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Original Article

Three dimensional anatomical microdissection of rat brain using fiber dissection technique

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ABSTRACT

Introduction: Using Klingler's fiber dissection technique, we aimed to demonstrate microdissections of the specific regions in the brain of rat which is undoubtly one of the mostly used animal in neuroscience researches.

Methods: Formalin fixed cerebral hemispheres of rat brains were dissected under operating microscope. Klingler's technique of fiber dissection was applied. Cortex, intrinsic anatomy and cranial nerves were studied. During and after dissection, photographs were taken and three dimensional pictures were obtained using a special software (Anamaker 3D©; available free from www.stereoeye.com, Tokyo, Japan).

Results: The anatomical relation of structures, seen in histological sections, was determined in our study. Hippocampus, thalamus and internal capsule, which are frequently studied, are explained with three dimensional fiber dissection technique. In rats, trigeminal nerve, olfactory nerve, hippocampus lying to the fornix and olfactory bulb lying to the frontal horn are more distinct when compared to humans.

Discussion: The microdissection of rat brain, to obtain needed structures accurately for experimental purposes, is an extremely important model. On this basis, our study serves the microsurgical anatomy of the rat brain for neuroscientists knowledge.

1. Introduction

In basic structure of central nervous system, white matter is composed of myelinated fibers that are classified into five functional categories: tracts in the brainstem, projection fibers passing up and down the neuraxis and connecting the cortex with caudal parts of the brain and spinal cord, association fibers interconnecting different cortical regions of the same hemisphere, limbic system tracts, and commissural fibers interconnecting the two hemispheres across the median plane.[1] Although it is complex and not completely elucidated, knowledge of the white matter organization is of neurosurgical significance. Although there are neuroanatomic texts, atlases and several studies that describe the fiber bundles,

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there is a lack of anatomic explanations and illustrations suitable to acquire an appropriate three dimensional knowledge for experimental practice.[2–6]

In 1935, Joseph Kingler, an anatomist in Basel, improved method of brain fixation and developed a technique that bears his name (Kingler's technique).[7,8] He dissected formalin-fixed brains with wooden spatulas. However, he froze and thawed the brains before dissection. The freezing method contributes to dissection and generally increases the distinction between the gray and white matter of the brain, even though the technique itself may not produce absolutely consistent results as Klingler himself acknowledged.[7] As a rule, however, the technique makes it easier to prepare dissections of the both fiber tracts, nuclei and serves literature for improving neuroanatomical knowledge in many studies on human cadavers.[5,6,9–11]

Microsurgical anatomy is important in humans for understanding the surgical anatomy. It is also important in rats to get correct specimens during neuroscience experiments. It is known that more than half of the animals used for neuroscience research are rodents (rats and mice) that are bred specifically for this purpose.[12] Microdissection of rat brain is frequently required for tissue evaluations like RNA or protein extraction and Western blot analysis. In neuroscientific studies, it is not uncommon to get a specimen that may contain some other structures than what was planned before experiment. Due to the practical conditions during the experiments, detailed examination is usually impossible. Quick removal of the intended brain region is essential to avoid enzymatic degradation of the tissue, and also to preserve the morphology of the tissue. For better morphology and staining results, the time period, from the removal of the tissue from body to place in an appropriate fixative environment, is critical and samples should not be let dry during dissection. In brief, knowledge of rat brain anatomy is extremely important to provide convenience for experimental neuroscience. Besides, it should also be made clear on histological grounds as well as anatomical basis before the microdissection of the tissue collection starts.

Our aim was to demonstrate a 3-D view of rat brain using a special technique in order to gain anatomical aspect with detailed knowledge concerning the main structures and tracts.

2. Material and methods

Adult male Wistar albino rats (weighing between 250 and 300 g) were used in the experiment. First group of rats ($n = 4$) were used for fixed brain dissections, second group of rats ($n = 4$) were used for histological evaluations. The animals were bred at the Marmara University Animal Research Laboratory. All experimental protocols were approved by the Marmara University School of Medicine Animal Care and Use Committee (permission number = 69.2010.mar).

The rats were decapitated under deep anesthesia by ether inhalation. The whole brain was rapidly removed from the skull. The brains were immersed in 10% formalin solution either for fixed brain tissue dissections or for histological investigations.

The first step in the preparation of specimens was the removal of the arachnoidal membrane and vascular structures under magnification (×6–×40). The fixed brains were turned to be frozen at −16 °C for 2 weeks.[13] Twenty-four hours after completion of the freezing process, the white fiber dissection method was started. The specimens were kept wet by occasional watering (alcohol) to avoid drying and to preserve the consistence of the tissues during dissection for better photographing. The fixed specimens were kept in 70% alcohol to avoid drying in-between dissection sessions. Surgical tools used for dissections were bone ronguer, clamp, scissors, forceps, microdissector, surgical knife, fine and self-shaped wooden spatulas. We dissected the rat brain both from medial to lateral and lateral to medial. Cranial nerves were also dissected bilaterally and their relation with cerebrum was demonstrated. This method was applied according to Klingler's fiber dissection technique.[13] Numerous digital photographs in each step were taken and with the use of specific software (Anamaker 3D©; available free from www.stereoeye.com, Tokyo, Japan), we fused the images to obtain an anaglyph image.[14,15]

For histological purposes, a brain cutting block, that permit the insertion of a standard razor blade into the cutting channels, was used to cut the brain coronally into slices. The brains were positioned on the cutting block such that the ventral surfaces looked upwards. Both the razor blade and the cutting block were kept on crushed ice during the process. The initial razor blade was placed in the channel at the most posterior aspects of the olfactory tubercles. At intervals of 2.0 mm the other razor blades were inserted along the caudal extent of the brain. The brain was thus divided into 11 sections (cranial to caudal). The razor blades were removed from the block. Coronal brain slices adhering to their surfaces were placed on a glass plate which was suspended on the crushed ice. The brain slices obtained were further processed for routine light microscopic evaluations. Tissues were dehydrated in graded ethanol series and cleared in toluene. They were embedded in paraffin and tissue sections (5 μm) and were cut by a rotary microtome. Finally, sections were stained with crystal violet to visualize general brain histology. Stained sections were examined and photographed under an Olympus BX51 photomicroscope (Tokyo, Japan) in order to verify the anatomical dissection routes (Fig. 1a–d).

3. Results

After the rats were decapitated, the head were turned posteriorly. When the skin was incised, periosteal tissue was seen over the bone. Large muscles, the superficial temporal muscles were dissected. After that, the bone was removed with a posterior to anterior approach. The occipital bones superiorly were cut by a bone ronguer in a forward direction to visualize the cerebellum in. After the occipital bone, superiorly came the interparietal bone that was a single bone located posterior to the two parietal bones. At the level of pons, clivus was noticed anteriorly. Removing the interparietal bone revealed a medial large vermis and the cerebellar hemispheres on two lateral sides. In this area, we could recognize the dentate nucleus which was the largest nucleus of the cerebellum.