

EXPERIMENTAL STUDY

The microscopical structure of the hippocampus in the rat

El Falougy H, Kubikova E, Benuska J

Institute of Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovakia.

hisham.elfalougy@fmed.uniba.sk

Abstract: The aim of this work was to study the rat's hippocampal formation by applying the light microscopic methods. The histological methods used to explore this region of the rat's brain were the Nissl technique, the Bielschowsky block impregnation method and the rapid Golgi technique.

In the Nissl preparations, we identified only three fields of the hippocampus proprius (CA1, CA3 and CA4). CA2 was distinguished in the Bielschowsky impregnated blocks. The rapid Golgi technique, according to the available literature, gives the best results by using the fresh samples. In this study, we reached good results by using formalin fixed sections. The layers of the hippocampal formation were differentiated. The pyramidal and granular cells were identified together with their axons and dendrites (*Fig. 9, Ref. 22*). Full Text (Free, PDF) www.bmj.sk.

Key words: archicortex, hippocampal formation, light microscopy, rat.

Abbreviations: CA1 – regio I cornus ammonis; CA2 – regio II cornus ammonis; CA3 – regio III cornus ammonis; CA4 – regio IV cornus ammonis.

The microscopical structure of the hippocampal formation has been studied intensively since the time of Cajal (1911) and his student Lorente de N6 (1934). They modified Golgi's method to increase its effectiveness and used it in studying the cellular architecture of the hippocampal formation in rodents (1, 2). By the middle of the last century the hippocampal formation started to be one of the most studied structures of the nervous system.

During the phylogenetic development from insectivores to primates, the topographical position of the hippocampus has undergone remarkable changes. As a consequence of these morphological changes, the hippocampus is found deeper and deeper in the temporal lobe (3).

The rat's hippocampal formation occupies a large portion of the ventroposterior and ventrolateral walls of the cerebral cortex (4). We can observe the C-shaped hippocampus proper after the removal of the thin entorhinal cortex. The ventromedial portion of the hippocampus proper is the tuberculum hippocampi, which is the homologous structure of the uncus gyri parahippocampalis in primates. The tubercle continues into the dentate gyrus, which extends dorsally under the corpus callosum to the septum. The caudal and ventral borders of the dentate gyrus are formed by the sulcus hippocampi. The fimbria hippocampi are observable

in the lateral border of the hippocampus. It continues under the corpus callosum as the fornix. Supracommissural hippocampus (the indusium griseum) extends over the corpus callosum to the anterior portion of the septum as thin strip of gray cortex (5, 6).

The hippocampal formation is subdivided into regions and fields according to the cell body location, cell body shape and size, proximal terminations, complex spines, distal branching characteristics and afferent and efferent projections (1). The hippocampus proprius, gyrus dentatus and subiculum are paradigm of simple cortex, consisting primarily of one basic cell type and its associated interneurons. These basic neurons are backed together in one layer of a three-layered structure, in contrast to six layers of neocortex (6). The dominant neurons in the hippocampus proprius and the subiculum are the pyramidal cells. The size and the density of these neurons are variable throughout the hippocampus proprius. This fact was used to differentiate the fields of the hippocampus proprius. Large number of spines was identified on the dendrites of the pyramidal cells (1). Dendritic spines are the major sites of excitatory synaptic transmission. Recent studies indicated that dendritic spines are highly dynamic structures. Alterations of the spines have been observed in physiological conditions such as memory and learning, and in pathological conditions such as epilepsy and hypoxia. The alteration of the dendritic arborization directly affects the number of the spines and thus the function of the neurons. It has been reported that the number of the dendritic spines in the CA1 pyramidal cells is reduced in aged rats and in Alzheimer's patients (7).

Granular neurons are the basic cells in the gyrus dentatus. The term used for the axons of the granular cells is the mossy fibers, because of their shape and course (8). In the hilum of the gyrus dentatus, 21 types of cells were identified (9).

While in the most brain regions neurogenesis is completed by birth, the dentate gyrus represent a notable exception in the

Institute of Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Address for correspondence: H. El Falougy, MD, Institute of Anatomy, Faculty of Medicine, Comenius University, Sasinkova 2, SK-811 08 Bratislava, Slovakia
Phone: +421.2.5937374

mammalian brain because its main neurons, the granular cells, proliferate during an extended period that begins during gestation and continues into the postnatal period. There are new findings suggesting that neurogenesis continues throughout the human life in the dentate gyrus (10). However the division of the hippocampal pyramidal cell division is completed before birth, the structure of the pyramidal cells is simple at birth. The pyramidal neuron undergoes process of postnatal maturation and proliferation of their axonal connections and dendrites (11).

To study the effect of pathological processes, such as hypoxia and ischemia, on the hippocampal formation, it is necessary to have anatomical knowledge of this region. Vulnerability of neurons in the hippocampal formation to the pathological processes is variable according the type of cells and the field, where these cells are placed. The goal of this work was to explore the fields, layers and cytoarchitectonic structure of the hippocampal formation.

Materials and methods

The material for this study was supplied by the Institute of Pharmacology in the Faculty of Medicine of Comenius University in Bratislava. The brains of six rats were dissected carefully and fast after a decapitation of animals were immersed in 10 % formalin solution.

After several weeks of fixation, the materials were processed by three different techniques. Two brains were used for each technique.

The brains of the first group were processed according to the Nissl technique (12). We used this method to illustrate the neural bodies and nuclei. The brains were cut in sections with 1–2 mm width of each in frontal and horizontal planes. The sections passed through the procedure of washing, dehydration, clearing, impregnation and embedding in paraffin. 10 µm sections were cut on a sliding microtome. The mounted sections were deparaffinized in xylene and transferred to 5 % formalin for five minutes. We washed the sections in 5 % acetic acid for five minutes, then briefly in distilled water. In the next step sections were stained for 30 minutes in 0.1 % toluidine blue solution in closed cell in thermostat at 37 °C, then the cell with sections were kept to cool at the room temperature. We washed the sections in distilled water and differentiated in 96 % alcohol until the background was relatively clear. Finally we washed the sections in absolute alcohol, cleared in xylene and cover slipped.

The second group of brains was processed by the Bielschowsky block impregnation method (13). This method was our choice in studying the neural projections and layers of the hippocampal formation. We cut 2–3 mm thick blocks in horizontal and frontal planes, and placed them in pyridine for 2 days. Blocks were washed in running tap water overnight, then washed in several changes of distilled water over 1 day. We treated the blocks in silver nitrate solution for 5 days in dark, and then washed in several changes of distilled water over 3 hours. The following step was a treatment in ammonical silver solution for 4 hours, then washing in distilled water (changed several times) for 2

hours. We reduced blocks in 20 % formalin for 12 hours, washed in water for 1 hour and paraffin processed. 20 µm sections were cut on a sliding microtome. The deparaffinized sections were placed for 15 min in 1 % gold chloride solution, followed by treatment in 5 % sodium thiosulphate for 2 minutes. In the last step sections were dehydrated, cleared and cover slipped.

Our last choice of staining method was the rapid Golgi technique, which was used profitably by Cajal. This method is used in observation of individual cells together with their projections (14, 15). We applied this impregnation method on the formalin fixed brains of the third group. We cut pieces not more than 4 mm in thickness in horizontal and frontal planes, and immersed them in osmium-dichromate solution at the room temperature for 7 days. The pieces were transferred to 0.75 % aqueous silver nitrate and kept for 24 hours in the dark. Then we placed the pieces back to the same osmium-dichromate solution used in the first step. They were left in this solution for 6 days. This step was followed by an immersion of the pieces in silver nitrate solution for 48 hours in the dark. Then we transferred them to a new osmium-dichromate solution for 5 days. Again we repeated the step with silver nitrate and increased the time solution for 3 days. The pieces were dehydrated in absolute alcohol for 5 minutes and embedded in soft paraffin matrix by pressing each piece gently into the melted paraffin. We made 200 µm sections on a sliding microtome. The pieces were transferred individually from the surface of knife to absolute alcohol for 15 minutes. Subsequently, we transferred the sections into oil of cloves for 15 minutes. Finally the sections were mounted on glass slides, washed in xylene and covered thinly by mounting medium.

Good sections from each group were signed and documented as photomicrographs by using Olympus digital camera.

Results and discussion

Under the term “hippocampal formation” we understand the complex of the gyrus dentatus, the hippocampus proprius, the subiculum and the area entorhinalis (16).

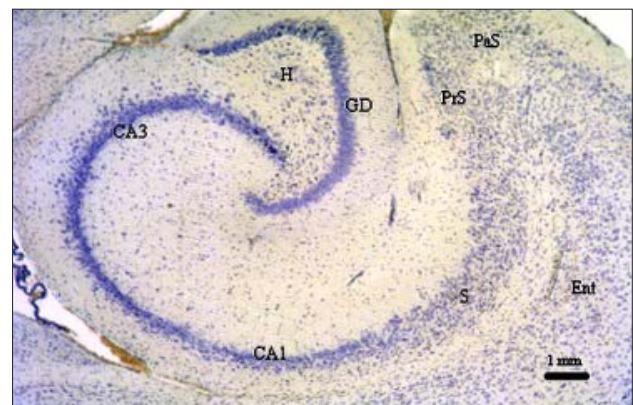


Fig. 1. Horizontal section of the rat brain, stained with the Nissl method. GD – gyrus dentatus, H – hilus fasciae dentatae, CA3 – regio III cornus ammonis, CA1 – regio I cornus ammonis, S – subiculum, PrS – pre-subiculum, PaS – parasubiculum, Ent – area entorhinalis.

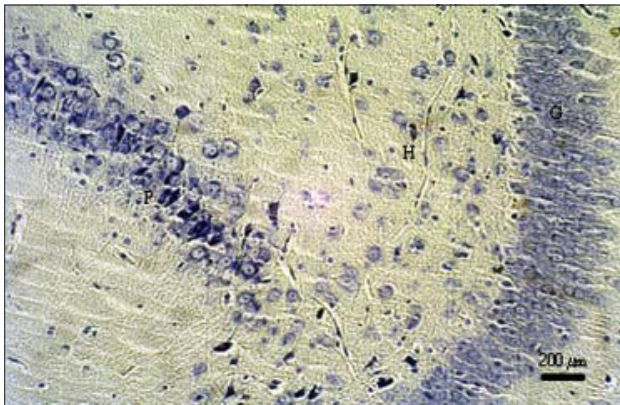


Fig. 2. Horizontal section of the rat brain illustrating the hilus of the gyrus dentatus, stained with the Nissl method. G – the granular cells of the gyrus dentatus, P – the pyramidal cells of CA3, H – hilus of the gyrus dentatus.

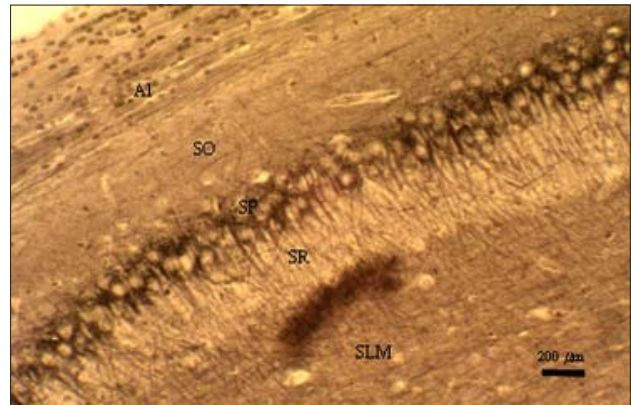


Fig. 4. Horizontal section of the rat brain illustrating layers of CA3, impregnated with the Bielschowsky method. AL – alveus, SO – stratum oriens, SP – stratum pyramidale, SR – stratum radiatum, SLM – stratum lacunosum-moleculare.

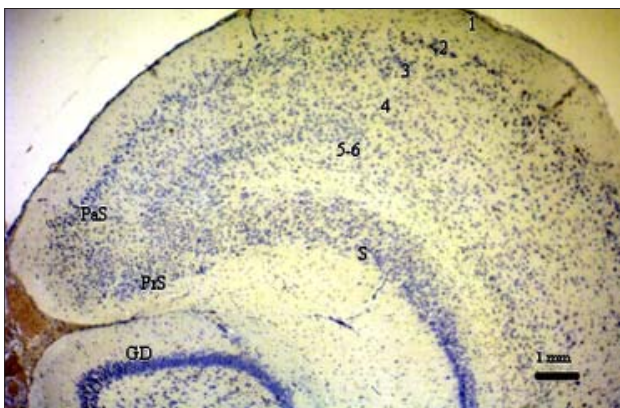


Fig. 3. Horizontal section of the rat brain illustrating the entorhinal cortex, stained with the Nissl method. GD – gyrus dentatus, S – subiculum, PrS – presubiculum, PaS – parasubiculum, 1–6 – layers of the entorhinal cortex.

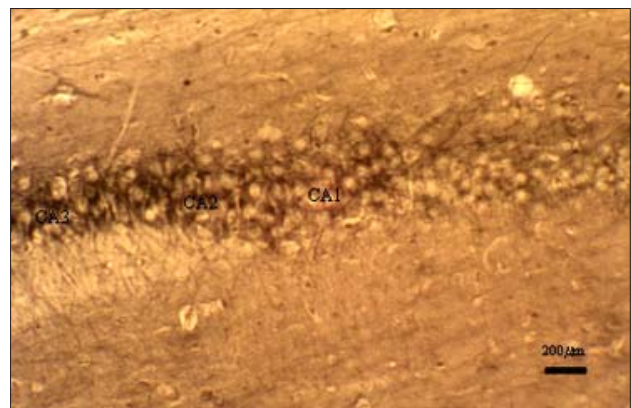


Fig. 5. Horizontal section of the rat brain illustrating CA2, impregnated with the Bielschowsky method. CA3 – regio III cornus ammonis, CA2 – regio II cornus ammonis, CA1 – regio I cornus ammonis.

All these structures can be observed in the horizontal section of the rat's brain stained by the Nissl technique. In the neurons stained by this method the cytoplasm is colored bright blue to bright violet, and the Nissl substance together with the nuclei are colored dark violet (Figs 1–3). The dentate gyrus is observed as coiled structure with opened concave part directed to the hippocampus proprius. The gyrus dentatus consists of three layers. Stratum moleculare is the superficial layer with scattered small nervous cells. Stratum granulare is the middle layer with packed oval shaped nervous cells, the granular cells. Stratum multiforme contains polymorphic nervous cells.

The hippocampus proprius is subdivided into 4 regions according density, size and branching of axons and dendrites of the pyramidal cells (CA1–CA4). Each of these regions consists of three layers: stratum moleculare, stratum pyramidale and stratum multiforme. The stratum pyramidale contains bodies of the pyramidal cells.

CA4 is the continuation of the CA3 in the concavity of the dentate gyrus. Hilus fasciae dentatae is the term used for the

complex of the pyramidal layer of CA4 and the stratum multiforme of the gyrus dentatus. CA3 is the region with large less densely packed cells. CA2 is a narrow transitional field between CA3 and CA1. We didn't distinguish the field CA2 in the sections stained by the Nissl technique. Densely packed medium sized cells characterize CA1.

The subiculum is the transitional field between the three-layered CA1 (archicortex) and the six layered area entorhinalis (neocortex). The subiculum is subdivided into: subiculum proprium, presubiculum and parasubiculum. The subiculum proprium is the field continuous with CA1 and the parasubiculum with the area entorhinalis. The presubiculum possess superficial layer of moderately packed cells. The parasubiculum has superficial layer with densely packed small cells. In both presubiculum and parasubiculum the superficial layer is overlying a deeper layer of small to medium sized cells similar to the deep layers of the area entorhinalis. The subiculum proprium instead of the superficial layer of cells contains loosely packed deep layer of pyramidal cells.

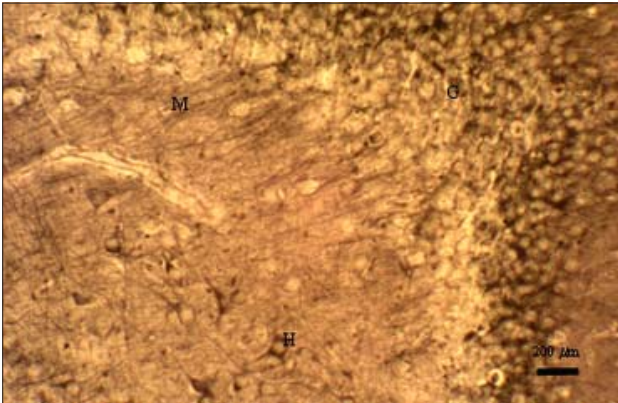


Fig. 6. Horizontal section of the rat brain illustrating the multifiform layer of the gyrus dentatus, impregnated with the Bielschowsky method. G – the granular cells, M – the mossy fibers, H – hilus of the gyrus dentatus.

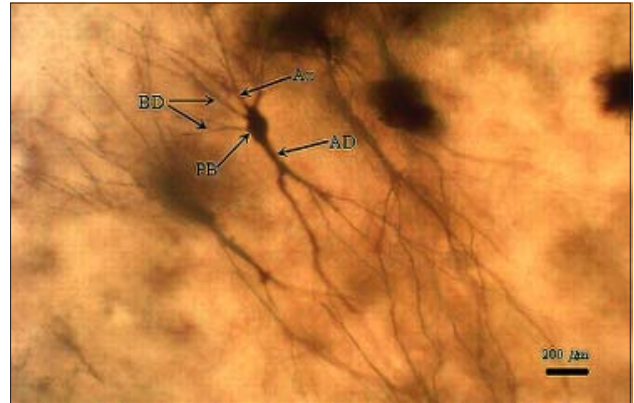


Fig. 8. Horizontal section of the rat brain viewing the structure of the pyramidal cell in CA3, impregnated with the Golgi method. PB – body of the pyramidal cell, Ax – axon of the pyramidal cell, AD – apical dendrite of the pyramidal cell, BD – basal dendrites of the pyramidal cell.

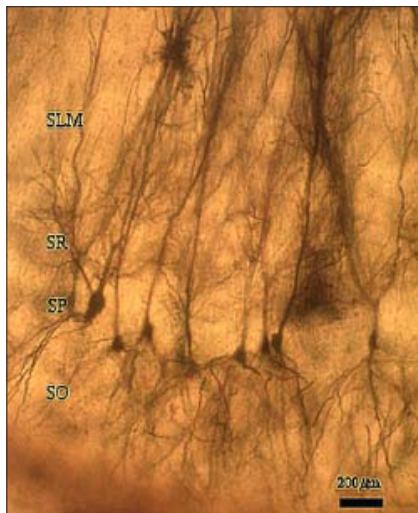


Fig. 7. Frontal section of the rat brain illustrating layers of the hippocampus proprius, impregnated with the Golgi method. SO – stratum oriens, SP – stratum pyramidale, SR – stratum radiatum, SLM – stratum lacunosum-moleculare.

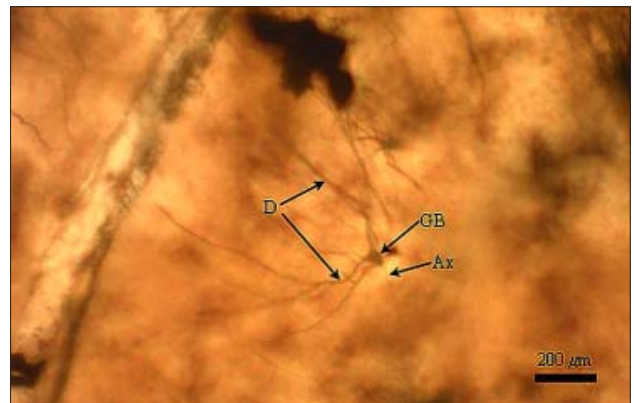


Fig. 9. Horizontal section of the rat brain viewing the structure of the granular cell in the gyrus dentatus, impregnated with the Golgi method. GB – body of granular cell, Ax – axon of the granular cell, D – dendrites of granular cells.

The area entorhinalis is composed of 6 layers. The most superficial layer (I) is the plexiform layer. The large stellate cells are located in the second layer (II). Medium sized pyramidal cells characterize the third layer (III). The fourth layer is a cell-sparse area with few scattered large pyramidal cells. The fifth (V) and the sixth layer (VI) are composed of small and medium sized cells.

In the brains of the second group, where we used the Bielschowsky impregnation method, the nerve fibers are stained in black and the neurons in brown (Figs 4–6). In these preparations, the dendrites of the granular cells in the gyrus dentatus are observable. We found here the polymorphic cells characterizing the hilus, too. The mossy fibers (axons of the granular cells) descend toward the CA3 field.

The fields of the hippocampus proprius are distinguishable. The CA2 lies as a narrow transitional field of cells between CA3 and CA1. Cajal subdivided the hippocampus proprius in 6 layers according to the position of the nervous cells bodies and ramification of the nervous fibers. These layers are recognized in the preparations of the second group. The alveus is the layer on the ventricular surface of the hippocampus. It contains the axons of the pyramidal cells. Stratum oriens is the second layer containing the basket cells. In the stratum pyramidale, we find bodies of neurons. The stratum radiatum consists of the dendrites of the pyramidal cells. In the stratum lacunosum-moleculare, the long apical dendrites of the pyramidal cells extend. The stratum radiatum and the stratum lacunosum-moleculare continue with the stratum moleculare of the subiculum.

The nerve cells, dendrites, axons and the terminal arborizations are stained dark brown against yellow background in the preparations impregnated by the rapid Golgi method (Figs 7–9). Only small number of nervous cells is stained. The cell body

together with its projections is impregnated, so we could study the cell as an individual unit. The bodies of the granular cells and its dendrites, which project to the molecular layer of the gyrus dentatus, are recognizable. The six layers of the cornu ammonis are identifiable. The pyramidal cells are characterized by the apical dendrites, which extend to the stratum radiatum and the stratum lacunosum-moleculare, and the basal dendrites extending to the stratum oriens. Axons of the pyramidal cells extend mainly to the alveus.

The rat hippocampal fields can be observed in the horizontal sections of the brain. In the Nissl stained sections, most authors rarely identify the CA2 (5). This finding was confirmed in the Nissl preparations used in this work. In the literature, the classical histological choice to distinguish the CA2 is the Golgi method, where the pyramidal cells of CA2 lacks the spiny thorns on the apical dendrites (17). In this work we identified CA2 by using the Bielschowsky impregnation method.

The best results of the rapid Golgi technique are reached by using fresh specimens (14). In our experiment we have obtained better results by using the formalin fixed sections, where the cytoarchitectonic structure of the hippocampus proprius and gyrus dentatus is observable.

Experimental studies proved that the hippocampus has a prominent role in the process of learning and memory (17, 18). Other important role of the hippocampus is its function in controlling the behavior related to food and appetite (19).

The experimental animal models proved that the pyramidal cells of the CA2 are the most affected hippocampal cells in the course of the Alzheimer's disease and the temporal lobe epilepsy (20, 21). The most vulnerable hippocampal cells to hypoxia are the pyramidal cells of CA1 and CA3 (22).

Based on these facts we can conclude that the changes of the hippocampus after pathological processes are mostly shown to be field-specific. Understanding mechanisms of these processes has to be based on the knowledge of the hippocampal cytoarchitecture. The present study describes and defines the hippocampal fields and their boundaries to conduct neuroanatomical studies on the hippocampus of the rat.

References

1. **Turner DA et al.** Morphological features of the entorhinal-hippocampal connection. *Prog Neurobiol* 1998; 55 (6): 537–562.
2. **Martin LA, Tan S, Goldowitz D.** Clonal architecture of the mouse hippocampus. *J Neurosci* 2002; 22 (9): 3520–3530.
3. **West MJ.** Stereological studies of the hippocampus: a comparison of the hippocampal subdivisions of diverse species including hedgehogs, laboratory rodents, wild mice and men. *Prog Brain Res* 1990; 83: 13–36.
4. **Bayer SA.** Hippocampal region. 335–352. In: Paxinos G (Ed). *The rat nervous system: forebrain and midbrain*. Sydney: Academic Press 1985; 1.
5. **Schwerdtfeger WK.** Structure and fiber connections of the hippocampus: a comparative study. *Adv Anat Embryol Cell Biol* 1984; 83: 1–74.
6. **O'Keefe J, Nadel L.** *The hippocampus as a cognitive map*. Oxford university press, 1978: 570 pp.
7. **Ruan YW et al.** Dendritic plasticity of CA1 pyramidal neurons after transient global ischemia. *Neuroscience* 2006; 140: 191–201.
8. **Hestings NB, Tanapat P, Gould E.** Neurogenesis in the adult mammalian brain. *Clin Neurosci Res* 2001; 1 (3): 175–182.
9. **Amaral DG.** A golgi study of cell types in the hilar region of the hippocampus in the rat. *J Comp Neurol* 1978; 182: 851–915.
10. **Gahr M et al.** What is the adaptive role of neurogenesis in adult birds? *Prog Brain Res* 2002; 138: 233–254.
11. **Seress L, Ribak CE.** Postnatal development of CA3 pyramidal neurons and their afferents in the Ammon's horn of rhesus monkeys. *Hippocampus* 1995; 5: 217–231.
12. **Vacek Z.** *Histológia a histologická technika. Díl 2: histologická technika*. Brno: Institut pre další vzdělávání pracovníků ve zdravotnictví, 1996: 184 pp.
13. **Cook HC.** *Manual of histological demonstration techniques*. London: Butterworths, 1974: 314 pp.
14. **Valverde F.** The Golgi method. A tool for comparative structural analyses. 12–31. In: Nauta WJH, Ebessson SOE (Eds). *Contemporary Research Methods in Neuroanatomy*. Berlin; Heidelberg; New York: Springer-Verlag, 1970.
15. **Baloyannis SJ.** The acoustic cortex in vascular dementia: a Golgi and electron microscope study. *J Neurol Sci* 2005; 229–230: 51–55.
16. **Künzle H, Radtke-Schuller S.** Hippocampal fields in the hedgehog tenrec: their architecture and major intrinsic connections. *Neurosci Res* 2001; 41 (3): 267–291.
17. **Keuker JIH, Rochford CDP, Witter MP, Fuchs E.** A cytoarchitectonic study of the hippocampal formation of the tree shrew (*Tupaia belangeri*). *J Chem Neuroanat* 2003; 26: 1–15.
18. **Barr WB, Goldberg E.** Pitfalls in the method of double dissociation: delineating the cognitive functions of the hippocampus. *Cortex* 2003; 39 (1): 153–157.
19. **Tracy AL, Jarrard LE, Davidson TL.** The hippocampus and motivation revisited: appetite and activity. *Behav Brain Res* 2001; 127 (1–2): 13–23.
20. **Kaufmann WA, Barnas U, Humpel C et al.** Synaptic loss reflected by secretoneurin-like immunoreactivity in the human hippocampus in Alzheimer's disease. *Europ J Neurosci* 1998; 10: 1084–1094.
21. **Leranth C, Ribak CE.** Calcium-binding proteins are concentrated in the CA2 field of the monkey hippocampus: a possible key to this region's resistance to epileptic damage. *Exp Brain Res* 1991; 85: 129–136.
22. **Johansen F.** Interneurons in rat hippocampus after cerebral ischemia. *Acta Neurol Scand Suppl* 1993; 150: 1–32.

Received November 5, 2007.

Accepted February 4, 2008.