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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.

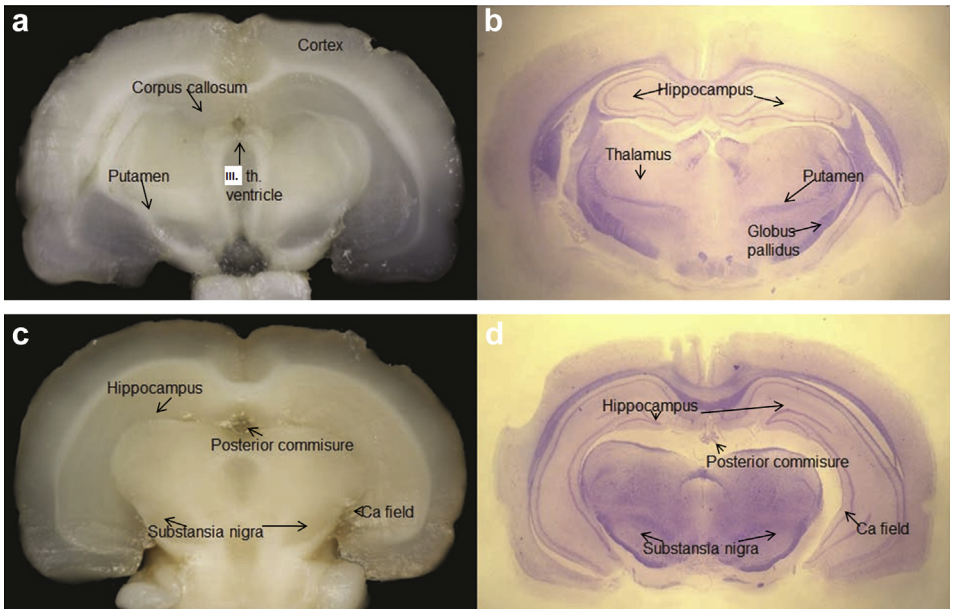

Fig. 1 – Representative coronal sections of the rat brain from two distinct levels. Macroscopic pictures of slices on the left and histological crystal violet stained (×10, original magnification) sections on the right.

Cerebellum was approximately one third of the whole brain under the occipital bone, some part being under the interparietal bone. At this level, foramen magnum could be seen while medulla passing in this area. Anterior to foramen magnum, occipital condyle could be visualized with large muscle groups.

Removing the parietal and frontal bones superiorly revealed the two cerebral hemispheres. The cerebral hemispheres were enclosed by the meninges, the protective connective tissue layers of the brain. The brain was apparent attached to the base of the skull by cranial nerves. Olfactory bulb extended into frontal lobe and the temporal lobe. Under the cerebrum the dura attachment at the tentorial and falx were recognized. After removing the dura, the seventh and eighth cranial nerves were present at the pontomedullar junction. Projecting superiorly, the two nerves conveyed to the acoustic canal, together (Fig. 2a). When we retracted the cerebellum and cerebrum superiorly the trigeminal nerve

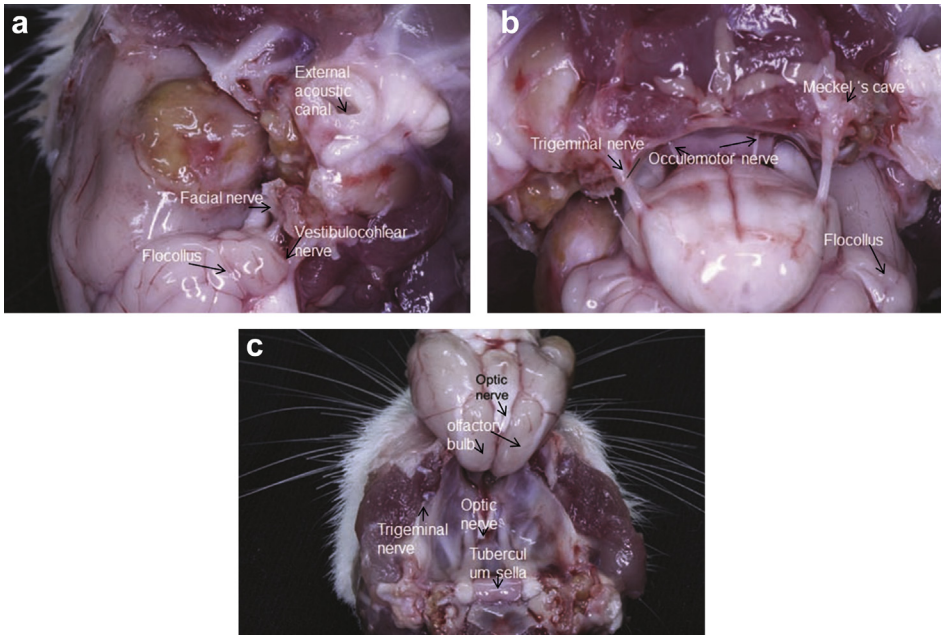

Fig. 2 – Removal of the brain from the skull. a. Facial and vestibulocochlear nerves. b. Ventral superior view of the medulla. c. Optic nerve dissected from the prechiasmatic region; tuberculum sella and olfactory bulbs are seen.

could be noticed. It gave three branches at the level of Meckel's cave and the branches of the trigeminal nerve radiating to the face were seen (Fig. 2b). At the anterior and superior part of the trigeminal nerve, the third and the fourth cranial nerves were detected. Trochlear nerve was very difficult to see but when we dissected carefully we were able to observe the nerve while projecting to superior orbital fissure (Fig. 2b). After cutting the trigeminal, the vestibular and the facial nerves, we saw the optic nerve and the pituitary gland. At the lateral side of the pituitary gland, mamillary body could be seen easily. After dissection of the frontal lobe and the cerebrum was superiorly retracted, the olfactory bulb, which was the largest nerve of the rat, was observed. Olfactory nerve projected to entorhinal cortex with a large bulb (Fig. 2c).

On the ventral aspect of the rat brain, from anterior to posterior the structures were olfactory nerve, olfactory bulb, optic nerve, hypothalamus, pyriform lobe, crus cerebri, trapezoid body, pons, trigeminal nerve and pyramidal tract. Olfactory bulb was located anterior to pyriform lobe. Optic chiasm was posterior to olfactory bulb and hypothalamus was posterior to optic chiasm. Crus cerebri was posterior to both the hypothalamus and the optic chiasm. Trigeminal nerve was located at the middle cerebellar peduncle. Pyramidal tract was located between the two trigeminal nerves (Fig. 3a).

At the dorsal view of the brain, large cerebrum tissue, cerebellum, pineal gland could be observed (Fig. 3b). Rat cerebrum had less sulcus and gyrus compared to human brain. Cerebellum was located posterior to the cerebrum. Pineal body was in-between the cerebrum and cerebellum. Olfactory nerve was present anterior to frontal lobe. Cerebellum had very big percentage volume compared to human cerebellum as shown in Fig. 3b–d.

In this step of the dissection, the brains were fixed in 10% formalin frozen at −16 °C for 2 weeks. After the brains were thawed, they were kept in 70% alcohol between the dissection sessions. After dissection of gray matter of frontal and temporal lobes, white matter was observed. Internal capsule covered the lateral wall of the lateral ventricle and hippocampus. Cortical fibers were seen in a superior to inferior fashion. Inferior to cortical fibers putamen was observed anterior to the frontal horn. Olfactory nerve and olfactory bulb situated inferomedial to cortical fibers (Fig. 3c).

Thalamus was located anterior and lateral to the hippocampus. Choroid plexus was laying anterior and medial to the thalamus and was observed with its pink color. Corpus callosum was at the midline and had fibers for communication between the two lobes (Fig. 3d).

Corpus striatum was anterior and lateral to the lateral ventricle. Hippocampus continued with the fornix at the midline and foramen Monro could be observed at the midline after removing stria medullaris. Thalamus and choroid plexus were located bilaterally. Thalamus was lateral to choroid plexus and lateral ventricle. After dissection of all gray matter from the dorsal surface and lateral surface of the cerebrum, hippocampus was detected conveying from the lateral to midline (Fig. 4a–d). Choroid plexus was present in-between the corpus striatum and the hippocampus. The olfactory nerve was anterior to the corpus striatum. Superior colliculus was located medial and posterior to the hippocampus as shown in Fig. 4b. Optic tract had lateral border that is called as lateral geniculate body, posterior to this, hippocampus could be visualized as shown in Fig. 4a.

Medial and lateral geniculate bodies located at the two lateral sides of the hippocampus. From anterior view optic striate, at the midline between the olfactory tubercles, could be observed. Optic striate continued with lateral geniculate body at the lateral side of the brain.

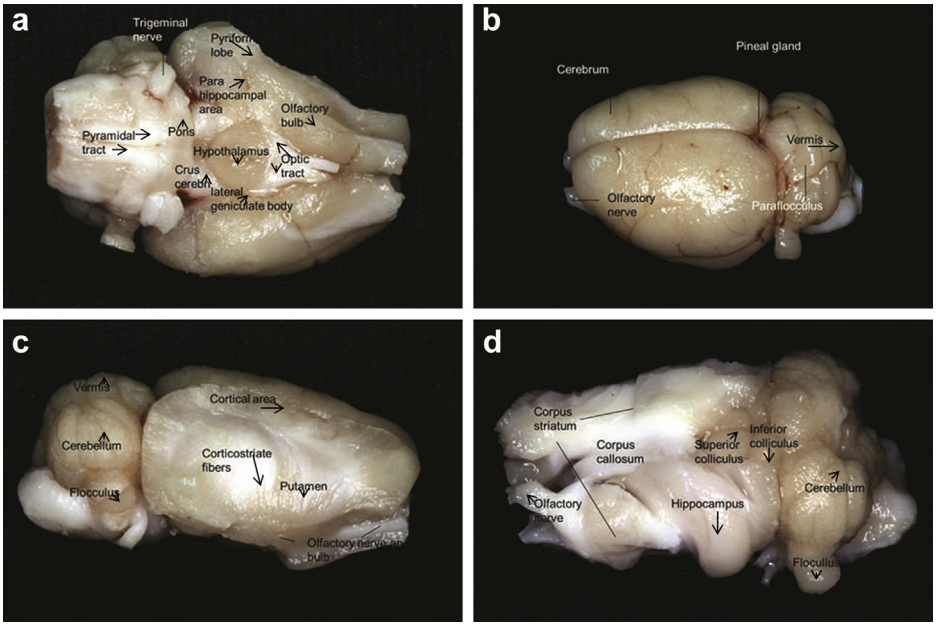

Fig. 3 – Before removal of the cortical gray matter. a. Ventral view of the rat brain. b. Dorsal view of the rat brain. c. Putamen is observed inferior with olfactory nerve and olfactory bulb are observed inferiomedial to cortical fibers. d. After dissection of all gray matter from the dorsal surface and lateral surface of the cerebrum.

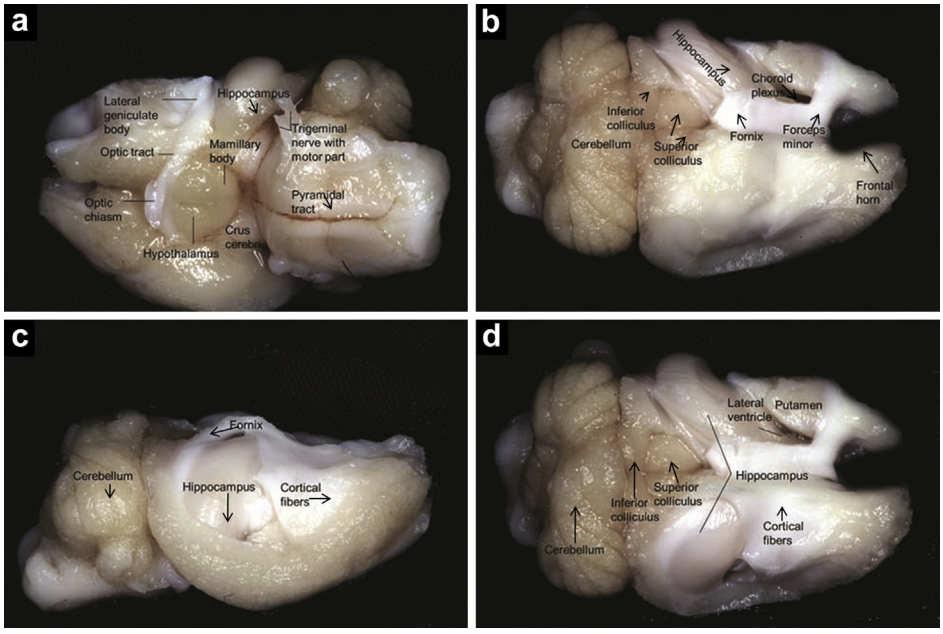

Fig. 4 – a. Hippocampus, trigeminal, crus cerebri and mamillary are seen. b. After bilateral cortical fibers are removed. c. Lateral view of rat brain. d. After dissection of cortical fibers, lateral ventricle is observed with its choroid plexus.

Cerebellum was dissected from the middle and inferior cerebellar peduncles. Lateral and medial geniculate bodies were superior to the middle and superior cerebellar peduncles. Superior cerebellar peduncle was superior to the middle cerebellar peduncle. At the dorsal view of the brain stem, after removal of the cerebellum, fourth ventricle and aquaductus cerebri were observed (Fig. 5a–b).

At the brain stem level we could observe the pyramidal tract anteriorly, gracilis cuneatus posteriorly and the trigeminal nerve arising from lateral and superior part of the pons. Vestibular and facial nerves arised from the lateral part of the medulla and were present superior to the pyramidal tract. Superior and inferior colliculi were larger parts of the pons and the mesencephalon. At the lower level of the brain stem; gracilis, cuneatus, obex, hypoglossal and vagal nuclei were seen.

Pituitary stalk was thin and was in pinkish color presented at the midline. Mamillary bodies were seen bilaterally. At the midline, we were able to observe the falx and after removing the falx, the corpus callosum. Lateral and inferior to the corpus callosum, fornix could be distinguished. In Fig. 6a–c, we can see the 3D demonstrations of whole rat brain.

4. Discussion

In this study, we described some important areas of the rat brain under microscope such as the superficial surface of the cortex, subcortical structures and cranial nerves. Anatomic dissection of rat brain is important for understanding the brain physiology and displays an important step in sample preparation for experimental proteomic (subcellular) or histopathological studies.

Important association, projection, and commissural fasciculi were identified anatomically using Klingler's fiber dissection technique in this study. The usefulness of this technique for the neurosurgeon has been demonstrated in several recent publications.[5,6,9,16–24] However, few of these articles used three-dimensional reconstruction to aid the reader in understanding the spatial relationships.[5,6,23] This

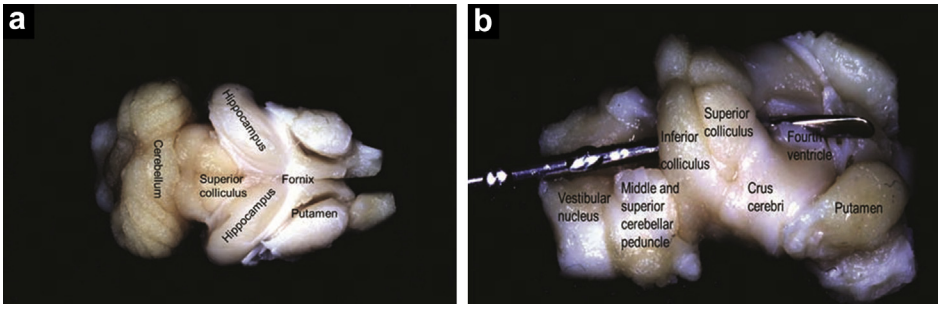

Fig. 5 – a. Dorsal view of the rat brain. b. Fourth ventricle is located posterior to the medulla and the pons. Third ventricle is connected to the fourth ventricle by aquaductus that is shown by dissector.

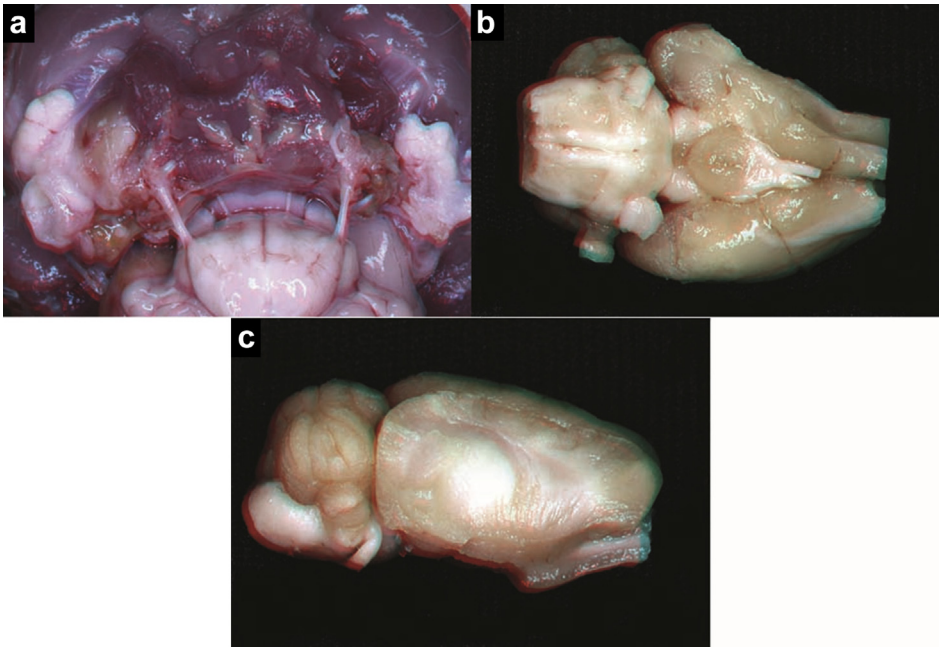

Fig. 6 – 3D vision of the some dissections.

method is applicable to pharmacological and behavioral studies which requires the dissection of numerous brains during short time intervals.[25] Our study created a collection of three-dimensional images that are useful in assimilating the important structures and topography and intricate relationships of the white fiber tracts in rat brain.

Fine dissection becomes possible in the deeper sites of the central nervous system using molded wooden spatulas of various sizes. However, the fiber dissection technique is limited because of the complex relations between fiber systems. The demonstration of one fiber system often results in the destruction of other fiber systems.[6] Enough anatomical preknowledge before beginning the dissection of fibers may reduce this possibility to some extent.

It is also predictable that the anatomical microdissection is difficult in rat brain because of its smaller size compared to human brain. Freezing helps us separate the fibers as explained in the technique.[13] Nevertheless, more thin and gentle spatulas are needed for dissection of that small brain. The fiber dissection technique would be much more difficult in younger rat brains due to incomplete myelinization and high water content which does not enable proper fixation.

The olfactory bulbs are paired, ovoid shaped structures forming the rostral end of the telencephalon. In many mammals, they occupy the foremost position in the skull. In humans and rats, the bulbs, displaced by the enlarged cerebrum, are relatively small and located under the ventral surface of frontal lobes. The olfactory bulb is a cortical structure and has a characteristic laminar organization. The first anatomical descriptions of the olfactory bulb were made in the second half of 19th century and summarized in the classic book of Ramon Cajal.[26]

The absolute size of the hippocampus increases steadily with phylogenetic development. However, it is known that it occupies a large portion in the forebrain of the rat. Similarly, our study revealed the large hippocampus underneath the posterior and temporal neocortex of rat. Previously, the hippocampus is divided into a dorsal portion, lying just behind the septum; a posterior portion where it begins to bend ventrally and laterally; and a ventral portion lying in the temporal part of the brain. The part of the hippocampus visible on its dorsal aspect is the hippocampus proper, while the fascia dentata is buried inside and on the bottom surface of the sausage shape. The fimbria is a large fiber tract which is visible on the lateral edge of the exposed hippocampus.[27] In the literature hypothalamus was observed extending posteriorly to the columns of the fornix and the mamilothalamic tracts and laterally to the talencephalic–diencephalic junction.[25] Thalamus was in-between hippocampus (anteriorly), crus cerebri and fimbria of hippocampus (medially), the medial lemniscus (posteriorly).[25]

The dissection of the hippocampus from the rat skull is frequently made medially through the lateral ventricle. However, paramedian or median approach at the high level cortex may also be used for dorsal hippocampal dissection and sagittal approach is recommended for fornix dissection. The whole bulk of the hippocampus with choroid plexus can be taken out by bilateral frontal approach, if dissection is continued to the posterior temporal area. Additionally, amygdala can also be dissected from the hippocampus and parahippocampal gyrus via the dissections performed from the inferior side. In the study of Heffner et al, they found that amygdala was including some portions of the entorhinal cortex.[25]

Although some are very thin and some are found embedded in the neural tissue of the cranium, the cranial nerves can also be removed under the light of good neuroanatomy knowledge. Removal of the brain from the skull leads to the optic and trigeminal nerves. They can be removed out holding the brain with the bayonet on one hand while

holding the skull on the other hand carefully avoiding the breaking away of the nerves. Trochlear nerve is very thin and fragile, but can be observed at the caudal side of the trigeminal nerve. Olfactory nerves occupy a wide region at the frontal ventral side of brain and extends to entorhinal cortex as an important part of the limbic system and gets in contact with uncinate fasciculus and hippocampal region. Considering the prominent, thick trigeminal nerve in rats, the optic, oculomotor, abducens, facial and vestibular nerves are less developed in rats compared to humans. It is not difficult to follow the branches of facial nerve in the rat from the bifurcation to the endpoints just beneath the skin, even without a microscope. Therefore, the rat facial nerve can be a good experimental material to be studied out. The parotid gland is large and covers the main trunk and the bifurcation of the facial nerve, yet the nerve does not pass through it, and the gland can be retracted off the nerve without any injury. After emerging from the cranium, the main trunk follows the external ear canal before branching. An average length of almost 5 mm provides an opportunity to study a thick nerve appropriately. Because of the shape of the cranium, the rat has very long buccal and mandibular branches, which may be suitable for experimental manipulations. However, the frontal branch is considerably short. Despite the possible variations, the standard deviation of the length of the nerve among animals is not high, which allows comparative studies without excessive dissections.

Vascular tufts, called choroid plexus, produce the cerebrospinal fluid within the ventricular cavities of adult brain. Lateral ventricles connect to the third ventricle by the interventricular foramina (of Monro). Continuing caudally, the cerebral aqueduct opens into the fourth ventricle. The fourth ventricle occupies the space dorsal to the pons and medulla and ventral to the cerebellum. CSF flows from the fourth ventricle to the subarachnoid space trough the median aperture (Magendie) and the lateral apertures (Luschka). Most of the CSF is produced by the choroid plexus of the lateral ventricles, although tufts of choroid plexus are found in the third and fourth ventricles as well. The structures that construct the ventricular system of the rat were similar to human according to our observations in the present study.

Eventhough we did not focus on substantia nigra in this study, it has a dark oval shape. It was described to be observed after a horizontal cut made at the brainstem at the level of the rhinal sulcus, dorsal edge of the nigra with removal of telencephalic tissue lateral to the brainstem.[25] The remaining tissue medial to substantia nigra was named as ventral tegmentum.

Human and rat brains have similarities in many ways. However, primitive brain is more developed in rats and the neocortex is more developed in humans. That's more likely because the frontal lobe is more mature and larger in humans, but temporal lobe and cerebellar lobes are larger in rats. The cerebellum is responsible for balance and posture which are more pronounced in rats besides the behavior under control of limbic system. The gyri of frontal lobe in humans is more pronounced, however we noticed the deep sulci in the large rat cerebellum in this study. More superficial fiber dissection route nested in the limbic system is observed in the rats in this study, compared to the complex white matter tissue in humans. Heffner et al depicted the central sulcus which is easily followed till the Sylvian fissue by fiber dissection technique in humans is observed more superficial in rats. The main structure of internal and external capsule is formed by cortical fibers, medial to which the putamen and the globus pallidus are located. These latter structures are prominent in rats. The caudate putamen location more delicately such as posterior to the anterior commissure, ventral to the corpus callosum and medial to the external capsule.[25] They also observed that caudate putamen was included with the globus pallidus. Structures that are followed easily in the anterior commissure and the relation of which is clearly seen with the olfactory bulb are more prominent in the rats compared to that of humans. The structures which are more clearly observed in humans, such as inferior frontooccipital fasciculus, inferior temporal fibers, internal capsule, Meyer's loop and sagittal stratum cannot be followed in rats using fiber dissection technique, but the complex structure of hippocampus and fornix is followed upon the medial area. However, the nuclei in the large cerebellum are distinguished in rats. Folia of the cerebellum, dentate nucleus, tonsillar and pineal region are all clearly identified. This finding of the present study once more shows the importance of balance in the rat.

5. Conclusions

This study, being the first demonstrative and explanatory study for rat brain dissection by fiber dissection technique in the literature, is important in order to learn approaches to specific structures in the rat brain to be used for various neuroscience experiments.

Conflicts of interest

All authors have none to declare.

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