Praca oryginalna *Original paper*

Evaluation of morphometric parameters of three types of oligodendrocytes in the periaqueductal grey matter of rats in different age groups

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Received 28.02.2014 *Accepted 27.06.2014*

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Summary

The aim of the study was to evaluate selected morphometric parameters of three types of oligodendrocytes on the basis of their morphology. A total of ten male Wistar rats in two age groups, 25 weeks and 140 weeks, were used for the experiments. After the rats were sacrificed by perfusion-fixation, the midbrains with the periaqueductal grey matter were collected and embedded in celloidin. Semi-thin sections were stained with methylene blue, analysed and photographed under a light microscope. The morphometric parameters taken into account were mean cell size, mean perimeter of soma in µm and mean area of soma in µm². In addition, the percentages of different types of oligodendrocytes in the measurement area were determined. The results obtained were analysed statistically. In the PAG of the 25-week-old rats oligodendrocytes with light cytoplasm predominated. Their average size was about 7 µm. In the 140-week-old individuals an increase was observed in the number of oligodendrocytes with medium and dark cytoplasm, and their average size was about 6.79 µm. The perimeters of the three types of oligodendrocytes were similar in the two age groups – about 20 µm. There were also no statistically significant differences in the surface area of the three types of oligodendrocytes in the 25- and 140-week-old rats. The average surface area of these cells was about 40 µm². Oligodendrocytes have been neglected in research on older individuals and should therefore be analysed in this regard.

Keywords: oligodendrocytes, morphometric parameters, periaqueductal grey matter, older rats

Glial cells, also called neuroglia, are one of the two components of nervous tissue, the other being neurons. There are four types of neuroglia in the mammalian central nervous system (CNS): oligodendrocytes (OL), astrocytes, microglia and ependymocytes (2, 19, 21). Increasing evidence suggests that neuroglia plays an active role in brain functions and their number in mammals is estimated to exceed that of neurons, accounting for about 90% of all brain cells in humans and about 65% in rodents (2, 6, 9, 10, 21, 22). Oligodendroglia, present in the white and grey matter of the brain and spinal cord, are round or oval cells with poorly branched processes (2, 6, 10, 21, 29). Their cell nuclei conform to the shape of the cell and have a darker colour due to the significant amount of heterochromatin located under the nuclear capsule. Del Rio-Hortega divided OL into 4 types on the basis of their morphological features, using silver staining. Types I and II have a small body and from 5 to 10 processes. Type III cells have larger bodies and fewer processes, and type IV cells are the largest and have no processes (2, 12, 15-17, 25, 32). OL are mainly localized near nerve fibres and capillaries, accompanying neurons and other glial cells. Due to the presence of processes extending from the cell body, OL are mainly thought to perform myelinating functions in the CNS. They are also involved in regulating the water and electrolyte balance in the brain, forming a scaffold for neurons, and are the main iron-accumulating cells in the CNS (2, 6, 10, 12, 21-23). Their morphology is adapted to myelination, which enables rapid transmission of nerve impulses through nerve fibres and constant control of myelin sheaths (5, 21-23). Most studies on the biology of OL involve research on rodents, because these cells are easily available and can be cultured. Using many genetically modified animals it is possible to learn the functions of individual cells in the CNS at an appropriate stage of disease development (2, 6). The morphology of OL is best known in well-myelinated areas of the brain in young animals. At the electron microscope level OL show a broad spectrum of morphological differences related to the density of their cytoplasm and nuclear chromatin. Three types of oligodendrocytes have been distinguished: those having light (OLl), medium (OLm) and dark cytoplasm (OLd) (15, 16). Electron density is linked to the age and metabolic activity of these cells and is closely correlated with the rate of myelination of nerve fibres. In ultrathin sections OL cytoplasm is relatively dark and contains numerous organelles. In contrast to astrocytes, OL do not contain either glial filaments or glycogen granules (21-23, 25). These cells have a complex cytoarchitecture taking the form of a network of microtubules performing important functions during transport of myelin proteins. The specificity of this cytoskeleton determines OL integrity and is an important factor for their survival. OL are a very important element of white matter and comprise about 75% of the entire population of neuroglial cells. OL are numerous in the foetus and neonate, but their number rapidly decreases as myelination progresses, and thus with age (1, 5, 14, 19, 20-24, 27). Glial disorders are implicated in many neurological and psychiatric diseases, and OL are particularly susceptible to damage in demyelinating diseases, such as multiple sclerosis (MS) (2, 6, 21-23, 28, 29). Morphological changes in oligodendrocytes involve the loss or degeneration of myelin proteins and proteins specific for oligodendrocytes, such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and myelin-associated glycoprotein (MAG). MAG plays a key role in the formation of myelin sheaths during the first stage of myelination and helps to maintain axon-oligodendrocyte contact in the mature myelin sheath (8, 9, 21-23). Previous studies of OL in the CNS have primarily focused on myelinating cells, while little is known about their morphology and functions after myelination is complete. Analysis of ultrathin sections of the optic nerve of 35-year-old monkeys showed an increase in the thickness of myelin sheaths, swelling in OL processes and an increase in the total number OL in comparison with young individuals. In contrast, similar studies on the ageing hippocampus in mice showed a decrease in the number of OL (1, 21-23, 27). Measurements of white matter volume in the human CNS have found an 11% reduction in comparison with young individuals. Magnetic resonance imaging (MRI) has shown that white matter volume decreases with age by about 28% in primates, whereas only a slight decrease in white matter volume was observed in the corpus callosum in monkeys. The decrease in white matter volume is thought to be closely connected with myelin loss by axons (9, 21-23). A 27% decrease in the total length of demyelinated fibres was observed in the optic nerve of rats and in the corpus callosum of monkeys (9, 21-23). In addition to numerous neurochemical changes, neuronal and glial changes occur during ageing, leading to neurodegeneration. Some authors have demonstrated an increase in the total number of different types of glial cells in the cerebral cortex in humans, monkeys and mice (1, 21-24, 27). An increase in the number of astrocytes was demonstrated in the cerebral cortex of ageing rats (29). In ultrathin sections inclusions were observed to appear with age in the cytoplasm of OL, astrocytes and microglia (19, 21-23). In older individuals OL form pairs or rows in the cerebral cortex, while in young animals they usually occur singly. No degenerative changes in the oligodendroglia and microglia were observed in the nuclei basales or brainstem of older dogs, while in the astrocytes astrogliosis was noted, manifested as accumulation of glial fibrillary acidic protein (GFAP) (5). The periaqueductal grey matter (PAG) is a well-myelinated region of grey matter in the CNS and has been described in many mammalian species. This region is involved in emotional and defensive reactions, memorization, urination, vocalization, and feelings of fear and anxiety. The PAG integrates behavioural responses to threats and stress stimuli (3, 4, 13, 18, 31). The PAG receives neural pathways and sends nerve fibres myelinated by OL to numerous areas of the brain, including the cerebral cortex, thalamus, hypothalamus, areas of the forebrain, brainstem nuclei and spinal cord. The PAG is one of the main components of the descending pain inhibitory system, in which opioids act to reduce pain activity. Due to connections between the PAG and the hypothalamus it is also involved in sexual behaviour and affects cardio-vascular activity.

The structure and function of oligodendrocytes in the CNS are as yet known only in young individuals, while there is little information on the morphology of the three types of OL in the PAG of older individuals. The available literature offers no data concerning the morphometric parameters of the three types of OL in the PAG. Morphometric examination yields more accurate results and enables morphological evaluation of CNS structures, and thus a better understanding of the mechanisms of their functioning. The aim of this study was to determine the morphology and morphometric parameters of the three types of OL in the PAG region of young and older animals at the light microscope level.

Material and methods

The study was carried out on 10 male Wistar rats in two age groups, with five 25-week-old rats in the first group and five 140-week-old rats in the second. The experiments on the animals were approved by the Second Local Ethics

Committee for Animal Experimentation in Lublin, Resolution No. 6/2011 of 15 February 2011.

The rats, under deep anaesthesia induced by 10% ketamine (100 mg/kg body weight), were perfused through the left ventricle with 50 ml 0.9% NaCl solution at 37°C. The perfusion was continued with 250 ml 1% glutaraldehyde and 1% paraformaldehyde in a 0.1 M phosphate buffer, pH 7.4, until vital functions had ceased. Then the skull was opened, the brain was removed, and fragments of midbrain containing PAG were excised. The material was fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.4, and re-fixed in a 2% solution of osmium tetroxide in a 0.1 M phosphate buffer, pH 7.4. Next the samples were dehydrated, embedded in Epon resin, cut in an ultramicrotome and stained with methylene blue solution (26). Semi-thin sections were analysed morphologically and photographed under an Olympus BX40 light microscope equipped with a Soft Imaging System (SIS) ColorView III-u digital camera. Morphometric parameters were measured and analysed with a calibrated system and the image was digitized using Soft Imaging System (SIS) Cell^D software. The oligodendrocytes selected for the analysis, with distinct cell nuclei, were measured and counted by the program. OL could be distinguished from other glial cells on the semi-thin sections by their round or oval shape and dark nuclei. Morphometric analysis of OL was performed under 40 × magnification and included cell size in µm, area of soma in μm^2, and perimeter and diameter of soma in µm. In addition, the percentages of the three types of OL in the PAG were determined in the two age groups. Statistical analysis was performed using the Statistica 10 software. All results were expressed as means ± standard error of the mean (SEM). Statistical differences between the groups were determined by Student's test and the level of statistical significance was set at $p < 0.05$.

Results and discussion

In the PAG of rats in the two age groups, i.e. 25- and 140-week-old animals, differentiation of cytoplasm density indicated the presence of three types of oligodendrocytes: light (OLl), medium (OLm) and dark (OLd).

Few OLd were observed on the semi-thin sections of the PAG of 25-week-old rats. Most numerous were OLl and OLm, located near blood vessels, neurons and astrocytes. In the 25-week-old individuals OLd were present in isolation or in the vicinity of OLm. OLl and OLm most frequently adhered to each other. OLm or OLl were observed near capillary blood vessels (Fig. 1). The results of the morphometric examination showed that in the 25-week-old rats the average cell size was about 6.5 µm for OLd, 7.09 µm for OLm, and 7.24 µm for OLl (Fig. 3). The average perimeter of the three types of OL was about 20 µm, while the average surface area was about 36 µm for OLd, 43 µm for OLm, and 45 µm for OLl (Fig. 4, 5). The percentage share of OLl was highest, about 58%, compared to about 24% OLm and about 18% OLd (Fig. 6).

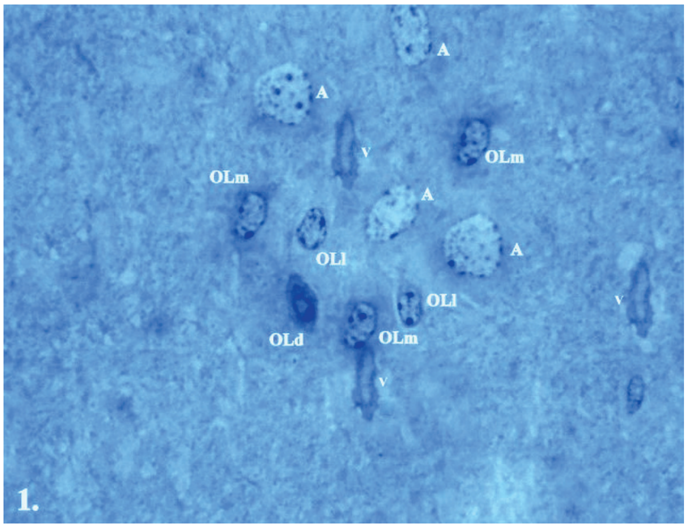

Fig. 1. Semi-thin section of the PAG of a 25-week-old rat
Explanations: OLl – oligodendrocytes with light cytoplasm, OLm – oligodendrocytes with medium cytoplasm, OLd – oligodendrocyte with dark cytoplasm, A – astrocytes, v – capillaries, magn. approx. 600 ×

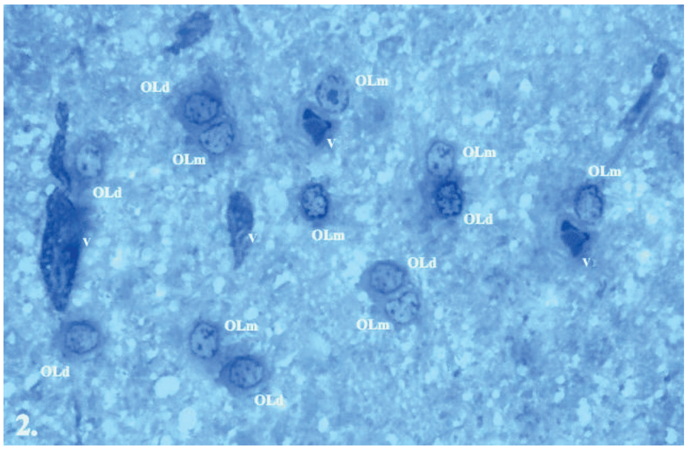

Fig. 2. Semi-thin section of the PAG of a 140-week-old rat
Explanations: OLm – oligodendrocytes with medium cytoplasm, OLd – oligodendrocyte with dark cytoplasm, A – astrocytes, v – capillaries, magn. approx. 600 ×

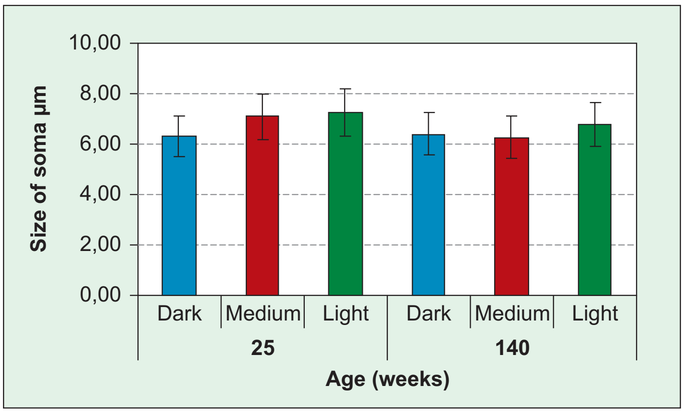

Fig. 3. Cell size of the three types of OL in the PAG of rats in 2 age groups

In the second age group, i.e. 140-week-old rats, OLm and OLd dominated in the PAG. The cells were located near blood vessels, neurons and astrocytes. Adjacent

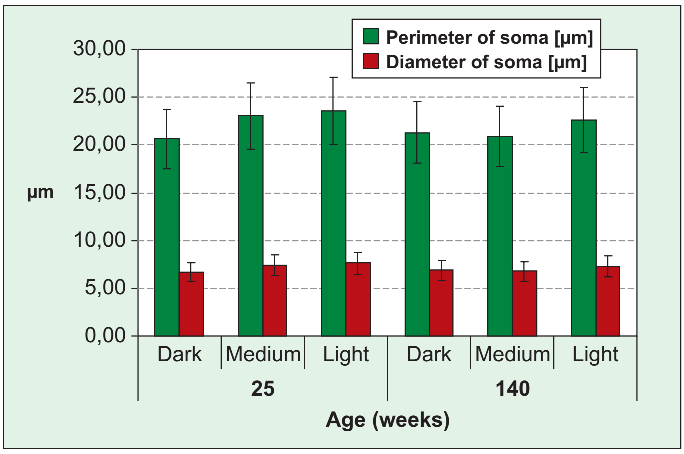

Fig. 4. Morphometric parameters of the three types of OL in the PAG of rats in 2 age groups (perimeter of soma in µm, diameter of soma in µm)

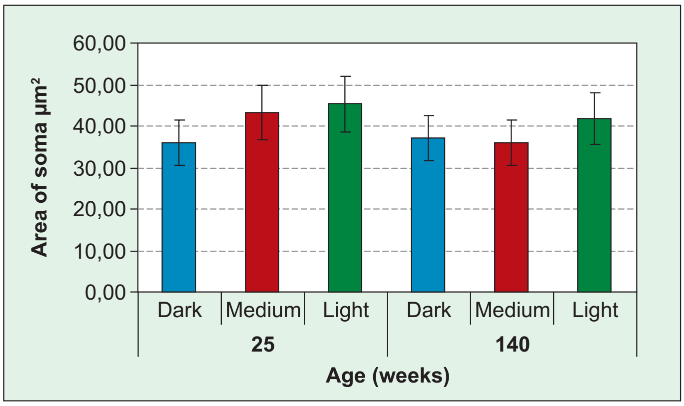

Fig. 5. Morphometric parameters of the three types of OL in the PAG of rats in 2 age groups (area of soma in µm²)

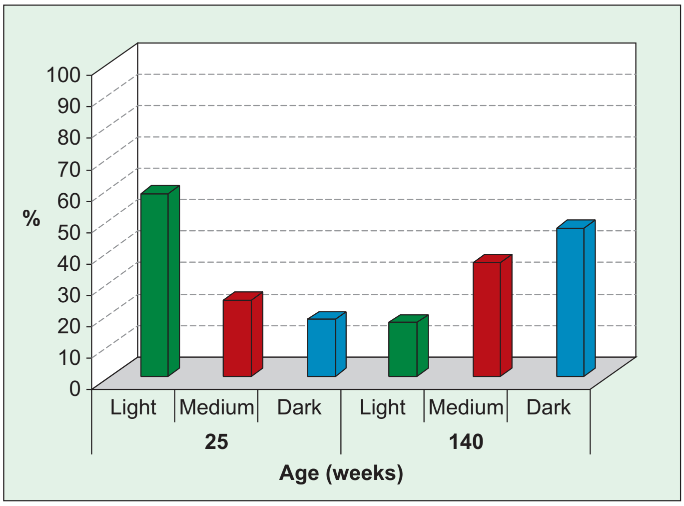

Fig. 6. Percentage shares of the three types of OL in the measurement area in the PAG of 25-week-old and 140-week-old rats

pairs of OLm and OLd were most frequently observed; while OLl were rarely present (Fig. 2). Morphometric examination determined that in the 140-week-old rats the average cell size was about 6.4 µm for OLd, 6.2 µm for OLm and 6.79 µm for OLl (Fig. 3). The average cell perimeter of the three types of OL was about 20 µm, while the average surface area of the cells in this age group was about 41.84 µm for OLl, 35.96 µm for OLm and 37.05 µm for OLd (Fig. 4, 5). In the PAG of the 140-week-old rats OLd were most prevalent, accounting for about 47% of OL, followed by OLm at about 36%, while the least numerous were OLl, at about 17% (Fig. 6).

In the present study examination of semi-thin sections of PAG under a light microscope revealed the presence of three types of cells, OLl, OLm and OLd, in both age groups of rats. Many studies of OL have focused on young individuals in which the myelination process has not been completed. Studies of OL in older individuals have mainly been conducted on monkeys, with a few observations carried out in humans, dogs and rodents. Particularly in monkeys cognitive abilities have been found to deteriorate with age (1, 5, 7, 11, 12, 17, 21-24, 30). OL in the cerebral cortex of 25-year-old monkeys have swollen processes and form characteristic pairs or rows, as in the PAG of older rats in the present study (21-23). In monkeys in which myelination is not complete and in the cerebral cortex of 4- and 10-year-old individuals, OL are most frequently localized near blood vessels and occur singly (21-23). The characteristic arrangement of OL in pairs may indicate a relationship between the arrangement of cells and their age (23). In the cerebral cortex of 25-year-old monkeys some OL bodies and processes have been found to contain inclusions of various electron density and size. Some authors treat them as specific structural markers associated with ageing (14). OL are thought to synthesize components of myelin sheaths which are renewed during ageing (27). In older monkeys OL processes joining together and simultaneous degeneration of myelin sheaths have been observed. Changes in the myelin sheaths lead to changes in nerve conduction, which may be one of the causes of ageing in primates (21). Ageing of the brain is characterized by morphological changes in the neurons and neuroglia, which in turn lead to neurochemical disorders and then to neurodegeneration. Quantitative and qualitative examinations of OLd in rats have showed varied dominance over other types of OL, depending on the area of the CNS and the age of the rats. Predominance of OLd was observed from the first month of life in the corpus callosum, from the third month in the cerebral cortex, and between the second and fifteenth month in the cerebellum (14). Percentage analyses of the three types of OL in the cerebral cortex have shown that their frequency of occurrence was dependent on the age of the rat. In young, 2-month-old individuals, cells of medium density were dominant (about 57%), with fewer light cells (about 25%), and the fewest with dark cytoplasm (about 17%). In contrast, in older, 32-month-old rats, a significant increase was noted in dark oligodendrocytes (about 41%) and a decrease in light cells (about 18%). The percentage of medium oligodendrocytes (about 53%) was similar to that observed in young individuals (8). In the corpus callosum of older rats dark oligodendrocytes predominated (about 52%), while the least numerous were light cells (about 12.3%). In the present study, the percentages of the three types of OL obtained in the PAG were similar to those noted in the corpus callosum of older individuals. A few authors have determined the size of particular types of OL in the cerebellum of rats, finding similar values for young and older animals (14). In the present study, morphometric analysis also did not reveal statistically significant differences in the size of each type of OL in the PAG of the two age groups. At the electron microscope level OL from the cerebral cortex of young and older monkeys was not found to differ in terms of the diameter of the cell nuclei of the three types of OL (21, 23). A study on the optic nerve and cerebral cortex in older monkeys showed that the number of OL may increase by up to 50% in comparison with young individuals (21). Such an increase in the number of OL is closely linked to myelination processes and leads to an increase in the number of myelin sheath segments. An increase in OL is usually accompanied by an increase in the number of microglial cells, whereas the number of astrocytes remains unchanged (21, 22). In contrast with ageing monkeys, other authors did not observe a change in the number of OL in the cerebral cortex of 36-month-old Wistar rats, but noted an increase in the number of astrocytes, which they suggest may result from neuron damage, hypoglycaemia or excessive amino acid secretion (20). Some speculate that the increase in the number of OL in monkeys is closely correlated with the participation of these cells in both the remyelination and demyelination of nerve fibres (21, 22). According to some authors OL are capable of mitotic divisions and can generate new cells called NG2, containing chondroitin sulphate proteoglycan. These occur both during the development process and in the mature CNS, but differ from OL (2, 6, 23, 33). New OL formed in the ageing brain are needed for remyelination and thus for rapid conduction of nerve fibres. During CNS damage and as a result of many neurodegenerative diseases OL undergo damage leading to their death (1, 6, 8-10). Due to its anatomical location and structure the PAG plays an important role in the stress response and adaptive functions. Owing to numerous connections with such areas as the cerebral cortex, corpus amygdaloideum, spinal cord, hypothalamus and brainstem nuclei, the PAG is associated with memory and learning processes and is involved in integrated reactions, including cardiovascular reactions, modulation of pain, thermoregulation, respiration, respiratory control, and urination. In addition, the PAG is implicated in neurodegenerative diseases such as Alzheimer's and Parkinson's disease or MS (3, 4, 13, 18, 31).

Morphological and morphometric examination of OL in different areas of the brain in the CNS are important in demyelinating diseases such as MS. Morphological changes in OL and myelin sheaths may play a significant role in cognitive impairment and may be associated with degeneration of myelin proteins and proteins specific for OL, such as MOBP, MBP, MOG and MAG. Changes in OL morphology may be induced by many factors, such as mutation, medicines, trauma and diseases. The development of methods determining OL morphology is an important direction of research leading to an understanding of the biology of these cells and their role in myelination and demyelination.

Conclusions:

1. The results obtained indicate that in the PAG of older, 140-week-old individuals oligodendrocytes with dark cytoplasm predominate, in contrast with young rats.

2. The size of the three types of OL was similar in the two age groups examined.

3. No significant differences between the two age groups were observed in the cell perimeter and area of any of the OL types.

4. OL have been neglected in research on older individuals and should therefore be analysed in this regard. In recent decades the involvement of these cells in the development of neurodegenerative diseases has not been fully explained, and knowledge of the biology of this neuroglia may contribute to an understanding of the mechanisms of various diseases of the central nervous system.

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