Praca oryginalna *Original paper*

Morphometrical analysis of neurons of corpus amygdaloideum's nucleus amygdaloideum lateralis in domestic pigs

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Morphometrical analysis of neurons of corpus amygdaloideum's nucleus amygdaloideum lateralis in domestic pigs

Summary

The aim of the research was to conduct morphometrical analysis during the process of maturation of nucleus amygdaloideum lateralis. The brain of domestic pigs of both sexes (taken during the following period of their life: from 7th to 15th week of intrauterine life, newborn animals, one-month and one-year-old animals) were used as the material for the examination. The brains were removed and processed conventionally by microscope. The preparations were colored according to Klüvera-Barrer's method and according to Nissel. Histological preparations obtained in this way were used for morphometrical analysis of the neurons of the corpus amygdaloideum's nucleus amygdaloideum lateralis. Morphometrical examinations were carried out by the Nikon Eclipse E-600 microscope compressed with a JVC TK-1380-E camera and a computer using morphometrical MULTI-SCAN-BASE 08.98 program.

The examination comprised the following parameters: the section area of nervous cells and the area of cell nucleus in μm^2; the nucleo-cellar rate in %; the average diameter of nervous cell in µm; the volume of nervous cells in μm^3; the number of neurons per 1 mm^2; the number of neurons per 1 mm^3. Morphometrical observation showed that in the 9th week of fetal life the cells forming the primary corpus amygdaloideum are of identical shape and size. The size of cell area of this period of fetal life fluctuates around 45 μm^2. In the 11th week of fetal life cells of the nucleus amygdaloideum lateralis are densely arranged, they have oval or polyhedral shape and contain a small amount of cytoplasm pretty evenly stained, in which Nissel's granules are visible. In the 12th week of a domestic pig's life cells of the nucleus amygdaloideum lateralis have a polyhedral, oval or pyramidal shape. In the subsequent periods of fetal life and new born animals, the appearance of cells composing the nucleus amygdaloideum laterais does not significantly change. In case of nucleus amygdaloideum lateralis nucleo-cellular rate fluctuates between 51 and 60%. The value of this rate decreases during development and maturation of neurons.

On the basis of the examined morphometrical parameters the author claims that the neurons attain morphological maturity at the end of fetal life.

Keywords: Corpus ammygdaloideum, brain, morphometry, pig

Corpus amygdaloideum is a cellular complex located in ventral part of rhinencephalon and its base lies nearly on the whole length of cortex of lobus piriformis. It consists of a series of nervous nuclei which form a body resembling an almond in shape. Morphologically one can divide it into two basic parts: nuclear structures and cortical structures. For a long time it has been the subject of numerous neuroanatomical, neurophysiological and histochemical examinations in many species. However, in the available literature there is no evidence of changes in the development and maturation of neurons forming nuclei of the corpus amygdaloideum in domestic pig (Polish Large White pig).

The cytoarchitecture of neurons forming individual nuclei of the corpus amygdaloideum has been quite well and thoroughly examined (1, 6, 11, 13, 14). The nucleus amygdaloideum lateralis and nucleus amygdaloideum baso-lateralis are phylogenetically younger parts of corpus amygdaloideum (7, 10) in relation to the remaining parts.

Nucleus amygdaloideum lateralis and nucleus amygdaloideum baso-lateralis have numerous efferent as well as afferent junctions with other brain parts. Nervous fibers connect them, above all, with various regions of the cerebral cortex and some subcortical structures of the brain. Efferent nervous fibers of the corpus amygdaloideum reach many regions of the neocortex and allocortex (2, 4, 8, 12, 17, 21, 22), such as: periolfactory cortex, hippocampal pad, intraolfactory cortex, insular agranular cortex, perilimbic cortex, motor and somatosensory cortex, pre-limbic cortex, cortex of fronto-orbital girgle, precentral cortex.

Efferent fibers from nuclei of the corpus amygdaloideum lateralis and baso-lateralis also reach some subcortical structures of the telencephalon (3, 9, 11), diencephalons and brain stem (18, 23), such as: Broc's diagonal band, ventral part of pale globe, semisupine nucleus and nuclei of hypothalamus.

Afferent junctions of the corpus amygdaloideum are numerous. They run from different regions of the allocortex and neocortex towards the nucleus amygdaloideum, i.e. lateral and baso-lateral. Afferent nervous fibers run from such regions as: medial cortex and lower surface of frontal lobe, olfactory cortex, temporal cortex, insular cortex, intraolfactory cortex and cortex of CA1 area of hippocampus (1, 5, 15, 19, 22, 24).

Afferent nervous cells also reach nucleus amygdaloideum, i.e. lateral and baso-lateral from the subcortical structures of the brain stem, hypothalamus, thalamus, lentiform loop, innominate matter, nuclei of septal area, and pale globe. The corpus amygdaloideum, apart from afferent and efferent nervous junctions with other brain structures, possesses a complex system of internal junctions. Junctions of the nucleus amygdaloideum lateralis and baso-lateralis are particularly interesting (3, 11, 16, 20).

Considering the significance of this structure responsible for many vital processes both in animals and humans it is necessary to examine the course of neuron development and maturation process of individual nuclei of the corpus amygdaloideum. Morphometric examinations are particularly important, which due to the introduction of methods ensuring more precise results warrant better understanding of the structure and function of the nervous system.

Material and methods

The examinations were carried out on the brains of domestic pigs of both sexes, divided into 3 groups. The first group (fetal) comprised 42 animals in the following periods of their fetal life: 9, 10, 11, 12, 13, 14 and 15th weeks. The second group comprised the postnatal period including 6 newborn animals (N_B). The third group consisted of postnatal animals, 1-month-old (6 animals) and one-year-old (6 animals). In the fetal groups the age determination procedure agreed with Baraldi's chart. The slides were stained according to Klüver and Barrera's method, with cresyl violet and luxol fast blue and also with methylene blue according to the modified Nissel's method.

Morphometric examinations were carried out with the use of a Nikon Eclipse E-600 microscope combined with a JVC TK-1380-E camera and a computer using a morphometric program Multi-Scan-Base 08.98. The examinations comprised the following parameters: 1. cell area and nucleus area μm^2; 2. nucleo-cellular rate %; 3. average cell diameter μm; 4. cell volume μm^3; 5. number of neurons per 1 mm^2; 6. number of neurons per 1 mm^3. Photographic documentation consists of macro and microphotographs taken with the use of a Olympus BX 40 microscope with a DP 10 digital camera.

Results and discussion

In the domestic pig the cellular complex of amygdala can be divided into 2 basic parts: nuclear structures and cortical structures of the corpus amygdaloideum. Nuclear structures of the corpus amygdaloideum phylogenetically homogenous in pigs form the nucleus amygdaloideum lateralis and nucleus amygdaloideum baso-lateralis. The nucleus amygdaloideum lateralis is located dorsally in relation to the nucleus amygdaloideum baso-lateralis.

The anterior end of the nucleus amygdaloideum baso-lateralis lies slightly backwards in relation to the anterior end of the nucleus amygdaloideum lateralis. The posterior end, on the other hand, lies on more or less the same level as the nucleus amygdaloideum lateralis. On most of its length, the nucleus amygdaloideum baso-lateralis occupies a great area in the medio-lateral part of the corpus amygdaloideum. Dorsally it borders with the nucleus amygdaloideum lateralis, laterally with fibers of the external capsule, ventro-laterally with the internal piriform nucleus, ventrally with the cortical nuclei of the corpus amygdaloideum.

A small bulge in the developing piriform lobe can distinctly be noticed in the 7th and 8th weeks of fetal life in the region at the base of the brain's hemisphere, its anterior end lies on the level of the chiasm of the optic nerve. On transverse cross-sections of brains coming from this period, the corpus amygdaloideum of the pig's fetus located internally in relation to the cortex of the piriform lobe is composed of a homogenous mass of maturing cells which radially penetrate from the dorsal direction deep inside this structure. This homogenous cell structure can be treated as the primary corpus amygdaloideum (16). The location of this cellular mass (primary corpus amygdaloideum) of the dorsal part of corpus amygdaloideum topographically corresponds to the localization – in later periods of fetal life – of nuclei of the baso-lateral group. In the 9th week of fetal life on the basis of microscopic observation one can conclude that all examined cell nuclei are more or less the same size. During this period of fetal life, some structures defined as cortico-medial in this part of the corpus amygdaloideum are present. These are phylogenetically older structures (5).

In the next week – i.e. 10th – of fetal life of the domestic pig the dorso-lateral part of the corpus amygdaloideum made up of, during earlier periods, homogenous cells undergoes distinct differentiation. Located dorsally and adhering to the internal surface of an already distinguishes band of nervous fibers forming external capsule, the group makes the nucleus amygdaloideum lateralis.

In the 11th week of fetal life cells of the nucleus amygdaloideum lateralis are densely arranged. They have an oval or polyhedral shape and contain a small amount of cytoplasm pretty evenly stained, in which Nissel's granules are visible.

In the 12th week of the domestic pig's life cells of the nucleus amygdaloideum lateralis have a polyhedral, oval or pyramidal shape. The cytoplasm of these cells is pretty intensively stained.

In the subsequent periods of fetal life and in new-born animals, the appearance of the cells composing the nucleus amygdaloideum lateralis do not significantly change. Thus, one can assume that the cells forming the above mentioned nuclei of the corpus amygdaloideum in the domestic pig are already morphologically mature in newborn animals.

Morphometric examinations were carried out on brains of newborn animals, fetuses and of one-month-old animals. The measurements carried out referred to the nucleus amygdaloideum lateralis and included the following parameters: 1. cell area and nucleus area µm^2; 2. nucleo-cellular rate – %; 3. average cell diameter – µm; 4. cell volume µm^3, 5. number of neurons per 1 mm^2; 6. number of neurons per mm^3.

In domestic pigs in which pregnancy lasts 112 days, one can observe the most advanced neurogenesis of the examined nuclei in the 9th week of fetal life, although in this period one does not observe the differentiation of nervous cells considering size, shape, and staining intensity in individual nuclear groups. The differentiation of this cellular complex in the fetus begins only in the 10th week (fig. 1).

The area of the neurons' cross-section increases significantly in the first examined weeks (9-12), then the parameters do not significantly change.

Cells forming the primary corpus amygdaloideum occurring in the 9th week of the domestic pig's fetal life are of the same shape and size.

The size of the cell area of this period of fetal life fluctuates around 45 µm^2 (fig. 1). In the 10th week of fetal life, cells, homogenous in the earlier period, undergo significant differentiation. From the homogenous cell mass, we can isolate two structures: nucleus amygdaloideum lateralis and nucleus amygdaloideum baso-lateralis. The cell differentiation process is connected with the simultaneous extension of the cell area. The size increase of this area is pretty significant in comparison with the previous period. In subsequent weeks of the domestic pig's fetal life, the process of increasing the cell area's size after another significant increase stabilizes with a slight deviation for "+" or "–". The process of another significant size increase of cell area forming the nucleus amygdaloideum lateralis was observed in the 11th week of fetal life (fig. 1). On the other hand, size parameters of the cell area of the nucleus amygdaloideum lateralis measured in the last weeks of fetal life and on the day of birth were very close to each other.

The area of the cell nucleus making up the primary corpus amygdaloideum and later nucleus amygdaloideum lateralis, just like the cell area, increased during the process of cell maturation. The nucleo-cellular rate, as one of the examined parameters, is strictly connected with parameters of cell area and nucleus area. In the case of the nucleus amygdaloideum lateralis the nucleo-cellular rate fluctuates between 51 and 60% (fig. 2). The value of this rate decreases during the development and maturation of neurons. On the basis of the conducted measurements of cell area and calculated nucleo-cellular rate one can conclude that the increased intensity of cell area during the process of neurogenesis corresponds to the increase of the cell area (fig. 3, 4). The

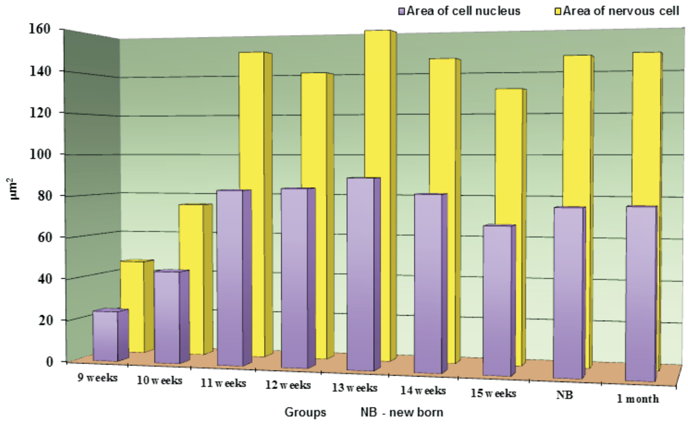

Fig. 1. The section area of nervous cell and the area of cell nucleus in µm^2

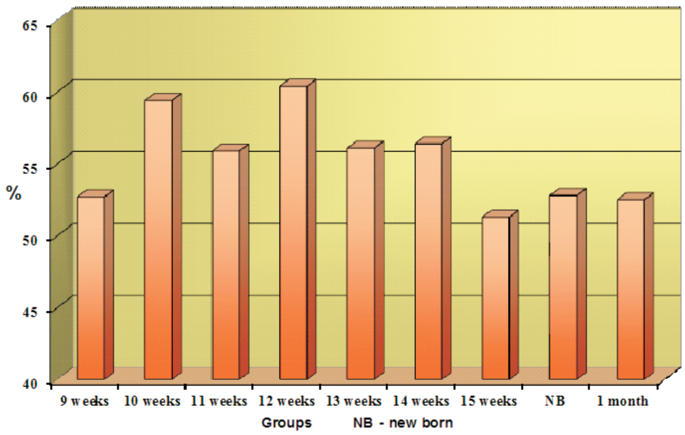

Fig. 2. The nucleo-cellar rate in %

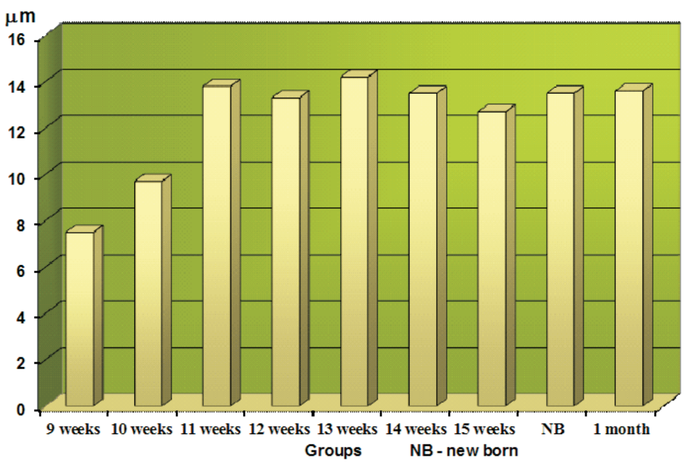

Fig. 3. The average diameter of nervous cell in µm

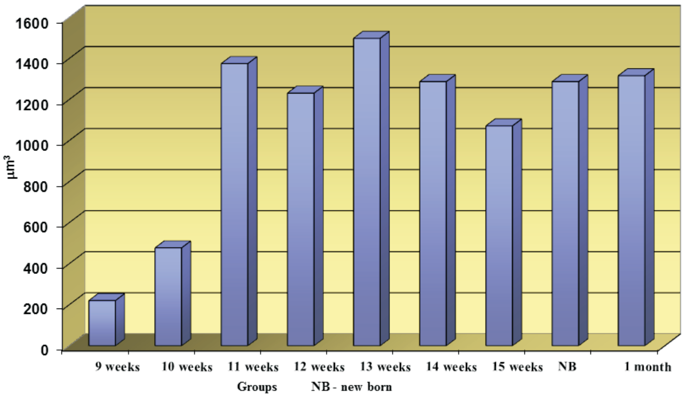

Fig. 4. The volume of nervous cell in µm³

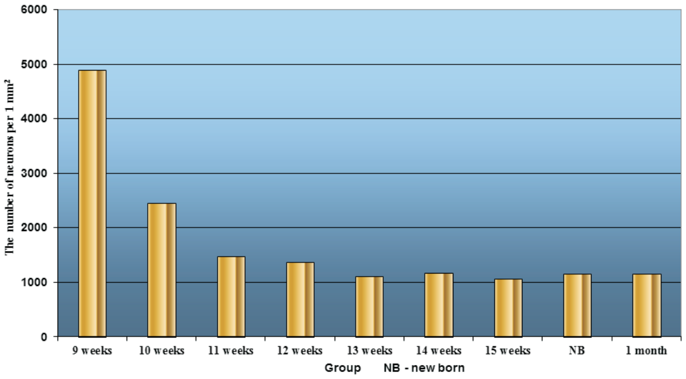

Fig. 5. The number of neurons per 1 mm²

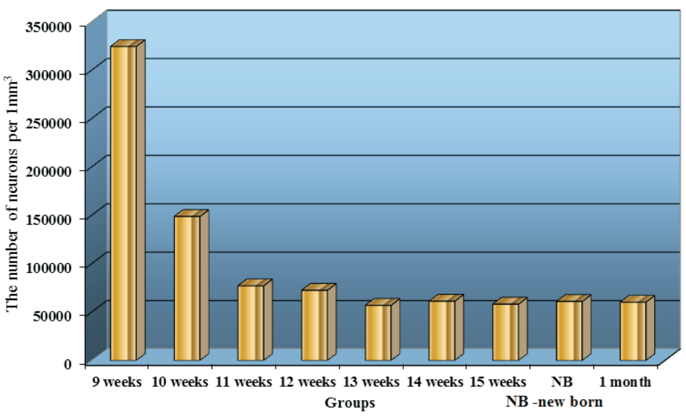

Fig. 6. The number of neurons per 1 mm³

size of an average diameter of a cell depends on the size of cell area. The values of this parameter agree in time with the increased intensity of cell area. The number of cells making primary corpus amygdaloideum and later corpus amygdaloideum lateralis occuring per 1 mm² of transverse cross-section of the examined structures of corpus amygdaloideum in the domestic pig's fetus, newborn animals and one-month-old animals changes significantly. The greatest number per 1 mm² was observed in the 9th week of fetal life (fig. 5). During subsequent periods of fetal life in this species the number of cells decreased a great deal. From the 11th week of fetal life till the day of birth the number of cells per 1 mm² of the cross-section of the examined structures stabilizes with slight deviances of "+" or "–".

During the development period of the examined nuclei of the corpus amydgaloideum the number of cells per 1 mm² of a cross-section was similar to the number of cells occurring per 1 mm³ (fig. 5, 6).

From the examined morphometrical parameters it can be inferred that neurons become morphologically mature at the end of fetal life. Detailed data of the carried out measurements are included in the enclosed diagrams.

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