRIs and slice images are semiautomatically segmented (Park et al., 2005b). Nine, the corresponding MRIs, slice images, and segmented images are used as the source of an atlas. Ten, the MRIs, slice images, and segmented images are used as the source of various 3D images, which are made by volume reconstruction or surface reconstruction. Eleven, the 3D images are virtually dissected with various methods (Tiede et al., 1993).
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LITERATURE CITED