Praca oryginalna *Original paper*

Morphological and histological analysis of the hippocampal formation in the American mink

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Received 13.09.2013 *Accepted 09.04.2014*

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Summary

The aim of the research was to characterize the topography and cytoarchitecture of the hippocampus in the American mink (Neovison vison). The hippocampal formation is a neural structure of the rhinencephalon which stretches from the splenium of the corpus callosum to the ventromedial angle of the cerebral hemisphere. The hippocampal formation is subdivided into regions, layers, and fields (CA1-CA4). The brains of six sexually mature American minks were used in the study. The material collected was mounted in paraffin, and the paraffin blocks were cut into slices. Morphological examinations were carried out with an Olympus BX40 light microscope. The dominant neurons in the hippocampus proper and the subiculum are pyramidal cells. The neurons are morphologically different in each of areas CA1-CA4. Small, loosely arranged neurons form 2-3 layers of cells. In areas CA1-CA3 the neurons are densely packed, forming 5-6 layers of cells. Most of them are pyramidal in shape, with large round or oval nuclei. Area CA4 contains loosely scattered cells of a pyramidal layer located near the entrance to the sinus of the dentate gyrus. The regio superior is a part of hippocampus adhering to the subiculum, whereas the regio inferior adheres to the dentate area.

Keywords: hippocampus, mink, pyramidal cells, morphology

The hippocampus is very sensitive to environmental factors influencing its development and functions. It is a highly plastic structure developing mainly during the postnatal period of life. The hippocampus in animals develops rapidly within the last trimester of pregnancy and the first few weeks or months of postnatal life, with species-related differences (13). Neural structures included in the limbic system are mainly located in the telencephalon, particularly in its ventral part, i.e. in the rhinencephalon. Some structures of the diencephalon are also currently thought to belong to the limbic system (1, 10, 14). Recent studies indicate that a structure of interest to neuroanatomists, physiologists, and histologists is the hippocampal formation (*formatio hippocampi*), defined by MacLean as 'the heart of the limbic system' (5, 14). The classical characterization and divisions of this region were modified by Blackstad (2, 5). Research carried out on the American mink (*Neovison vison*) was based on the division of the hippocampus into 3 basic areas: the subiculum, the regio superior, and the regio inerior (8, 16). According to many histological studies the hippocampal formation consists of the subiculum, the hippocampus proper, and the dentate gyrus with a system of nerve fibres and the entorhinal cortex. The trisynaptic circuit, which is the main hippocampal information-processing unit, connects the dentate gyrus, CA3, CA1, and entorhinal cortex (13). All these structures are classified as the same type of cortex – archicortex (12). Previous studies on mink have revealed that there is a genetic component in the predisposition of these animals to abnormal behaviour, such as stereotypic behaviour, fur-chewing, fur-licking and hair-plucking. It has been documented that abnormal behaviour may result from a dysfunction of the central nervous system related to morphological and functional changes in the hippocampal neurons (15).

The most important factors influencing hippocampal neurogenesis include stress, ageing, physical exercise, and hippocampus-dependent learning (13).

In view of the lack of basic data on the topography of the hippocampal formation and the morphological features of neuron populations making up individual fields, layers, and areas of the hippocampus, the aim of this study was to describe the results of histological examinations performed on healthy one-year-old American minks (*Neovison vison*).

Material and methods

The investigation was carried out on 6 adult female American minks (*Neovison vison*), which were put to sleep with 10% ketamine (100 mg/kg of body weight) at the age of 12 months. Following craniotomy, the brain was removed, and slices containing the hippocampus were dissected. The hippocampus was fixed in 4% buffered formalin, dehydrated in ethyl alcohol and embedded in paraffin blocks, which were cut into 10-µm-thick slices and stained with cresyl violet according to Klüver and Barrera (11), as well as by a routine technique with Hematoxylin and Eosin (H+E). Next, the hippocampi were analysed and photographed with an Olympus BX40 light microscope connected to an Olympus Color View IIIu digital camera.

Results and discussion

The hippocampus is located closest to the lateral ventricle of the brain. The characteristic curved shape of the hippocampal formation makes it possible to differentiate the dorsal, ventral and medial parts of the formation. The dorsal and ventral parts are oriented rostrally and pass into the medial part at the back. The hippocampal fissure separates the ventral hippocampus from the brain stem (5, 8). The hippocampus of mink consists of two major parts: the hippocampus proper (Ammon's horn, or the Cornu Ammonis), and the dentate gyrus (DG). The hippocampus proper consists of four fields: CA1, CA2, CA3, and CA4 (Fig. 1). Each field consists of 3 distinct layers: stratum oriens (SO), stratum pyramidale (SP), and stratum radiate (SR). This division was based on differences in the morphology of pyramidal cells, which are the principal neurons of the hippocampal cortex. The hippocampus of mink consists of the following neural structures: the subiculum (Sb), the regio superior (Rs), and the regio inferior (Ri) (Fig. 2). The most peripheral part of the hippocampus is the subiculum, which connects it with the neocortex. It has a preserved laminar structure, with a marginal layer and cellular layers I and II. These layers are represented by nerve fibres and glial cells. Cellular layer I of the subiculum is formed of relatively well stained neurons of average size, whereas cellular layer II consists of small, multipolar, triangular or oval cells that are slightly less intensively stained. The regio superior is located dorsally with respect to the dentate area and consists of 5 layers. The stratum oriens is the most external layer, made up of numerous small neurocytes with nerve fibres and glial cells. The second layer is the stratum pyramidale, which is composed of different numbers of layers over its length in the various hippocampal areas. In CA1, for example, there are 3-4 layers, compared to 5-6 in CA3. Differences in the size, shape, and intensity of neuron staining make it possible to determine the boundaries between regions. Frontally, the regio superior passes into the subiculum, and from the other side into the regio inferior. The boundary with the subiculum is made clearly visible by intensively stained cells arranged in layers. This region is composed of small and medium-sized, densely packed nerve cells. In CA1 the cells are loosely scattered, whereas in CA2 and CA3/CA4 they adhere closely to one another (Fig. 3 A, B, C, D). The stratum radiatum is a narrow band of small, loosely scattered nerve cells, between which the neuroglia is localized. There is a relatively thin stratum lacunosum behind the stratum radiatum, composed of single neu-

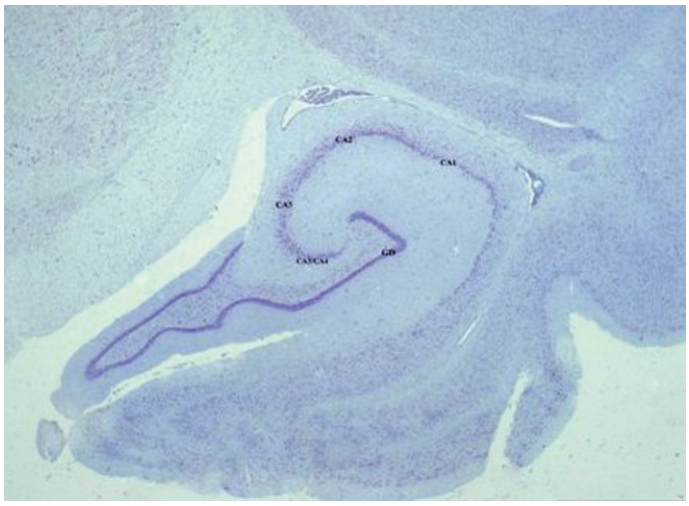

Fig. 1. Structure and topography of the hippocampus and gyrus dentatus in the American mink. Staining according to Klüver and Barrera's method (mag. approx. 100×)

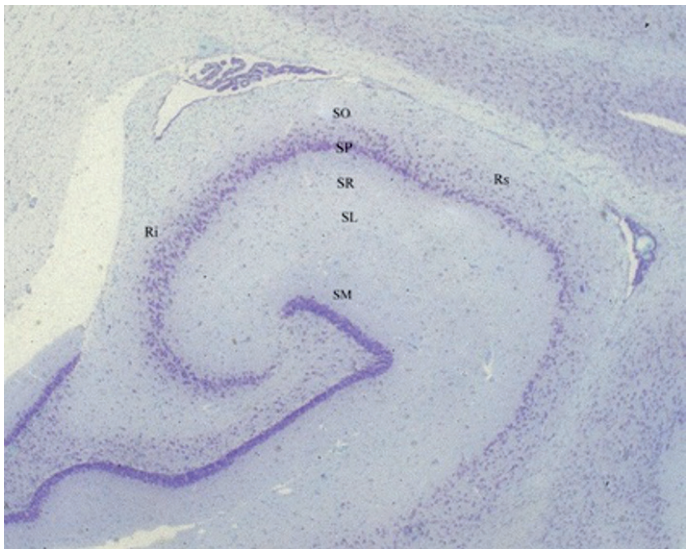

Fig. 2. Structure and topography of the hippocampus and gyrus dentatus in the American mink. Staining according to Klüver and Barrera's method (mag. approx. 200×)

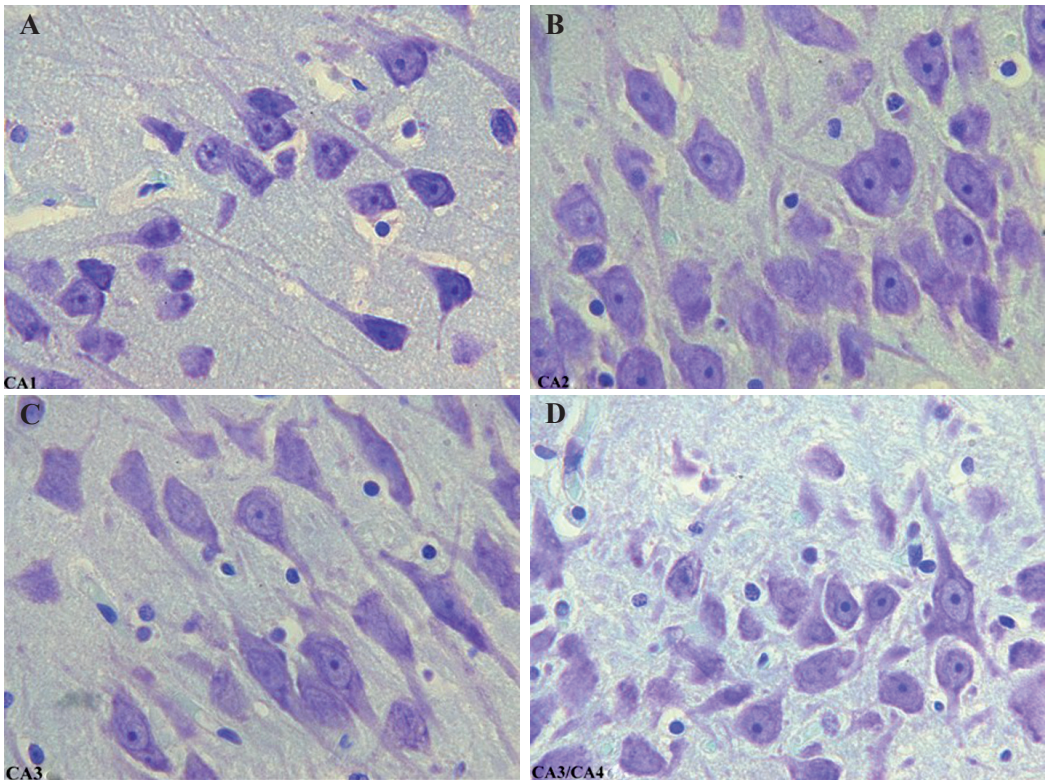

Fig. 3. Pyramidal cells in CA1 (A), CA2 (B), CA3 (C), CA3/CA4 (D). Staining according to Klüver and Barrera's method (mag. approx. 600×)

rons, numerous nerve fibres, and glial cells. The last layer of the regio superior is the stratum moleculare. Single small and medium-sized neurons occur between a dense network formed of numerous plexus of nerve fibres and accompanying glial cells. The regio inferior is located between the regio superior and the hilus of the dentate gyrus. Transversely, it has the shape of a crescent whose terminal arm faces the dentate area. The regio inferior, like the regio superior, has a laminar structure and consists of the stratum oriens (SO), stratum pyramidale (SP), stratum radiatum (SR), stratum lacunosum (SL), and stratum moleculare (SM) (Fig. 2).

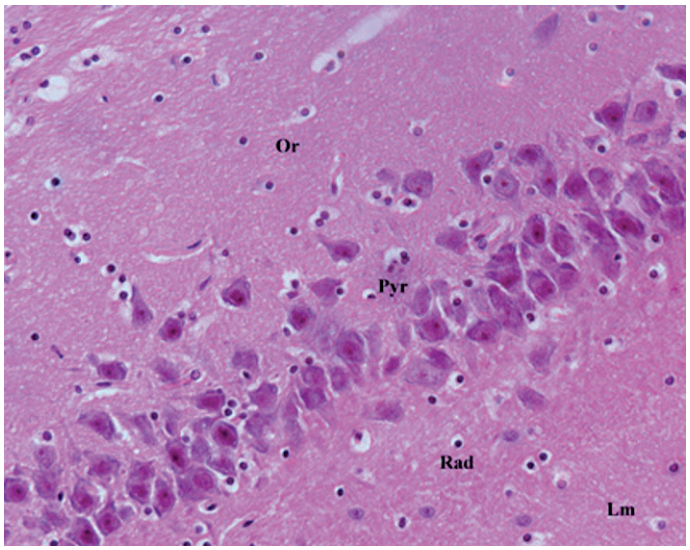

Fig. 4. Structure of the hippocampal formation in the American mink. Staining according to the H+E method (mag. approx. 400×)

The stratum oriens slightly narrows the lumen of the lateral ventricle of the brain. In the external part of this layer nerve fibres compose the alveus of the hippocampus, which passes into the fimbria of the hippocampus. There are numerous glial cells here, and between them single small, triangular or multipolar neurons. The stratum pyramidale is made up of large nerve cells whose size and intensity of staining make it possible to demarcate the boundary between the regio superior and regio inferior. This layer has the characteristic appearance of a crescent over the entire length of the regio superior. Pyramidal neurons of triangular or multilateral shape are larger and more intensively stained in comparison to the stratum pyramidale of the regio superior. The cells are smaller and more densely packed near the hilus (Hil) of the dentate area. The stratum radiatum mainly consists of glial cells, small, loosely arranged granular neurons, and between these, single, medium-sized nerve cells. The stratum lacunosum and stratum moleculare are of the same form as the regio superior.

Various types of neurons make up the cell population in fields CA1, CA2, CA3, and CA4 (Fig. 3 A, B, C, D). Because of the number of processes extending from the cell body, hippocampal neurons are included among multipolar neurons. The cell body has various

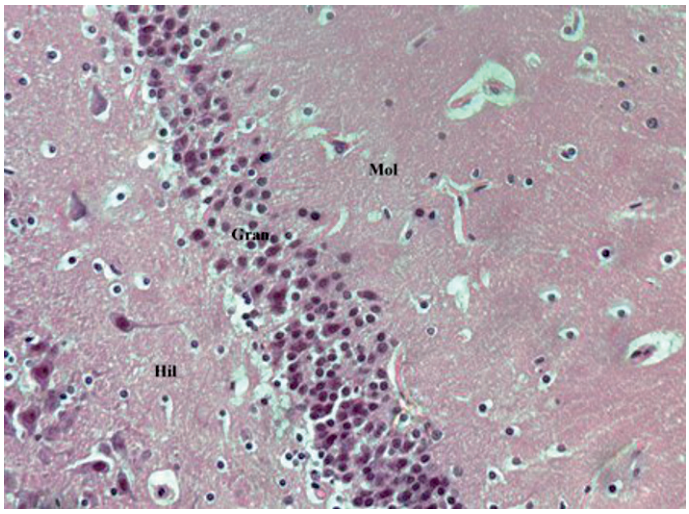

Fig. 5. Structure of the gyrus dentatus in the American mink. Staining according to the H+E method (mag. approx. 400×)

forms: pyramidal, which is the most prevalent shape, oval, round, granular, extended, stellate, fusiform, multilateral or triangular. The hippocampal neurons form bands located close to one another or loosely scattered. Pyramidal neurons are generally the largest cells. There is a centrally located cell nucleus together with the nucleolus in the perikaryon. The nucleus stains more brightly in slides than the neuroplasm and occupies a larger part of the neuron body. The neuroplasm stains intensively due to the presence of Nissl bodies. These cells are close to one another and sometimes even occur in pairs. There are visible dendrites with Nissl bodies that are less intensively stained than in the perikaryon. Lateral branching of dendrites is observed in some neurons. Oval neurons, like pyramidal cells, form a numerous population of hippocampal cells. Their body has a round shape with a large cell nucleus localized centrally in the perikaryon or more medially or eccentrically. In comparison to pyramidal neurons, the cell nuclei stain less intensively in slides due to the small amount of nuclear chromatin. The neuroplasm of these cells stains more brightly because of the presence of Nissl bodies. In contrast to pyramidal neurons, dendrites are not visible. The cells are more loosely arranged and form narrow bands of adjacent cells or wider ones, with a slightly looser arrangement. Oval neurons are generally smaller than pyramidal ones. The remaining types of nerve cells occur between the types of neurons mentioned above. The nucleus is located centrally or sometimes an eccentric location can be observed. The nucleus is paler than the perikaryon of the neuron. There are Nissl bodies present, causing the nerve cell bodies to stain violet in the neuroplasm. Stellate and triangular cells are the same size as pyramidal neurons. They are close to one another or loosely arranged. There are fewer dendrites visible in the neurons in comparison to pyramidal neurons. Nerve fibres, capillary blood vessels, and numerous intensively stained nuclei of glial cells are present between the neurons. The fields of the hippocampus proper are distinguishable. The stratum pyramidale contains the bodies of the pyramidal cells. Field CA1 is a narrow zone consisting of medium-sized cells. Fields CA2 and CA3 together constitute a wide band. CA4 is the continuation of CA3 in the hilus of the dentate gyrus (Fig. 1, 3 A, B, C, D). The hippocampus has been examined in humans and numerous experimental animals (2-7, 9, 15, 16). The research indicates that the divisions made by Blackstad in the rat are the most justified (2). The most important layer of the hippocampus in the American mink (*Neovison vison*) is the stratum pyramidale, as in the cow and the rat (2, 18). In mink, medium-sized and large cells of this layer are intensively stained over the entire length of the regio superior and regio inferior, and are the most visible. According to Rosa (3, 17), the layer of pyramidal cells in sheep located in C1 is divided into sub-layers L and B, consisting of loosely arranged pyramidal cells. In mink, this layer has not been divided according to this criterion. As in the rat, the regio superior in mink is also a homogenous cortical structure (2). Research conducted on pigs (9) shows that the cells of the subiculum are arranged in 2 layers: a medial and an internal layer. Such a division has not been observed in the American mink (*Neovison vison*). There is no information on the topography and morphology of the hippocampus in mink in recent literature. The results presented in this paper may provide an innovative look at the morphology and cytoarchitecture of the hippocampus in the American mink (*Neovison vison*). Due to increased interest in the limbic system in conjunction with CNS diseases, further research concerning the morphometry of this region of the CNS in the American mink (*Neovison vison*) is required.

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