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Morphometric Parameters of Pyramidal Cells in CA1-CA4 Fields in the Hippocampus of Arctic Fox (*Vulpes lagopus*)

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The aim of the study was a quantitative examination of neurons of hippocampal subfields (CA1-CA4) in mature male Arctic fox (*Vulpes lagopus; syn. Alopex lagopus*). The preparations were dyed using cresyl violet. Histological preparations were used to morphometricaly analyze the neurons of hippocampus. This analysis included the following parameters: average size of cells in μm, periphery of cells in μm, average cell area in μm^2, percentage of cells in area and size of the largest and smallest cells in μm in CA1-CA4 fields. Morphometric observations show that the cells involved in hippocampal formation in polar fox in all layers CA1-CA4 differ in size, shape, cell area and nucleus area. The size of the cell area in CA3 is the largest and fluctuates around 249.4 μm^2, whereas in CA2 the cell area is 184.1 μm^2. The cells of the CA2 field are densely arranged, pyramidal and contain a small amount of cytoplasm; their size fluctuates. Cells of CA2 and CA4 had the largest diameter of about 23.6 μm, whereas cells of the CA3 field had the smallest diameter of about 8.3 μm.

Key words: Hippocampus, fox, morphometric parameters.

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The hippocampus is phylogenetically one of the oldest brain structures. This macroscopically curved part of the hippocampus bears some resemblance to horns and is called Ammon's horn (*cornu ammonis* – CA). It is composed of cortical structures such as the *subiculum*, *proper hippocampus* and *dental gyrus*. *Proper hippocampus* consists of four fields: CA1-CA4. Anatomical division into CA1-CA4 fields was first proposed by LORENTE DE NÓ in 1934 and this division is still used today (LORENTE DE NÓ 1933). CA1-CA4 fields consist of 3 layers: *stratum pyramidale*, *stratum radiatum* and *stratum lacunosum-moleculare* (EUSTACHIEWICZ & ŁUSZCZEWSKA 1999). This differentiation was primarily based on morphological differences of pyramidal cells which constitute the main population of hippocampal neurons (VIDA 2010). Apart from pyramidal neurons, there are also glial cells in the hippocampus. The junctions of the hippocampus with other nerve structures have been well examined and defined due to involvement in the control of animal behavior. In addition to the amygdala, the hippocampus is the most important structure that manages the processes of learning and memory. It is involved in behavioral reactions of the body such as reproduction, food intake, endocrine responses and many others (LORENTE DE NÓ 1933). Many diseases of the central nervous system (CNS) are accompanied by neuron damage of the hippocampus. One of many examples is ischemia, concerning damage of neurons in the CA1

field (MOSSAKOWSKI *et al*. 1989). Neurodegenerative diseases such as Alzheimer's disease, schizophrenia, bovine spongiform encephalopathy (BSE) or Parkinson's disease are other examples of neuronal damage in the hippocampus (PENFIELD & MATHIESON 1974; SIMIĆ *et al*. 1997; RAĞBETLI *et al*. 2010; LEE *et al*. 2013). The hippocampus has been the subject of many anatomical, histological as well as physiological studies in human and many species of mammals (SIMIĆ *et al*. 1997; INSAUSTI *et al*. 1998; KAUFMANN *et al*. 1998; EUSTACHIEWICZ & ŁUSZCZEWSKA 1999; COULIN *et al*. 2001; EL FALOUGY *et al*. 2008; SPALDING *et al*. 2013; RAĞBETLI *et al*. 2010). Morphological evaluation of the hippocampus *in vivo* may be performed with the use of non-invasive techniques such as magnetic resonance (MR) providing data on voxel-based morphometry and relatively small resolution (BIEDERMANN *et al*. 2012; LUDERS *et al*. 2013). Postmortal evaluation of hipocampal morphology is much more precise than MR and may be performed with different magnifications and staining methods. However, no morphometric measurements have been carried out in Arctic fox (*Vulpes lagopus*; syn. *Alopex lagopus*). Thus, the aim of the present study was to evaluate quantitatively various morphometric parameters in neurons of the pyramidal layer in each of the CA1-CA4 fields of the hippocampus using a computerized image analyzer.

Material and Methods

The study was performed in accordance to individual approval for experiments on foxes number 28/2013. Experimental material for histological analyses was obtained in accordance to local law regulations.

The examinations were carried out on 6 adult male Arctic foxes (*Vulpes lagopus*; syn. *Alopex lagopus*). The brains were removed from skulls and immediately placed into 10% buffered formalin for immersion fixation for at least 3 months. The obtained hippocampus was fixed in formalin, dehydrated in ethyl alcohol and embedded in paraffin blocks. The paraffin sections were cut in a microtome and then stained with cresyl violet according to Klüver and Barrera's method (KLÜVER & BARRERA 1953). All sections were analyzed cytoarchitectonically and morphometrically with a calibrated image analysis system that consisted of a computer equipped with morphometric software Cell D Soft Imaging System (SIS) with an attached digital camera Colorview IIIu (Soft Imaging System).

The morphometric parameters of neurons

Morphometric analysis was based on measurements of pyramidal cells in the fields CA1-CA4 of the hippocampus of Arctic fox (*Vulpes lagopus*; syn. *Alopex lagopus*). Each neuron was characterized by a set of morphometric parameters: length (μm; the long axis of the soma), width (μm; the short axis of the soma), size of neurons (μm), area of soma (μm^2), area of nucleus (μm^2), cell diameter (μm), cell perimeter (μm) and percentage of neurons (%).

Statistical analysis

The values are presented as means ± SEM. The obtained results were statistically evaluated using Student's *t*-test. Significant differences were assumed for $\alpha = 0.05$. The coefficient of error was calculated to evaluate the precision of all measurements.

Results

The values of morphometric parameters concerning each of the CA1-CA4 fields in the hippocampus of Arctic fox are shown in Figs 2, 3, 4 and Table 1. The values obtained do not show significant differences. However, there is a slight tendency for fluctuation within the area of soma, area of nucleus, cell diameter, cell perimeter, length of

Table 1

Morphometric parameters in the hippocampal fields CA1-CA4

	Fields of hippocampus			
	CA1	CA2	CA3	CA4
Length of neurons (μm)	19.2 ± 1.59	24.3 ± 0.75	18.6 ± 1.3	17.2 ± 0.76
Width of neurons (μm)	12.9 ± 0.51	15.2 ± 0.57	12.2 ± 1.2	10.4 ± 1.1

Values are means ± SEM.

neurons and width of neurons, size, periphery and cell surface area. The neuron population of CA1-CA4 areas includes mainly pyramidal neurons with numerous dendrites. The percentage of neurons in individual CA1-CA4 areas of the pyramidal layer did not show statistically significant differences. Classical divisions of this structure of rhinencephalon were developed by LORENTE DE NÓ (1933), who divided the hippocampal cortex into 4 fields denoting them with the symbols CA1-CA4. The examinations carried out on Arctic fox show that the criteria of division into fields CA1-CA4 correspond to those of LORENTE DE NÓ (1933). The hippocampus was divided into regions and fields in relation to the localization of nerve cell bodies, their sizes and distributions. The dominating cells of the hippocampus in the pyramidal layer are mainly pyramidal neurons with many dendrites (Fig. 1). In the CA1 field the size of neurons reached a value of about 16.2 μm, the cell diameter was 18.03 μm while the cell perimeter was 54.1 μm. The neurons of this region are characterized by average size, are loosely arranged and their perikaryons are weakly stained. The area of soma was about 234.7 μm^2, whereas the area of nucleus was 153.8 μm^2 (Figs 2 and 3). The nerve cells of the CA2 field are densely packed and form a few layers with visible dendrites extending from their bodies. Neurons attain a size of about 14.4 μm, a diameter of 15.2 μm and perimeter of 47.6 μm. The area of soma was about 184.1 μm^2, whereas the area of the nucleus was about 125.1 μm^2 (Figs 2 and 3). In the CA3 field there are small, oval neurons with intensively stained and densely packed perikaryons. The majority of neurons showed a size of about 13.0 μm, diameter of 14.5 μm and perimeter of 45.1 μm. The area of soma reached about 164.9 μm^2, while the area of the nucleus attained 108.1 μm^2 (Figs 2 and 3). In the CA4 field neurons are longer and more weakly stained in comparison with the CA2 and CA3 fields. Their sizes, diameters and perimeters attained approximately 16.5 μm, 19.1 μm and 55.6 μm, respectively (Figs 2 and 3). The area of soma oscillated around 249.4 μm^2, while average value of surface area of the nucleus was about

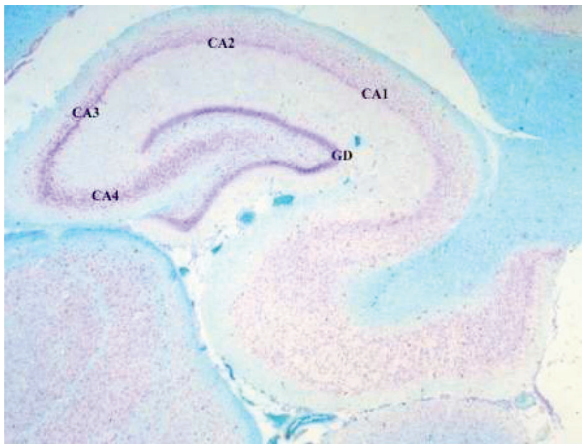

Fig. 1. Structure and topography fields of hippocampus (CA1-CA4) and gyrus dentatus in Arctic fox (*Vulpes lagopus*; syn. *Alopex lagopus*). Staining according to Klüver and Barrera's method (mag. approx 100x).

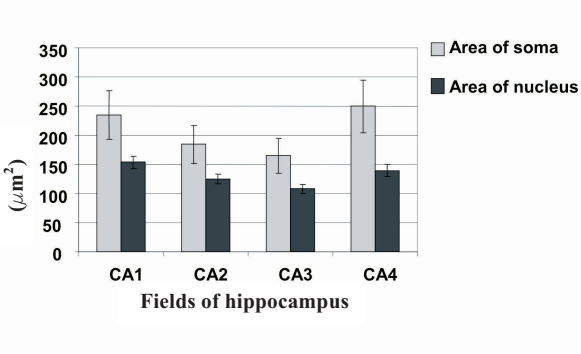

Fig. 2. Morphometric parameters of neurons (area of soma in μm^2, area of nucleus in μm^2) in the hippocampal fields CA1-CA4.

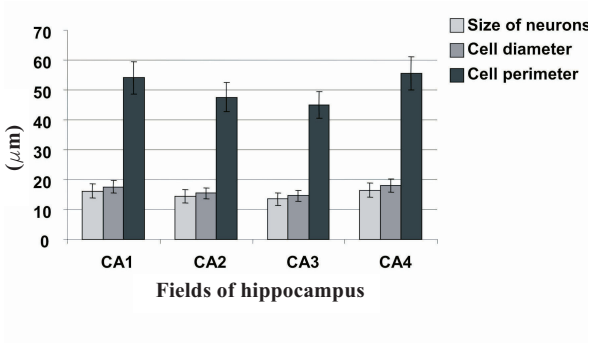

Fig. 3. Morphometric parameters of neurons (size of neurons in μm, cell diameter in μm, cell perimeter in μm) in the hippocampal fields CA1-CA4.

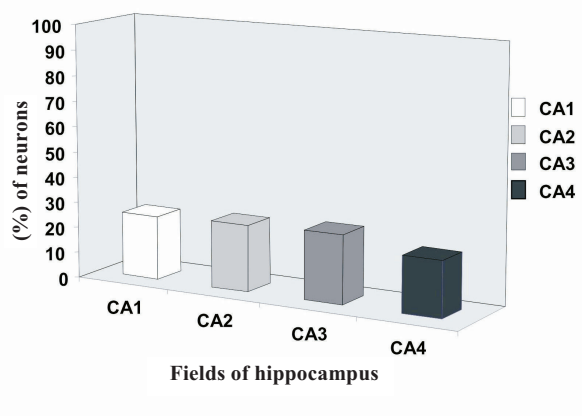

Fig. 4. Percentages of neurons in the hippocampal fields CA1-CA4.

139.6 μm^2. The percentage of the total population of neurons in CA1-CA4 fields of the hippocampus showed that the highest percentage of the total population is within the CA3 field, whereas the lowest was observed in the CA4 field (Fig. 4). The results show that in each of the CA1-CA4 fields of the hippocampus in Arctic fox there are no statistically significant differences between the examined parameters. The graphs were drawn on the basis of the results from each hippocampal field of adult fox. The smallest length of neurons in the CA4 fields was 17.2 μm whilst the neurons in CA2 were the longest. The average length of neurons in fields CA1, CA2, CA3 and CA4 was not significantly different (P>0.05). The smallest width of neurons in the CA4 fields was 10.4 μm whilst the neurons in CA2 were the longest. The average length of neurons in fields CA1, CA2, CA3 and CA4 was not significantly differentiated (P>0.05) (Table 1).

Discussion

Morphometric parameters have also been described in different structures of the limbic system in human, rabbit, rat, *Chinchilla lanigera*, guinea pig, dog, monkey, gerbil and common shrew (PENFIELD & MATHIESON, 1974; KAUFMANN *et al*. 1998; MOSSAKOWSKI *et al*. 1989; RAPP *et al*. 1999; RÓWNIAK *et al*. 2007; EL FALOUGY *et al*. 2008; RAĞBETLI *et al*. 2010; NAJDZION *et al*. 2011; NAJDZION *et al*. 2012; WASILEWSKA *et al*. 2012; SPALDING *et al*. 2013). Many authors have shown in nucleus amygdaloideum in rabbit, rat and chinchilla claustrum and in hippocampus a clear increase in the number of neurons combined with an increase of cell volume in various regions of the limbic system (KOWIAŃSKI *et al*. 1999; RÓWNIAK *et al*. 2004, 2007; NAJDZION *et al*. 2011; NAJDZION *et al*. 2012; WASILEWSKA *et al*. 2012). Statistically significant differences of each morphometric parameter in the fields CA1-CA4 of hippocampus of pyramidal cells were demonstrated in human, dog, rat and mouse (INSAUSTI *et al*. 1998; KAUFMANN *et al*. 1998; COULIN *et al*. 2001; EL FALOUGY *et al*. 2008; RAĞBETLI *et al*. 2010; BIEDERMANN *et al*. 2012; NAJDZION *et al*. 2012; SPALDING *et al*. 2013). The authors have demonstrated gender differences in the number of pyramidal neurons in the examined fields (NAJDZION *et al*. 2011). The evaluation of the number of neurons is associated with the size and body mass of the examined species. In adult mouse, for example, the number of neurons in the hippocampus is approximately 8354.70 (INSAUSTI *et al*. 1998; COULIN *et al*. 2001), whereas in human there are about 19 million (SIMIĆ *et al*. 1997; WEST & GUNDERSEN 1990). In neurodegenerative diseases, many authors report a decrease in the number of neurons in various fields, e.g. pyramidal cells in the CA2 field are the most susceptible to Alzheimer's disease, epilepsy, dementia, schizophrenia, major depression and chronic stress, whereas cells of the CA1 and CA3 fields are the most vulnerable during hypoxia (WEST & GUNDERSEN 1990; LERANTH & RIBAK 1991; SIMIĆ *et al*. 1997; KAUFMANN *et al*. 1998; LEE *et al*. 2013). Morphometric examinations of the CNS provide a valuable opportunity for monitoring the effects of various environmental and pharmacological factors on structure and function, and a better understanding of morphology and the changes taking place in CNS.

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