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Case report

Topographic anatomy of the subthalamic nucleus localized by high-resolution human brain atlas superimposing digital images of cross-sectioned surfaces and histological images of microscopic sections from frozen cadaveric brains

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ABSTRACT

Despite the recent advent of neuro-radiographic techniques, creating a 'perfect' human brain atlas providing precise and consistent images with minimal distortion is practically difficult. In this study, we created a new human brain atlas from cadaveric brains with serial sections of 50 μm thickness covering the entire basal ganglia. Human cerebral hemispheres were obtained from 10 donated cadavers and fixed in 10% formalin solution, cut in a block measuring 50 mm × 30 mm × 50 mm around the midpoint of the anterior and posterior commissures and frozen at −40 °C. Each block was cut into 50-μm-thick sections on the freezing microtome and the cross-sectioned surface was photographed. Simultaneously, every 10th slice from one sagittal hemisphere was sampled and stained using the Kluver-Barrera method. Prepared slides were photographed under light microscopy, and data from digital images of the cross-sectioned surface (DICSS) and digital images from microscopic sections (DIMS) were processed. Gray areas on DICSS largely represented areas of dense cellularity, and around subthalamic nucleus (STN), the zona incerta and field of Forel were clearly distinguishable on the anterosuperior side, as was the substantia nigra on the caudal side. DICSS successfully delineated the anatomical structure identical to the STN and surrounding contiguous nuclei. This new brain atlas will allow elucidation of anatomy that cannot be clearly disclosed from modern radiographic imaging or is very difficult to analyze with spatially inconsistent histological sections, and will contribute to further progress in anatomical studies of the human basal ganglia.

1. Introduction

During the last two decades, deep brain stimulation (DBS) has gained wide use for patients with various movement disorders, including tremor, Parkinson's disease, and dystonia [1,2]. Selective modulation of activities in the targeted nucleus can contribute to disclosure of not only the pathophysiology of diseases but also the role of basal ganglia nuclei in the human brain [3–6]. For scientific investigation of the human basal ganglia, global comprehension of the topographic anatomy is essential to determine which part a recorded neuronal activity belongs to, and during stereotactic surgery for DBS, precise measurement of coordinates will directly influence the surgical outcomes [7–9]. Numerous radiographic techniques have been developed for use in magnetic resonance imaging (MRI) to better delineate the targeting nucleus, the subthalamic nucleus (STN), on radiographic images [10,11]. However, multi-sectioned atlases from fixed cadaveric brains still have played an important role [12,13], and many researchers and neurosurgeons engaged in stereotactic functional neurosurgery have

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been applying such atlases by digitizing, adjusting the magnification, and superimposing the resulting images on MR images from their patients to correct inter-individual difference in shape, size, and position [14,15]. While those atlases precisely demarcate structures in the basal ganglia, the anatomical sections are spatially sparse and distorted as a consequence of the staining processes. Since such inconsistencies make these images unsuitable for rendering into 3-dimensional models [16,17], creating a 'perfect' human brain atlas to provide precise and consistent images with minimal distortion is practically difficult.

In this study, we further advanced the anatomic atlas using serial sections of 50 μm thickness covering the entirety of human basal ganglia. Its usefulness in stereotactic surgery and the prospects for further scientific research are discussed.

2. Methods

2.1. Preparation of cadaveric brain tissues

Japanese human cerebral hemispheres were obtained from 10 donated cadavers (age range, 73–95 years; median, 80 years) and were fixed in 10% formalin solution for more than 4 weeks. Specimens were separated into right and left hemispheres on the midplane, and the anterior commissure (AC) and posterior commissure (PC) were identified in the mid-sagittal plane. The AC-PC line and mid-AC-PC point were identified, and the central part of each hemisphere was cut into a block measuring 50 mm × 30 mm × 50 mm, centering around the mid-AC-PC point, in the antero-posterior direction perpendicular to the AC-PC line, in the lateral direction parallel to the midplane, and in the rostro-caudal direction perpendicular to the AC-PC line and midplane, respectively. Those blocks sufficiently contained the basal ganglia, midbrain, and upper pons, which were immersed in phosphate buffer with glycerin for 2 weeks and frozen at -40 °C for 4 weeks.

2.2. Digital images from cross-sectioned surface of the frozen block

Each frozen block was cut into 50-μm-thick sections on the freezing microtome on which the digital camera was mounted at precisely the same distance from the surface of the sectioned block. Every time a tissue slice was cut on the microtome, each cross-sectioned surface was automatically photographed [Fig. 1A] and recorded in the same format (4064 pixels × 2704 pixels, 31.4 MB/photo, JPEG format). Images were transferred to a computer and edited on Adobe Photoshop software (Adobe Systems, Ltd., San Jose, CA, USA) to accentuate the gray-white contrast [Fig. 1B]. Digital images of the cross-sectioned surface (DICSS) were used to create the 2-dimensional atlas and 3-dimensionally reconstructed (3DR) models.

2.3. Digital images from histological slices

As the frozen block was cut and photographed, every 10th slice from one sagittal hemisphere was sampled and stained using the Kluver-Barrera (KB) method [Fig. 1C]. The prepared slides were photographed under light microscopy, and data from digital images from microscopic sections (DIMS) were processed on the software. DIMS were then superimposed onto the corresponding DICSS, and distortions were corrected by adjusting the magnification of the images independently in 2 directions (rostro-caudal and lateral directions in coronal images, and antero-posterior and rostro-caudal directions in sagittal images) to merge the contours of each image [Fig. 1D].

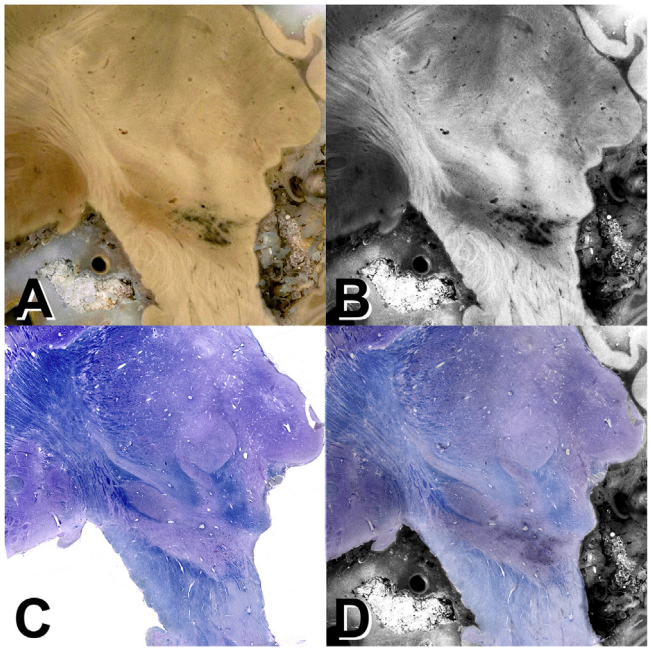

Fig. 1. The surface of frozen human brain in sagittal section was photographed (A) and edited in gray-white images (B). DICSS were used to create a 2-dimensional atlas. Simultaneously, sliced sections were sampled and stained with the KB method (C). Prepared slides were photographed under light microscopy, and data from DIMS were processed on the image software. DIMS were superimposed on the corresponding DICSS, and distortions were corrected by adjusting the magnification of images independently in 2 directions (rostro-caudal and lateral directions in coronal images, antero-posterior and rostro-caudal directions in sagittal images) to merge the contours of each image (D).

2.4. Comparison of gray-white contrast on DICSS with neuron-myelin contrast on DIMS

To verify whether DICSS correctly disclosed the area of specific nuclei in the basal ganglia, we compared gray-white contrast on DICSS to neuron-myelin contrast on DIMS around the STN, and consistency of the anatomy was assessed by comparing the borders of these nuclei between DICSS and DIMS.

2.5. Localization of STN on 3DR models

Data of DICSS were processed using the TRI system (RATOC System Engineering, Tokyo, Japan) to create 3DR models. The coordinates of STN borders were calculated on the basis of the 3 axes centering on the AC-PC line, and distances to the anterior, posterior, medial, lateral, rostral, and caudal borders were measured from each plane, including the mid-AC-PC point (MCP). The central point of the STN was defined as the intersection of mid-planes between the anterior and posterior borders, medial and lateral borders, and dorsal and ventral borders.

3. Results

Anatomical slices were successfully made from 10 frozen blocks of cerebral hemispheres. Nine blocks were sliced horizontally or vertically to the midplane including the AC-PC line to construct the conventional 3 sections (3 axial slices, 3 coronal slices, and 3 sagittal slices) [Figs. 2–4]. During DBS surgery, we obtained the series of T2-weighted MR images tilted 4° to the coronal plane, in which the image including the STN was chosen. The trajectory tilted 10° from the mid-plane, sufficiently passing through the STN, was planned on images [5,18,19]. One block was cut in the

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