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Serial Slice Images and Segmented Images of the Brainstem for Recognizing the Stereoscopic Morphology of its Nuclei and Tracts

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The purpose of this research is to present the serial slice images and segmented images of the human brainstem to make the three-dimensional (3D) images, which are helpful in recognizing stereoscopic morphology of the brainstem components.

A brainstem was taken out from a cadaver. The brainstem was embedded with paraffin to make brainstem block. The brainstem block was serially sectioned and digitalized to make slice images. In the slice images, 28 brainstem components including several nuclei and tracts were segmented to make segmented images. The segmented images were volume-reconstructed to make 3D images.

One hundred forty-three couples of serial slice images and segmented images with 0.5 mm intervals, 360 × 288 resolution, 0.125 mm pixel size, and 8 bits gray were achieved. 3D images of the brainstem components were sectioned and rotated. The serial slice images and segmented images were verified by the result that coronal images, sagittal images, and 3D images of the brainstem were not distorted.

The serial slice images and segmented images of the brainstem, which were prepared in this research, will be presented to the world. The images are expected to be used for other researchers to make 3D images and virtual dissection software which are helpful in recognizing stereoscopic morphology of the brainstem components.

Key words : Serial slice images, Segmented images, Brainstem, Stereoscopic morphology, Nuclei Tracts, Three-dimensional images

Introduction

The human brainstem includes several components such as nuclei and tracts which are important not only for the conduction of motor and sensory stimuli but also for the maintenance of life. For accurate diagnosis and treatment of the brainstem diseases, stereoscopic location and shape of the brainstem components should be recognized. For simply understanding the stereoscopic morphology, the brainstem of cadaver can not be dissected along its nuclei and tracts; the brainstem atlases including two-dimensional pictures require much effort by the medical students and doctors (DeArmond et al. 1976, Jennes et al. 1995). If three-dimensional (3D) image of the brainstem is made and sectioned at free angles; and the 3D images of the brainstem components are selected to display

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and rotated at free angles, stereoscopic morphology of the brainstem components can be easily recognized. For the reason, in other researches, computed tomographs and magnetic resonance images of the brainstem were scanned; however they were not enough to identify the brainstem components (Funahashi et al. 1989, Marx et al. 2004). It was tried that pons and medulla oblongata were serially sectioned to make serial slices; however, midbrain was not involved and the serial slices did not keep good quality and were not stained, so that nuclei and tracts in the serial slices were difficult to identify (Axer et al. 2002).

The purpose of this research is to present the serial slice images and segmented images of the human brainstem for making the 3D images, which are helpful in recognizing stereoscopic morphology of the brainstem components.

Materials and Methods

1. Serial sectioning of the brainstem to make slices

A brainstem was taken out from a cadaver. A donated Korean cadaver (53 years old male) was perfused with 10% formalin solution through the

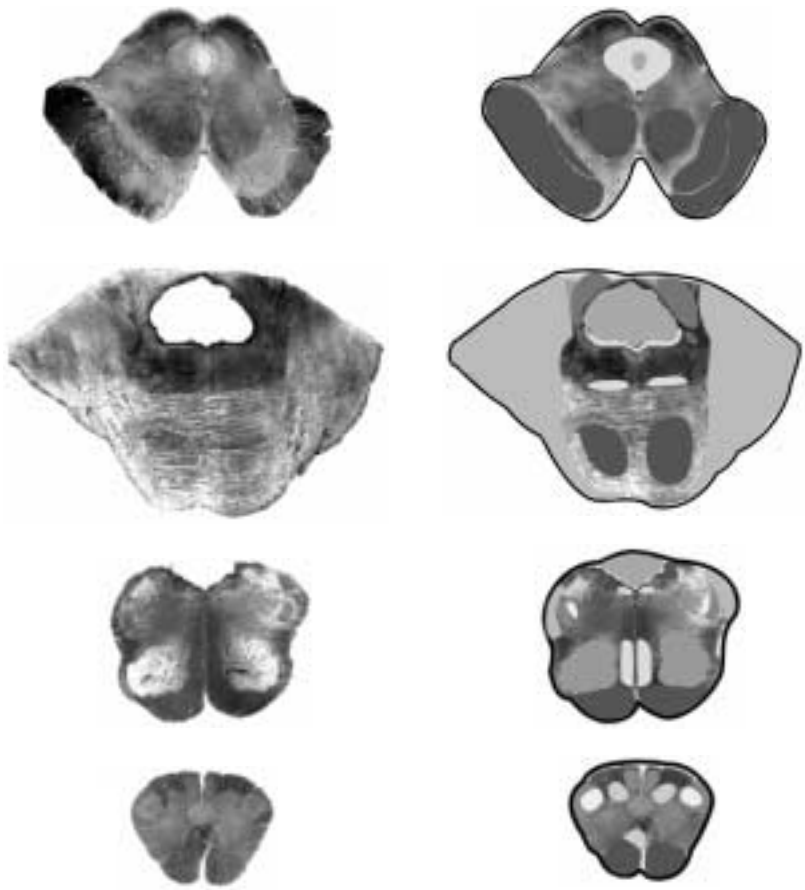

Fig. 1. Slice images (left column) and segmented images (right column) of the midbrain, pons, rostral medulla oblongata, and caudal medulla oblongata (from top to bottom).

femoral artery. After carefully extracting the brain (Felle et al. 1995), the whole brainstem consisting of midbrain, pons, and medulla oblongata was acquired.

Two brainstem blocks were made. After dehydration, the brainstem was transversely divided into upper and lower halves using a knife. The upper and lower halves were embedded with paraffin to make two blocks.

Two brainstem blocks were serially sectioned to make 143 slices. Both blocks were serially sectioned using a sliding microtome (HM 400, Microm™) in the transverse direction. Total height of both blocks was 71.5 mm, and thickness of the serial slices was 0.02 mm, so that total number of the serial slices was 3,575. One slice out of 25 serial slices was regularly selected and put on the slide glass for further experiment, so that number and interval of the selected slices became 143 and 0.5 mm, respectively.

2. Staining and digitalizing of the slices to make slice images

After each slice was Pal-Weigert stained, each slice was printed on a photographic paper (A4 size) with a consistent enlarging scale (five times) using an enlarger to make 143 slice photographs.

Table 1. Features of 143 couples of slice images and segmented images

Images	Color depth	One file size	Total file size
Slice images	8 bits gray	101.3 Kbytes	14.1 Mbytes
Segmented images	24 bits color	303.9 Kbytes	42.3 Mbytes

Intervals: 0.5 mm, Resolution: 360 × 288, Pixel size: 0.125 mm, File format: tag image file format (TIFF).

Each slice photograph was digitalized to make a slice image using a scanner (Scanjet 4c, Hewlett-Packard™). At this time, field of view and resolution was adjusted to be 225 × 180 and 360 × 288 mm, respectively, so that pixel size of the slice image was 0.625 mm. But the actual pixel size was 0.125 mm because the enlarging scale was five times during photographical printing. Color depth and file format of the slice image was adjusted to be 8 bits gray and tag image file format (TIFF), respectively (Fig. 1, Table 1).

3. Aligning of the slice images by using a median line and by examining the coronal and sagittal slice images

The slice images were aligned by using a median line. On the Adobe Photoshop (version 7.0, Adobe™),

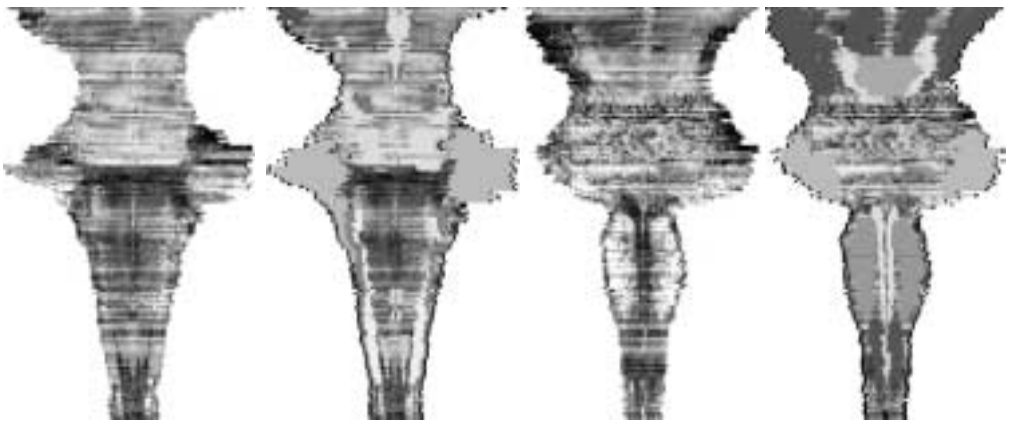

Fig. 2. Corresponding coronal slice images and coronal segmented images.

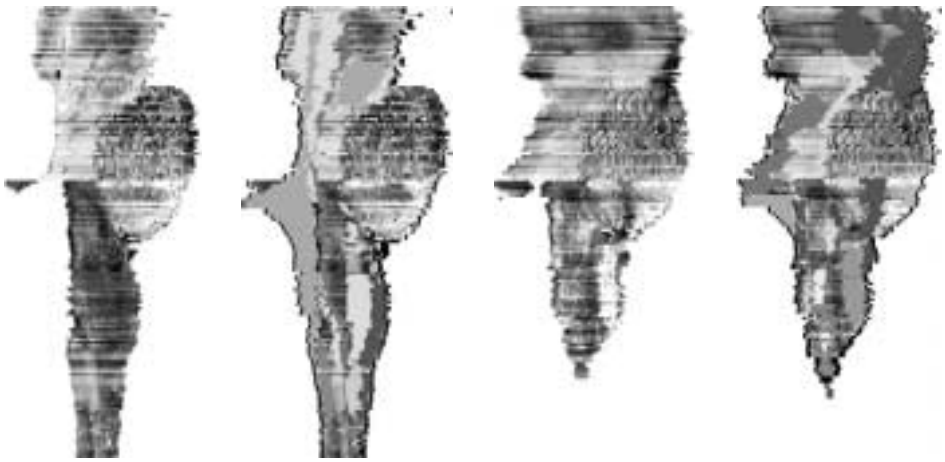

Fig. 3. Corresponding sagittal slice images and sagittal segmented images.

Table 2. Twenty-eight brainstem components, which are segmented

Classification	Components
Surface, Ventricle	Brainstem surface, Mesencephalic aqueduct, Fourth ventricle, Central canal
Related to spinal nerve	Gracile nuclei, Cuneate nuclei, Medial lemnisci, Corticospinal tracts, Pyramidal decussation
Related to cranial nerve	Oculomotor nuclei, Spinal nuclei of trigeminal nerve, Spinal tracts of trigeminal nerve, Abducens nuclei, Facial nuclei, Medial vestibular nuclei, Superior vestibular nuclei, Lateral vestibular nuclei, Lateral lemnisci, Hypoglossal nuclei
Remaining	Periaqueductal gray matter, Red nuclei, Substantiae nigrae, Medial longitudinal fasciculi, Inferior olivary nuclei, Superior cerebellar peduncles, Decussation of superior cerebellar peduncles, Middle cerebellar peduncles, Inferior cerebellar peduncles

a median line, which was never moved, was superimposed on all slice images. Each slice image was adequately rotated and moved right or left until the slice image was symmetric with respect to the median line.

The slice images were aligned by examining coronal and sagittal slice images. After stacking, slice images, coronal and sagittal images were made (Park et al. 2005). In the coronal and sagittal images, the zigzag brainstem surface, which was caused by incorrect alignment, were found. The incorrectly aligned slice images were adequately moved ventrally or dorsally until the coronal and sagittal images showed smooth surface (Figs. 2, 3).

4. Segmentation of 28 brainstem components in the slice images to make segmented images

Twenty-eight brainstem components were decided to segment in the slice images. In the case that the brainstem components are bilateral, both bilateral components were decided to segment (Table 2).

The brainstem components in the photographic papers were identified by referring to the brainstem atlases (DeArmond et al. 1976, Jennes et al. 1995). On the papers, contours of the brainstem components were drawn using a pen by the medical experts (Fig. 4).

By referring to the photographic papers, the brain-

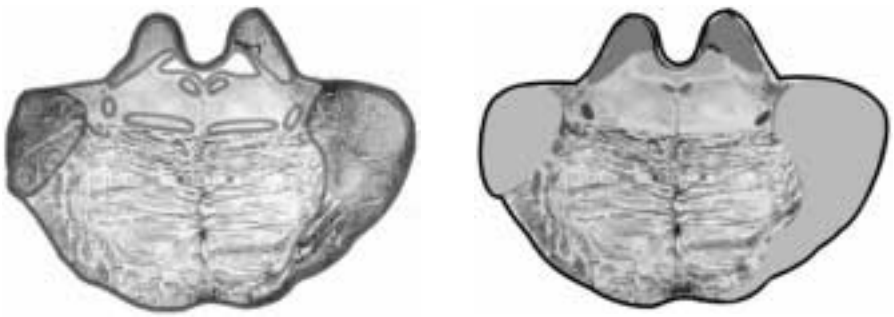

Fig. 4. Photographic paper, where contours of the brainstem components are manually drawn (left), and corresponding segmented image (right).

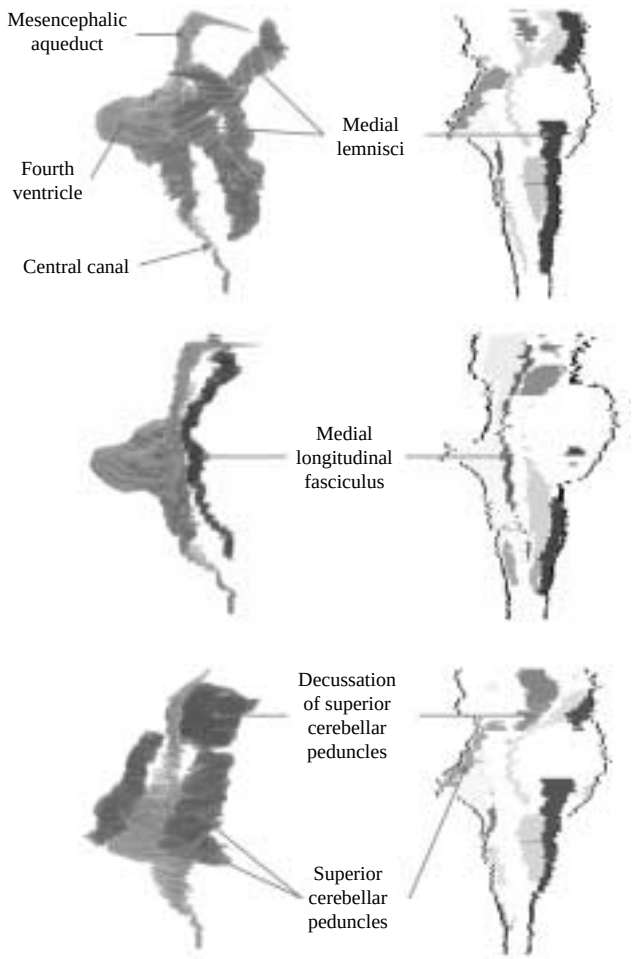

Fig. 5. Rotated 3D images of the selected brainstem components (left column) and sectioned 3D images of the brainstem (right column).

stem components were manually segmented in the slice images to make 143 segmented images. It was done on the CorelDRAW (version 10, Corel Corporation™) by the medical experts. Then the contours of each brainstem component were filled with a specific color to make 143 segmented images. At this time, similar colors were not used for the neighboring brainstem components. Resolution (360 × 288), pixel size (0.125 mm), and file format (TIFF) of the segmented images were adjusted to be the same as those of the slice images. But color depth of the segmented images was adjusted to be 24 bits color for representing the segmentation colors (Figs. 1, 4) (Table 1).

The segmented images were verified and corrected by examining the coronal and sagittal segmented images. After making coronal and sagittal images of the segmented images, zigzag contours of the brainstem components, which were caused by incorrect segmentation, were found. The incorrect segmented images were corrected until the coronal and sagittal images showed smooth contours (Figs. 2, 3).

5. Volume reconstruction of the segmented images to make 3D images

All segmented images were stacked in sequence and subsequently volume-reconstructed to make a 3D image using Visual C^{++} programing language (version 6.0). The 3D image was sectioned at free angles to display the sectional segmented images. The 3D images of the selected brainstem components were displayed and rotated at free angles. The 3D images were examined to find the stereoscopic location and shape of some brainstem components (Fig. 5).

Results

One hundred forty-three couples of the slice images and segmented images were made. Interval and pixel size of the images was 0.5 and 0.125 mm, respectively (Table 1).

The slice images were transverse and parallel with each other; they had constant intervals; and they were aligned (Fig. 1). It was confirmed by the result that coronal and sagittal, images were not distorted (Figs. 2, 3).

The segmented images were satisfactory. Because of the coronal and sagittal segmented images, incorrect segmentation could be easily found and corrected (Figs. 2, 3). The correct segmented images were confirmed by the result that 3D image of each brainstem component showed smooth contour (Fig. 5).

The 3D images of the brainstem could be sectioned and rotated. The 3D image of the brainstem could be sectioned at free angles to display the sectional segmented images, in which each brainstem component could be easily identified with the specific segmentation color. 3D images of any combinations of the brainstem components could be selected to display and rotated at free angles. In the rotated 3D images, not only stereoscopic shape of each brainstem component but also stereoscopic locational relationship of the neighboring brainstem components could be easily identified (Fig. 5).

Through examination of the 3D images, the stereoscopic location and shape of some brainstem components were found as follows: Medial lemnisci, which curved ventrally in the midbrain and medulla oblongata and dorsally in the pons, looked like an M-character. Medial longitudinal fasciculus curved dorsally to be close to the fourth ventricle but far from the mesencephalic aqueduct and central canal. Bilateral superior cerebellar peduncles ascended ventrally in the pons to become the decussation of superior cerebellar peduncles in the midbrain (Fig. 5).

Discussion

For accurate diagnosis and treatment of human

brainstem diseases, stereoscopic morphology of the brainstem components should be recognized. For example, when the extent of a lesion in the brainstem is identified on MRIs, it is important to know the stereoscopic location and shape of the brainstem surface, red nuclei, substantiae nigrae, and inferior olivary nuclei, which are landmarks on the brainstem MRIs (Hirsch et al. 1989). Also, when an electrode is inserted around the brainstem during stereotaxic surgery, it is important to know the stereoscopic locations of the vital nuclei and tracts, which should not be injured (Afshar and Dykes 1982). Furthermore, when central electroauditory prosthesis is implanted into the cochlear nuclei, it is important to know the stereoscopic locations of the vestibular nuclei and spinothalamic tracts into which the prosthesis should not be implanted (Terr and Edgerton 1985a, b, Sinha et al. 1987, Terr and Sinha 1987, Mobley et al. 1995).

The stereoscopic morphology of the human brainstem components could be easily recognized if 3D images of the brainstem are made of the serial slice images and segmented images. In other researches, those images of the rat brainstem were prepared to make 3D images, which can be used for neuroscience researches (German and Manaye 1993, Brevik et al. 2001).

To make good quality of 3D images, the serial slice images and segmented images of the brainstem need to be prepared on the following principles.

First, the serial slice images of the whole brainstem need to be prepared. In this research, the whole brainstem consisting of the midbrain, pons, and medulla oblongata were used as material. In the next research, to make 3D images of the entire course of motor and sensory pathways, the serial slice images from the telencephalon to spinal cord need to be prepared.

Second, the serial slice images need to be transverse, parallel with each other, and they need to have constant intervals. In this research, to make the serial slice images transverse, longitudinal direction of the brainstem was kept in mind while dividing, embedding, and serial sectioning the brainstem. To make the serial slice images parallel to each other and to maintain their intervals constant, the precise sliding microtome was used while sectioning the brainstem.

Third, the serial slice images need to be aligned. In this research, the median line, coronal images, and sagittal images were used (Figs. 2, 3). In the next research, alignment rods need to be inserted into the brainstem block; corresponding MRIs of the brainstem block need to be scanned; or alignment software needs to be composed (Axer et al. 2002).

Fourth, the serial slice images need to show apparent brainstem components. In this research, serial sectioning of the brainstem block, Pal-Weigert staining and digitalization (pixel size: 0.125 mm) of the serial slices were performed in the best conditions (Fig. 1).

Fifth, the correct segmented images of many brainstem components need to be prepared. In this research, almost important brainstem components, which could be identified in the serial slice images, were segmented (Table 2). In addition, manual segmentation was performed by the medical experts; at this time, not only the brainstem atlas (DeArmond et al. 1976, Jennes et al. 1995) but also the photographic papers, where contours of the brainstem components were already drawn, were used (Fig. 4). Furthermore, the segmented images were verified and corrected using the coronal and sagittal images (Figs. 2, 3). In the next research, to reduce segmentation time and human errors, semiautomatic segmentation needs to be performed (Park et al. 2005).

Sixth, the slice images and segmented images need to be finally verified by making and examining the 3D images. In this research, volume reconstruction was performed to enable the 3D images to be sectioned (Fig. 5). In the next research, surface reconstruction needs to be performed to reduce the file size of the 3D images and enhance the rotating speed of the 3D

images. Also, virtual dissection software with multiple functions needs to be composed (Höhne et al. 1992, Tiede et al. 1993).

The serial slice images and segmented images of the brainstem, which were prepared in this research (Table 1), will be presented to the world. The images are expected to be used for other researchers to make 3D images and virtual dissection software, which are helpful in recognizing the stereoscopic morphology of the brainstem components.

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뇌줄기 신경핵과 신경로의 입체 생김새를 깨닫기 위한 뇌줄기의 연속절편영상과 구역화영상

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간추림 : 사람 뇌줄기의 연속절편영상과 구역화영상을 만든 다음에 쌓고 부피재구성해서 3차원영상을 만들면 뇌줄기의 입체 생김새를 깨닫는 데 도움 된다. 이 연구의 목적은 사람 뇌줄기의 연속절편영상과 구역화영상을 만든 다음에 퍼뜨려서 다른 연구자가 뇌줄기의 3차원영상을 만드는 데 도움 주는 것이다.

시신에서 뇌줄기를 꺼내서 파라핀으로 포매한 다음에 연속절단해서 뇌줄기 절편을 만들었다. 이 절편을 컴퓨터에 입력해서 연속절편영상을 만들었다. 연속절편영상에서 보이는 뇌줄기의 구조물 28개를 구역화해서 구역화영상을 만들었다. 구역화영상을 쌓고 부피재구성해서 3차원영상을 만들었다.

연속절편영상과 구역화영상을 143쌍 만들었다(간격 0.5 mm, 해상도 360 × 288, 화소 크기 0.125 mm, 빛깔 8 bits gray). 뇌줄기 구조물의 3차원영상을 잘라서 보고 돌려서 보았으며, 이 결과로 연속절편영상과 구역화영상에 별 문제가 없는 것을 확인하였다.

이 연구에서 만든 뇌줄기의 연속절편영상과 구역화영상을 퍼뜨리면, 다른 연구자가 이 영상을 써서 뇌줄기의 3차원영상과 가상해부 소프트웨어를 만들 수 있고, 뇌줄기의 입체 생김새를 깨닫는 데 도움 될 것이다.

찾아보기 낱말 : 연속절편영상, 구역화영상, 뇌줄기, 입체 생김새, 신경핵, 신경로, 3차원영상

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