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Distribution of various types of oligodendrocytes and cellular localisation of iron in the frontal cortex of the adult rat

Agata Wawrzyniak-Gacek

Department of Histology and Embryology, University of Agriculture, Lublin, Poland

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Oligodendrocytes, called oligodendroglia, are present in the white and grey substance of the CNS. They constitute a heterogeneous group, in which 3 types of these cells have been distinguished: those of light, medium and dark cytoplasm. They locate themselves at the neurones and blood vessels. The number of light oligodendrocytes decrease with age, while the number of dark oligodendrocytes increase. In histochemical studies the product of the reaction located itself in the body and processes, indicating the participation of these cells in the metabolism of iron.

key words: frontal cortex, oligodendrocytes, iron, rat

INTRODUCTION

Among different kinds of glia, oligodendrocytes, also called oligodendroglia, constitute the most numerous population [1, 5, 6, 8, 9, 11, 22, 28]. The studies of Ogawa et al. [26, 27] on the level of light microscope revealed their different locations in the central nervous system (CNS). As was revealed by studies conducted by various authors [23, 24, 33] with the use of an electron microscope, they constitute a heterogeneous group of cells, which show certain common features e.g. regular-shaped oval or spherical nucleus, typical cytoplasmic organelle with extremely well-developed rough endoplasmic reticulum and a specifically located Golgi apparatus, not only in the perinuclear area, but also in the protoplasmic processes. The heterogeneity of these cells manifests itself in the occurrence of oligodendrocytes with light, medium and dark cytoplasm.

Histochemical studies reveal that oligodendrocytes in mammals are the main cells of the CNS containing iron [2, 3, 12, 13]. The first studies reported the presence of iron in weakly myelinised areas of the CNS, and further research revealed the presence of iron in the cells of this kind of glia, also in strongly myelinised areas [10, 18, 29, 32, 34, 35].

The presence of iron in oligodendrocytes was also linked to GABA metabolism, because the increase of its contents was observed in the areas where this neurotransmitter is responsible for neural activity.

Thus, it seems interesting to investigate the distribution of various types of oligodendrocytes and the presence of iron in them in the weakly myelinised areas of rat's brain, i.e. in the frontal cortex.

MATERIAL AND METHODS

Male Wistar rats aged 90 days (9 specimens), 120 days (9 specimens) and 150 days (9 specimens) were used in this study. Three specimens of animals were assigned to each method used. The following methods, which in this paper are assumed to be complementary to one another, were used in the studies:

1) Method of nervous tissue impregnation with the use of potassium-silver cyanide according to Ogawa et al. [26, 27], which allowed for observation of oligodendrocytes in the area of the frontal cortex of the brain on the level of light microscope.

Address for correspondence: Agata Wawrzyniak-Gacek, Department of Histology and Embryology, University of Agriculture, ul. Akademicka 12, 20–033 Lublin, Poland, tel: +48 81 445 69 72, fax: +48 81 445 67 41

2) Method of observation in an electron microscope according to Sato [31] in order to recognise different types of oligodendrocytes, i.e. those of light, medium and dark cytoplasm, as well as their situation in relation to other structures present in the examined area of the rat's brain.

3) Histochemical method of detecting the presence of iron ions in oligodendrocytes, according to Le Vine et al. [17].

Impregnation of the nervous tissue with silver salts

The animals under general anaesthesia were perfused through the left ventricle with fluid of the following composition: 0.8% maltose, 0.4% glucose and 0.8% solution of NaCl at a temperature of 37°C. Afterwards, the perfusion samples of cortex were collected and put into 10% formalin solutions for 24 hours. The frontal cortex was cut on a freezing microtome into 30-μm-thick sections and transferred into distilled water with a few drops of formalin added. Then the sections were put onto slides and transferred into 5% water solution of potassium-silver cyanide for 24 hours at a temperature of 37°C. The sections were rinsed with distilled water and ammoniacal pyridine solution prepared according to the following procedure: the concentrated ammonia was added drop by drop to the 10% solution of silver nitrate ($AgNO_3$) until a dark brown precipitate appeared. After the precipitate had been filtered, 3 drops of pyridine were added to the obtained solution. Next, the sections were reduced with 10% formalin, rinsed with distilled water and fixed in 5% sodium thiosulphate. At the further stage the sections were dehydrated by passing them through a range of alcohols and mounted with Cedax resin.

Preparing specimens for observation in an electron microscope

Perfusion was carried out in narcosis through the left ventricle: first with 0.9% NaCl solution at a temperature of 37°C, then it was continued with 1% solution of glutaraldehyde and 1% paraformaldehyde in 0.1% phosphatic buffer (pH = 7.4). After the perfusion, small samples of the frontal cortex were collected and fixed in 2% glutaraldehyde solution in 0.1 M phosphatic buffer (pH = 7.4) at room temperature, and fixed again with 1% solution of OsO_4 in 0.1 M phosphatic buffer (pH = 7.4). The fixed tissue specimens were dehydrated in ethanol and embedded in Epon resin. Ultra-thin sections were double stained with uranyl acetate and lead citrate according to the procedure described by Sato [31]. Observation of ultra-thin sections was carried out in Tesla BS-500 electron microscope. Attention was paid to the number of particular types of oligodendroglia per 100 observed cells in order to prepare histograms.

Detecting the presence of iron ions by means of histochemical method

The histochemical method of iron ion detection was based on the procedure which had been described by Bunting and modified by Nguyen-Legros et al. [25]. The animals were deeply anaesthetized and perfusion was carried out with 10% formalin solution, the brains were prepared and the area of the brain frontal cortex was sampled. Frozen 70-μm-thick sections were taken from these samples. The sections were transferred into distilled water, put onto slides and dried at room temperature. Then they were transferred for 30 minutes to the freshly prepared mixture containing 0.25 N HCl, 2% potassium ferricyanide ($K_3Fe(CN)_6$) and 8% Triton X 100. The sections were then rinsed in the PBS solution for 30 min. The rinsed sections were incubated in 60 mg 3.3' diaminobenzidine (DAB) in 300 ml 0.01 M TRIS HCl (pH = 7.6) with the addition of 600 μl of 30% H_2O_2. Incubation was conducted for three hours in complete darkness at room temperature.

After incubation, the sections were again rinsed in PBS solution for 30 minutes. Next, the tissue was dehydrated and mounted in Cedax resin. Then they were observed and photographed in light microscope.

RESULTS

Impregnation of the nervous tissue with silver salts

The application of the method of impregnating the sections with silver salts allowed for observation of the distribution and location of oligodendrocytes in relation to structures present in the frontal cortex. In all age groups, i.e. in animals which were 90, 120 and 150 days old, the oligodendroglia distribution model was similar. Oligodendrocytes, stained with silver salts, were unevenly distributed. In the area of the frontal cortex, oligodendrocytes were noticeable mainly near the neurones and in the vicinity of the capillary blood vessels (Fig. 1).

Observation of types and distribution of oligodendrocytes on the level of electron microscope

Studies on the level of the electron microscope allowed for observation of three types of oligodendrocytes: those of light, medium and dark cytoplasm,

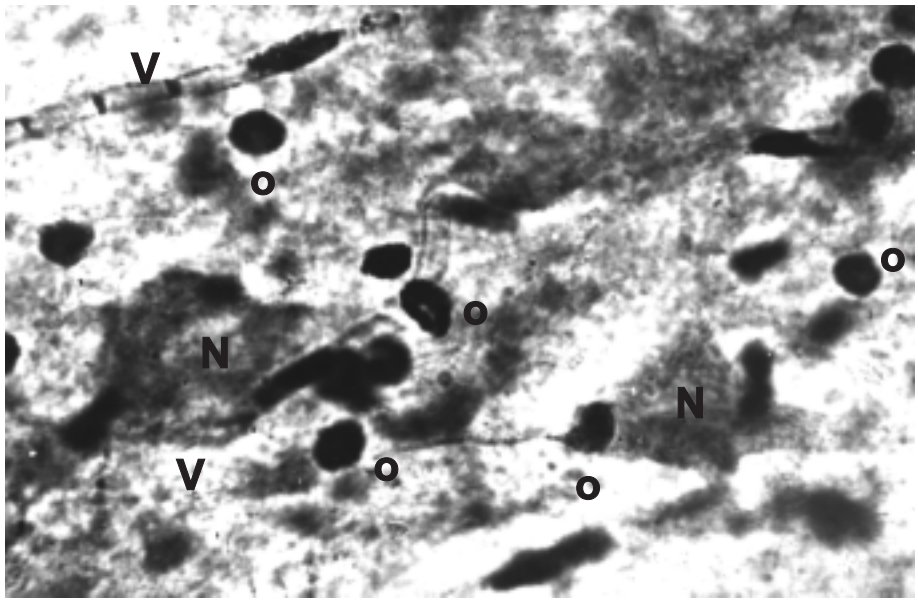

Figure 1. Frontal cortex of a 150-day old rat, stained by means of the silver method. Oligodendrocytes (o) are localised at neurones (N) and blood vessels (V); × 1200.

as well as of their location near characteristic structures that are present in the frontal cortex.

In 90-day old individuals mainly oligodendrocytes with medium cytoplasm were observed. Less numerous were those with light cytoplasm, and those with dark cytoplasm occurred sporadically (Fig. 2). Similar types of oligodendrocytes were observed in 120-day-old individuals. In this age group in the examined area there was an increase of population of oligodendrocytes with dark cytoplasm (Fig. 2). In the frontal cortex of 150-day old rats there occurred mainly oligodendrocytes with medium and dark cytoplasm, while oligodendrocytes with light cytoplasm were the least numerous (Fig. 2). In all examined age groups there were no significant differences in the location of oligodendrocytes in the area of the frontal cortex. Three types of these glia cells were found at the neurones (Fig. 3–5), blood vessels, (Fig. 6), and scarce nerve fibres.

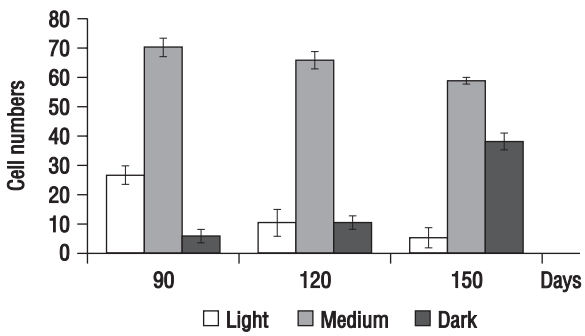

Figure 2. The proportion of various oligodendrocyte types per 100 cells observed in an electron microscope in the frontal cortex of a rat's brain.

Histochemical detection of the presence of iron ions in oligodendrocytes

As a result of histochemical reaction to the presence of iron, a brown reaction product was observed, which located itself in the cytoplasm and in oligodendrocyte processes in the area of rat's frontal cortex in all age groups A slight reaction to the presence of iron was also visible among the oligodendrocytes of this area (Fig. 7).

DISCUSSION

The location of oligodendrocytes at neurones and blood vessels in the examined cortex area is similar to the results obtained by Ogawa et al. [27] and Le Vine et al. [19] on the level of light microscopy.

Observations in the electron microscope allowed for the isolation of 3 types of oligodendrocytes with different cytoplasm density in the studied area in all age groups. Oligodendrocytes with medium cytoplasm constituted the largest population, and the number of cells with dark cytoplasm increased with the age of individuals, and in 150-day-old animals such cells were the most numerous. The greatest number of oligodendrocytes with light cytoplasm

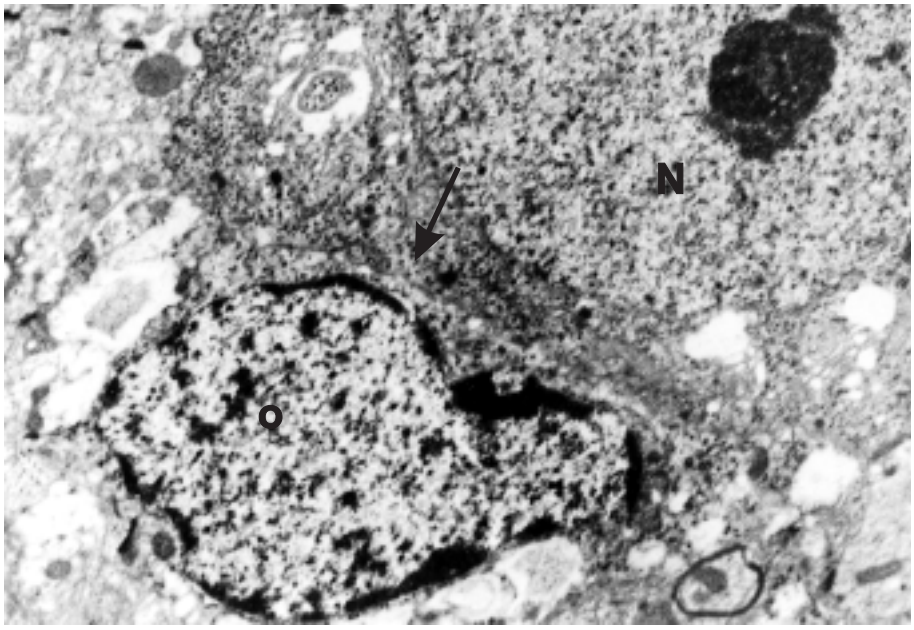

Figure 3. Electronogram from the frontal cortex of a 90-day-old rat presents a light-cytoplasm oligodendrocyte (o) in direct vicinity (arrow) of a neurone (N); × 8000.

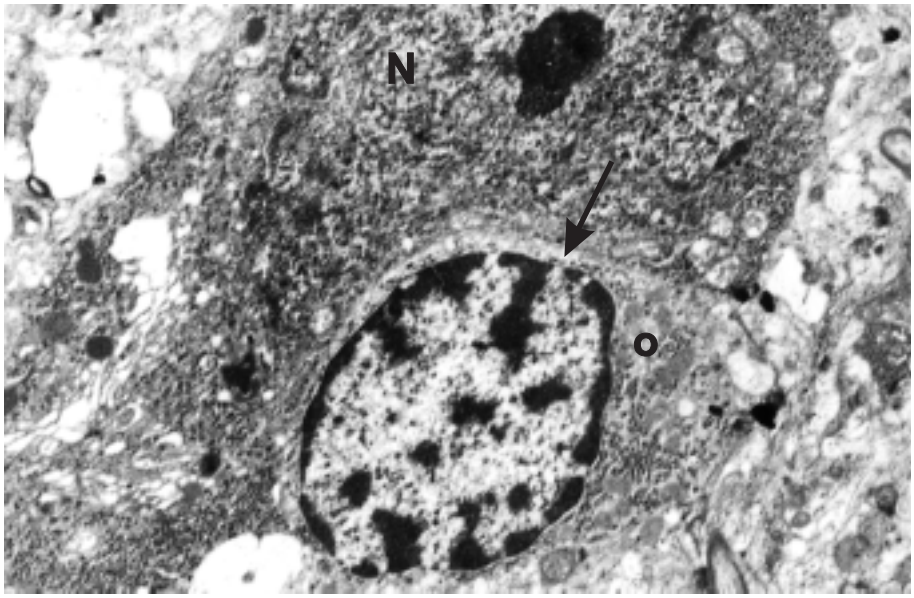

Figure 4. Electronogram from the frontal cortex of a 120-day-old rat, presenting a medium-cytoplasm oligodendrocyte (o) in direct vicinity (arrow) of a neurone (N); × 8000.

was observed in the frontal cortex of the youngest group, and their number decreased with age. This indicates the possibility of the transformation of oligodendrocytes with light cytoplasm into cells with medium and then with dark cytoplasm. The studies of other authors confirm this as well [20, 21, 23].

The observations of ultrathin sections confirmed the similar pattern of oligodendrocyte distribution, which had been observed in the light microscope. All three types of oligodendrocytes were located directly at the neurones and capillary blood vessels or in their close vicinity, and less often at the nerve fibres. The location of these cells at blood vessels indicates that they are the next kind of glia, after astrocytes, which come into contact with blood vessels [14].

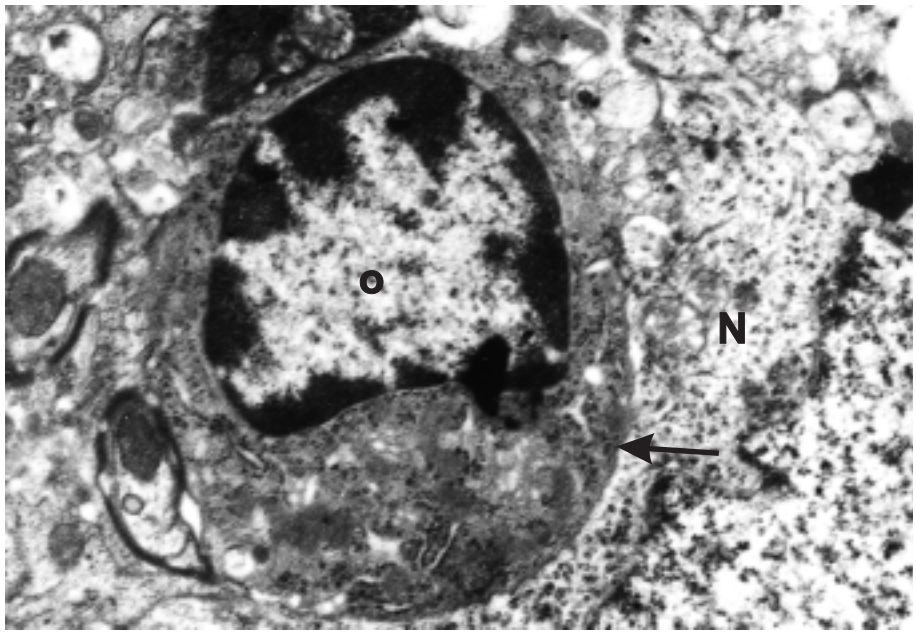

Figure 5. Electronogram from the frontal cortex of a 150-day-old rat. An oligodendrocyte (o) with dark cytoplasm in direct vicinity (arrow) of a neurone (N); × 10000.

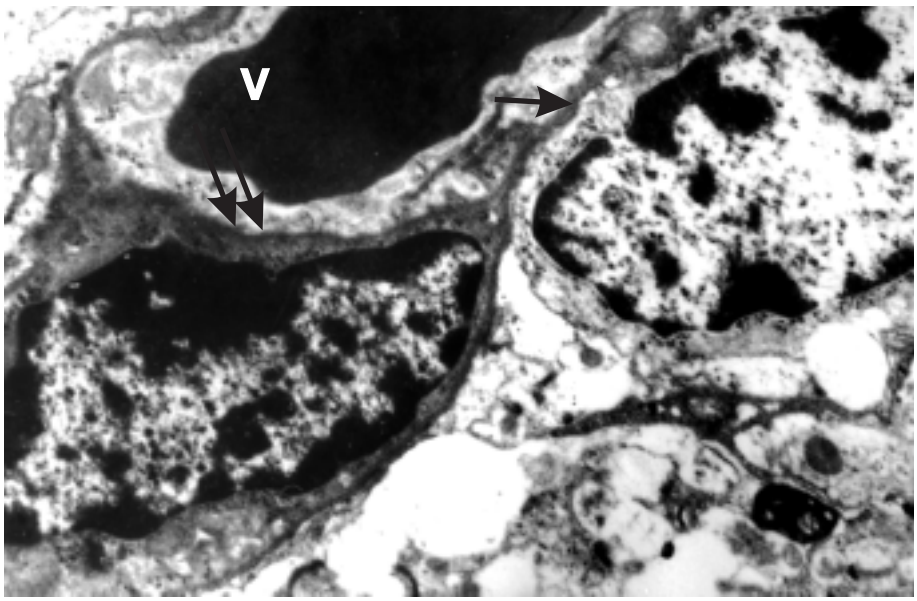

Figure 6. Electronogram from the frontal cortex of a 150-day-old rat shows an oligodendrocyte with light (arrow) and dark cytoplasm (double arrows) in direct vicinity of a blood vessel (V); × 10000.

In the studied area, oligodendrocytes contained the product of histochemical reaction that indicating the presence of iron in their cytoplasm and processes. These findings are consistent with the results obtained by Le Vine et al. [19]. Iron is a component of many neuronal enzymes. It is also a component of enzymes involved in fat metabolism [7, 16]. Transferrin and ferritin are engaged in maintaining iron homeostasis in the brain. The studies of Benkovic et al. [4] on the level of light microscope revealed the presence of iron, transferrin and ferritin in the oligodendrocytes of rat's brain cortex, which were situated near neurones and blood vessels. The studies of Jefferies et al. [15] demonstrate that the endothelial cells of the brain blood vessels have receptors for transferrin and may mediate in the transport of

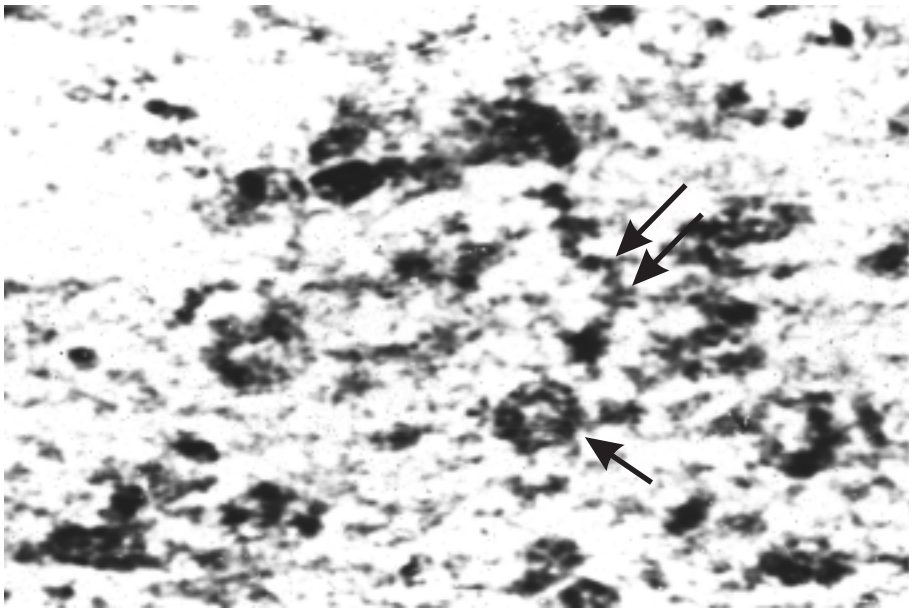

Figure 7. The deeper layers of the frontal cortex of a 120-day-old rat. The product of the histochemical reaction to the presence of iron occurs in the cytoplasm and oligodendrocyte processes, (arrow) as well as between these cells (double arrows); × 1400.

transferrin or iron through the blood-brain barrier, into other structures of the brain. Another source of iron can be ferritin, which binds about 1/3 of the total amount of iron in the brain, which had been revealed in this particular type of glia [30]. In the light of these authors' studies, and of our own research as well, it might be supposed that oligodendrocytes which contact themselves with blood vessels take part in the transport of transferin into other structures of the brain. Oligodendrocytes which place themselves at the neurones may be a source of iron, which is indispensable for the metabolic processes of these neurones [36].

The cause of the occurrence of oligodendrocytes with various cytoplasm densities has not been determined so far. It might be connected with the accumulation of iron and iron binding substances in the cytoplasm of oligodendrocytes. However, this needs further research.

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