ISSN 0006-2979, Biochemistry (Moscow), 2025, Vol. 90, No. 12, pp. 2027-2040 © The Author(s) 2025. This article is an open access publication.
Russian Text © The Author(s), 2025, published in Biokhimiya, 2025, Vol. 90, No. 12, pp. 2139-2152.

Mitochondria in Developing Brain: Contribution of Deviations to Higher Susceptibility to Neurodegeneration in Latter Periods of Life

Natalia A. Stefanova[1,a]*, Natalia A. Muraleva[1], Diana V. Sityaeva[1], Mikhail A. Tyumentsev[2], and Nataliya G. Kolosova[1]

[1]*Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, 630090 Novosibirsk, Russia*

[2]*University of Tennessee Health Science Center, Memphis, Tennessee 38163, USA*

[a]*e-mail: stefanovan@mail.ru*

Received September 18, 2025
Revised October 8, 2025
Accepted October 11, 2025

Abstract—It has been proven that the preclinical period of the sporadic (>95% of cases) form of Alzheimer's disease (AD) can last for decades, but the question of when the disease begins to develop and what contributes to it remains open. It is hypothesized that vulnerabilities to AD may be influenced by anatomical and functional brain parameters formed early in life. This is supported by our research on the senescence-accelerated OXYS rats – a unique model of AD. The delayed brain maturation observed in these rats is associated with insufficient glial support, a key regulator of neural network function, and the development of AD signs in the OXYS rats is preceded and accompanied by the mitochondrial dysfunction. This raises the question of whether the structural and functional features of mitochondria could influence brain maturation and thus determine predisposition to the later development of AD signs. In this study, we compared mitochondrial biogenesis, their trafficking, and structural state in the neuronal cell bodies, axonal and dendritic processes, as well as activity of the mitochondrial dynamics processes in the prefrontal cortex and hippocampus of OXYS and Wistar rats (control) during the period of brain maturation completion (from birth to 20 days of age). Changes in the number and ultrastructural parameters of mitochondria were compared with the parameters of dynamics processes, assessed by the frequency of mitochondria undergoing fusion or fission, the content of the key biogenesis protein PGC-1α, and proteins mediating mitochondrial dynamics (mitofusins Mfn1 and Mfn2, dynamin-1-like protein DRP1). In OXYS rats, deviations in formation of the mitochondrial apparatus in the early postnatal period were identified, which may contribute to the delayed brain maturation of these rats, promote mitochondrial dysfunction, reduce synaptic density, and ultimately lead to the neuronal death and development of the early neurodegenerative changes.

DOI: 10.1134/S0006297925602874

Keywords: mitochondria, early postnatal period, neurodegeneration, Alzheimer's disease, senescence-accelerated OXYS rats

INTRODUCTION

By 2050, global population of individuals aged 60 and older is expected to reach approximately 2 billion (WHO, 2018), with over 150 million people suffering from Alzheimer's disease (AD), which is becoming the leading cause of senile dementia [1]. Consequently, the relevance of elucidating molecular and biological prerequisites and mechanisms of AD development, as well as developing early diagnostic and preventive methods based on this knowledge, is only increasing. Modern diagnostic methods have established that the preclinical period of the sporadic (>95% of cases) form of AD can last for decades, but

* To whom correspondence should be addressed.

the question of when the disease begins to develop and what contributes to it remains a subject of debate [2].

According to the results of epidemiological and experimental studies in recent years, the prerequisites for reduced cognitive abilities in later life and accelerated aging – the main risk factor for AD development – may already form in the early postnatal period, when the brain development is completed [3-10]. This is also supported by our research on the senescence-accelerated OXYS rats – a unique model of sporadic AD. We identified features of brain maturation in OXYS rats during the early postnatal period (from birth to 20 days of age) that could serve as prerequisites for the development of early neurodegenerative changes [11-13]. In particular, we showed that the completion of brain development in OXYS rats occurs against the background of reduced astrocytic and microglial support in the hippocampus and prefrontal cortex – key regulators of neural network function. Insufficient glial support may be the cause of the reduced efficiency of interneuronal contact formation observed in OXYS rats, allowing it to be considered a key event on the path to the later development of AD signs [14]. At the same time, we previously showed that the development of AD signs in the OXYS rats is preceded and accompanied by the age-related mitochondrial dysfunction [15-18]. Thus, we confirmed validity of the "mitochondrial cascade hypothesis" [19, 20], according to which pathogenesis of the sporadic AD is based on the age-related mitochondrial dysfunction. Our studies using OXYS rats also allowed us to raise the question on whether the genetically determined structural and functional features of mitochondria could influence the process of brain maturation in the early postnatal period and thus determine predisposition to the later development of AD.

This study aims to investigate the features of mitochondrial biogenesis, their structural state in the neuronal cell bodies, axonal and dendritic processes, as well as activity of mitochondrial dynamics and trafficking processes in the prefrontal cortex and hippocampus of OXYS rats during the early postnatal period, and to assess their possible contribution to the development of early neurodegenerative changes in the future.

MATERIALS AND METHODS

Animals. The study was conducted with male Wistar and OXYS rats at the Shared Research Facility "Gene Pools of Laboratory Animals" of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences. Animals were kept under standard laboratory conditions (22 ± 2°C and a 12-h light/dark cycle) in cages (57 × 36 × 20 cm) with free access to water and standard granulated food for laboratory animals (BioPro, Russia).

Electron microscopy. Rats were euthanized with carbon dioxide and decapitated. Prefrontal cortex and hippocampus were isolated, from which tissue samples of cubic shape (2 × 2 × 2 mm) were cut. The isolated brain fragments were fixed in a buffer (2.5% glutaraldehyde, 1.5% paraformaldehyde, 0.1 M cacodylate buffer) for 1 h at room temperature (RT), washed twice in a buffer, post-fixed with a 1% aqueous solution of osmium tetroxide containing several crystals of potassium ferricyanide ($K_3[Fe(CN)_6]$) for 1 h at RT, and incubated in a 1% aqueous solution of uranyl acetate overnight. The next day, the samples were dehydrated in a series of ethanol and acetone solutions and embedded in an Epon 812 resin (Electron Microscopy Sciences, USA). Complete polymerization of the samples was achieved by maintaining them at 60°C for 3 days. Ultrathin sections (65 nm) were obtained using a Leica Ultracut EM UC6 ultramicrotome (Leica Microsystems GmbH, Germany). Sections were examined using a JEOL JEM 1400 electron microscope (JEOL Ltd., Japan) at the Shared Research Facility for Microscopic Analysis of Biological Objects (ICG SB RAS, Novosibirsk, Russia) at magnifications of 2800× and 3500× (neuronal body) and 12,000× (neuropil). Identification of brain structures (Layer IV of the prefrontal cortex (Bregma 4.68 – Bregma 3.72 mm) and the CA1 field of the hippocampus (Bregma – 2.28 – Bregma – 3.60 mm)) was performed according to Paxinos and Watson [21].

To assess structural and functional parameters of mitochondria in OXYS and Wistar rats (n = 3), 40-60 pyramidal neurons of the prefrontal cortex (at ages 0, 7, 14, and 20 days) and hippocampus (at ages 7, 14, and 20 days) were analyzed. To assess parameters of mitochondria in the neuropil of the prefrontal cortex and hippocampus of OXYS and Wistar rats (n = 3), 3-4 sections were obtained from each animal, and 15 photographs were taken for each section, totaling 45-60 photographs per rat. Statistical analysis was performed based on averaged data for each section from each animal (n = 12-15 per group). Using ImageJ software, ultrastructure of mitochondria, their quantity, and localization in neuronal bodies, axonal and dendritic processes, as well as activity of mitochondrial dynamics processes, were evaluated. The following criteria were used to assess ultrastructure of mitochondria: 1) intact mitochondria – intact cristae, mitochondrial membranes, absence of degranulation, and hydration of the mitochondrial matrix; 2) moderately altered mitochondria – partially disrupted cristae structure, degranulation, hydration of the mitochondrial matrix with formation of vacuolar "cavities" within the mitochondrion, visually estimated

to reach about 15-50% of its area; 3) severely altered mitochondria – extremely disrupted cristae structure: abnormally thinned cristae, loss of original appearance; degranulation, hydration of the mitochondrial matrix more than 50% of the mitochondrial area; in this case, mitochondria are significantly enlarged ("swollen")/loss of integrity ("rupture") of the outer mitochondrial membrane [15].

Western blot analysis. Samples of the prefrontal cortex and hippocampus of Wistar and OXYS rats at ages 0, 7, 14, and 20 days (n = 5-6) were homogenized using a RIPA lysis buffer with addition of protease and phosphatase inhibitors (Sigma-Aldrich, USA). Concentration of total protein was determined using bicinchoninic acid (BCA) (Thermo Fisher Scientific, USA). Proteins were separated by electrophoresis in a 10% polyacrylamide gel, transferred to a nitrocellulose membrane (Bio-Rad, USA), and blocked with 5% bovine serum albumin in 10 mM phosphate-buffered saline (pH 7.4) for 1 h. The membrane was next incubated at 4°C overnight with primary antibodies against PGC-1α, Mitofusin 1, Mitofusin 2, Drp1, beta-actin (ab54481, ab57602, ab50838, ab56788, ab1801, Abcam, USA; 1 : 1000), Miro1, Miro2, Trak1, and Trak2 (PA572835, PA596182, PA570029, MA527606, Invitrogen, USA; 1 : 1000) and for 1 h with secondary anti-rabbit and anti-mouse antibodies (ab6721 and ab97046; Abcam; 1 : 5000). Signal intensity was detected using a ChemiDoc MP imaging system (Bio-Rad) and evaluated using ImageJ software (NIH, USA).

Statistical analysis. Statistical analysis was performed using the Statistica 10.0 software package (Statsoft, USA). Factorial analysis of variance (ANOVA) with post hoc comparison of group means (Newman–Keuls test) was used. Data are presented as M ± S.E.M. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Mitochondrial biogenesis, quantity, and structural state in the early postnatal period. Mitochondria are dynamic structures with close relationship between their morphology and functionality. Their number, size, and shape are determined by the processes of biogenesis, fission, fusion, and mitophagy; they actively interact with other organelles, forming membrane contacts [22]. The state of mitochondrial biogenesis in neurons of the prefrontal cortex and hippocampus of OXYS and Wistar rats in the early postnatal period was assessed based on the level of the key protein PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) [23]. ANOVA analysis showed that the level of PGC-1α depended on the genotype of the animals and was lower in OXYS rats in the prefrontal cortex ($F_{1,32} = 8.26$, $p < 0.008$; Fig. 1, a and c) and hippocampus ($F_{1,32} = 14.13$, $p < 0.001$; Fig. 1, b and d). According to the comparison of the group means, the level of PGC-1α was significantly lower in OXYS rats in both brain structures ($p < 0.05$) at birth and at 7 days of age – in the hippocampus ($p < 0.05$).

The efficiency of mitochondrial apparatus formation in the pyramidal neurons of the prefrontal cortex and hippocampus of OXYS and Wistar rats in the early postnatal period was assessed by their quantity and structural state using electron microscopy (EM) (Fig. 1e). The number of mitochondria in the neuronal bodies of the prefrontal cortex of both rat strains changed unidirectionally during this period ($F_{3,143} = 3.6$, $p < 0.02$; Fig. 1f). In the hippocampus, it depended on the genotype ($F_{1,316} = 7.99$, $p < 0.005$) and age ($F_{2,316} = 5.8$, $p < 0.004$; Fig. 1g) of the animals. In Wistar rats, the number of mitochondria was maximal at 7 days of age and decreased by two weeks of age ($p < 0.001$). In OXYS rats, on the contrary, the number of mitochondria in the neuronal bodies was minimal at 7 days of age and increased by 20 days of age ($p < 0.01$). As a result, density of the neuronal mitochondria in OXYS rats at 7 days of age ($p < 0.01$) was lower, and at 14 and 20 days of age – higher ($p < 0.01$ and $p < 0.001$, respectively) compared to the age-matched Wistar rats.

ANOVA analysis showed that the number of mitochondria in the neuropil (axonal and dendritic processes) of the prefrontal cortex and hippocampus (Fig. 1, h and i) depended on the genotype ($F_{1,91} = 13.2$, $p < 0.0005$ and $F_{1,66} = 18.5$, $p < 0.0001$, respectively) and increased with age in the rats of both strains ($F_{3,91} = 136.4$, $p < 0.0001$ and $F_{2,66} = 90.1$, $p < 0.0001$, respectively). However, in OXYS rats, this parameter was higher at 14 days of age in the hippocampus and at 20 days of age in both brain structures ($p < 0.01$ for all) compared to Wistar rats.

Assessment of mitochondria by their structural state. Evaluation of the mitochondria based on their structural state revealed that in the prefrontal cortex of the rats from both strains during the early postnatal period the specific content of organelles with intact ultrastructure changed unidirectionally ($F_{3,313} = 26.7$, $p < 0.0001$) and was maximal at 14 days of age and minimal at 20 days of age (Fig. 2a). The content of mitochondria with moderately altered ultrastructure also depended solely on age ($F_{3,313} = 18.6$, $p < 0.0001$): in both Wistar and OXYS rats this parameter decreased by two weeks of age and tripled by 20 days of age. The specific content of organelles with pronounced alterations decreased in both rat strains by one week of age and increased by 20 days of age ($F_{3,313} = 13.4$, $p < 0.0001$) and was higher in OXYS rats at 7 and 20 days of age ($F_{1,313} = 6.1$, $p < 0.02$).

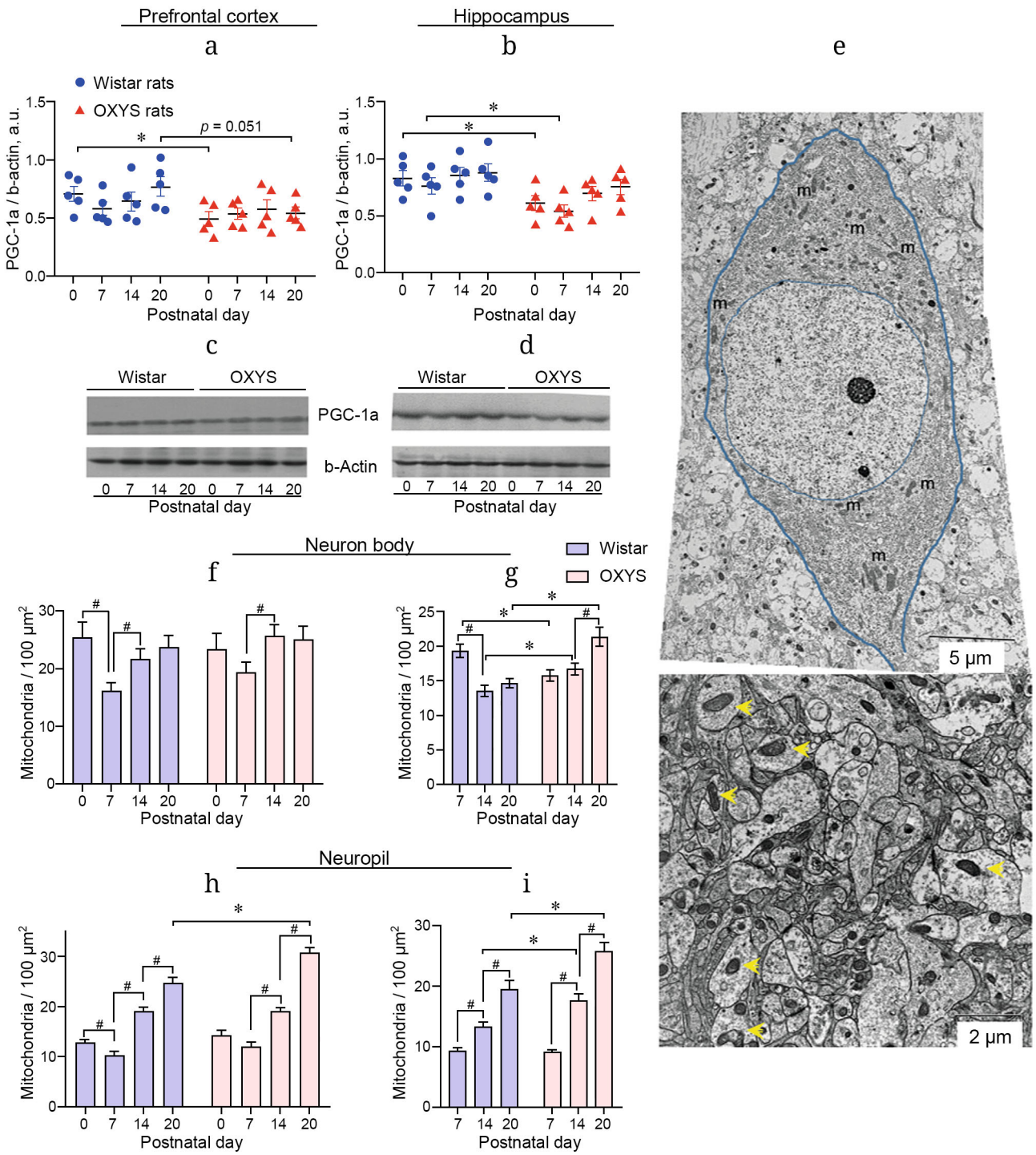

Fig. 1. Content of PGC-1α protein in the prefrontal cortex (PFC) (a) and hippocampus (b) of Wistar and OXYS rats during the postnatal completion of brain maturation. Representative blot images in the PFC (c) and hippocampus (d). Beta-actin was used as a control. Representative EM images of a pyramidal neuron (e, upper panel) and neuropil (e, lower panel). Mitochondria (m) are located in the neuron cytoplasm. The neuron body is surrounded by neuropil; mitochondria are visible in axonal and dendritic processes (yellow arrows). Numbers of mitochondria per 100 μm^2 of neuron cytoplasm and in the neuropil in the PFC (f, h, respectively) and hippocampus (g, i, respectively) are shown. Data are presented as M ± S.E.M. * Significant differences compared to the age-matched Wistar rats; # compared to the previous age. Differences were considered statistically significant at $p < 0.05$.

In the hippocampus of both rat strains (Fig. 2b), the specific content of organelles with intact ultrastructure was maximal at one week of age, decreased by 20 days of age ($F_{2,193} = 89.2$, $p < 0.0001$), and was lower in OXYS rats ($F_{1,193} = 4.8$, $p < 0.03$). The content of mitochondria with moderately altered ultrastruc-

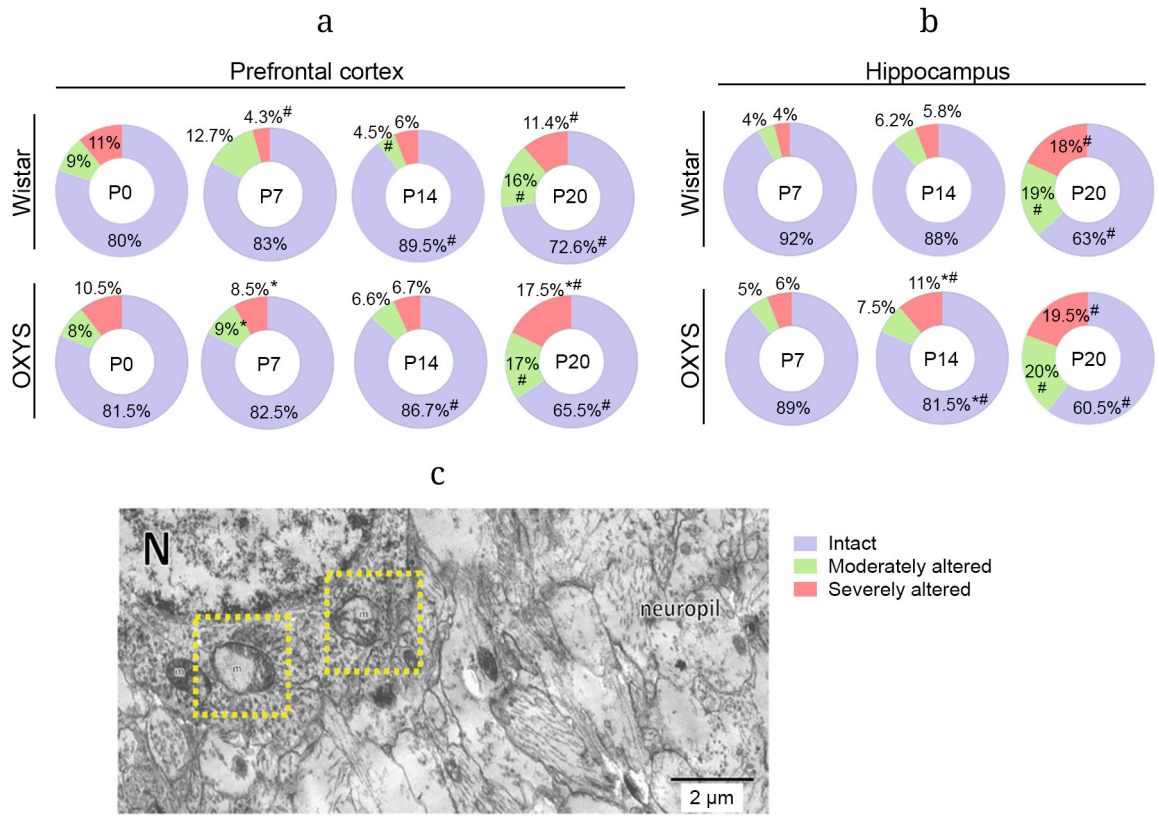

Fig. 2. Specific content of mitochondria in the neurons by the degree of their structural degradation in the prefrontal cortex (a) and hippocampus (b). Representative electron microscopy (EM) image of a pyramidal neuron (N) and neuropil (neuropil) in the CA1 field of the hippocampus of the 14-day-old OXYS rats (c). Mitochondria (m) with moderately pronounced destructive changes (yellow dashed line). Data are presented as M ± S.E.M. * Significant differences compared to the age-matched Wistar rats; # compared to the previous age. Differences were considered statistically significant at $p < 0.05$.

ture (Fig. 2c) depended only on age ($F_{2,193} = 61.6$, $p < 0.0001$): in both Wistar and OXYS rats, this parameter increased by 20 days of age. The specific content of organelles with pronounced alterations was higher in OXYS rats ($F_{1,193} = 6.6$, $p < 0.02$) and increased by 20 days of age in both rat strains ($F_{2,193} = 54.8$, $p < 0.0001$).

Mitochondrial dynamics in the early postnatal period. Processes of mitochondrial dynamics were assessed by the presence of contacting mitochondria undergoing fusion or fission (Fig. 3a). It was found that the specific content of such mitochondria in the neuronal bodies of the prefrontal cortex did not depend on age ($F_{3,143} = 0.05$, $p = 0.98$) and was significantly lower in OXYS rats in comparison with Wistar rats at 14 and 20 days of age ($p < 0.05$; Fig. 3b), indicating significant decrease in the intensity of mitochondrial dynamics. In the hippocampus of both rat strains, the proportion of contacting mitochondria was maximal at 7 days of age and decreased by two weeks of age ($F_{2,315} = 3.9$, $p < 0.05$; Fig. 3c). In the neuropil, this parameter depended only on age – it decreased by 14 days of age and increased by 20 days of age in the prefrontal cortex ($F_{3,91} = 5.3$, $p < 0.003$; Fig. 3d) and, conversely, decreased by 20 days of age in the hippocampus ($F_{2,66} = 3.4$, $p < 0.05$; Fig. 3e) of both rat strains.

Proteins Mfn1 and Mfn2, which are directly involved in the regulation of mitochondrial dynamics, play a key role in the process of mitochondrial fusion, while Drp1 is necessary for their fission and mitophagy [24]. Assessment of the changes in their content during the early postnatal period in the brains of OXYS and Wistar rats was performed using Western blot analysis.

In the prefrontal cortex (Fig. 4a), the content of Mfn1 changed unidirectionally with age in the rats of both strains ($F_{3,40} = 6.069$; $p < 0.002$), with the level significantly increasing by 14 days of age and decreasing by 20 days of age in Wistar rats ($p < 0.002$ for both). The content of Mfn2 depended on genotype of the animals ($F_{1,40} = 40.2$; $p < 0.001$): in OXYS rats, its level was lower at birth, at 7 days, and at 20 days of age, but higher at 14 days of age compared to the age-matched Wistar rats ($p < 0.001$, $p < 0.012$, $p < 0.001$, and $p < 0.009$, respectively).

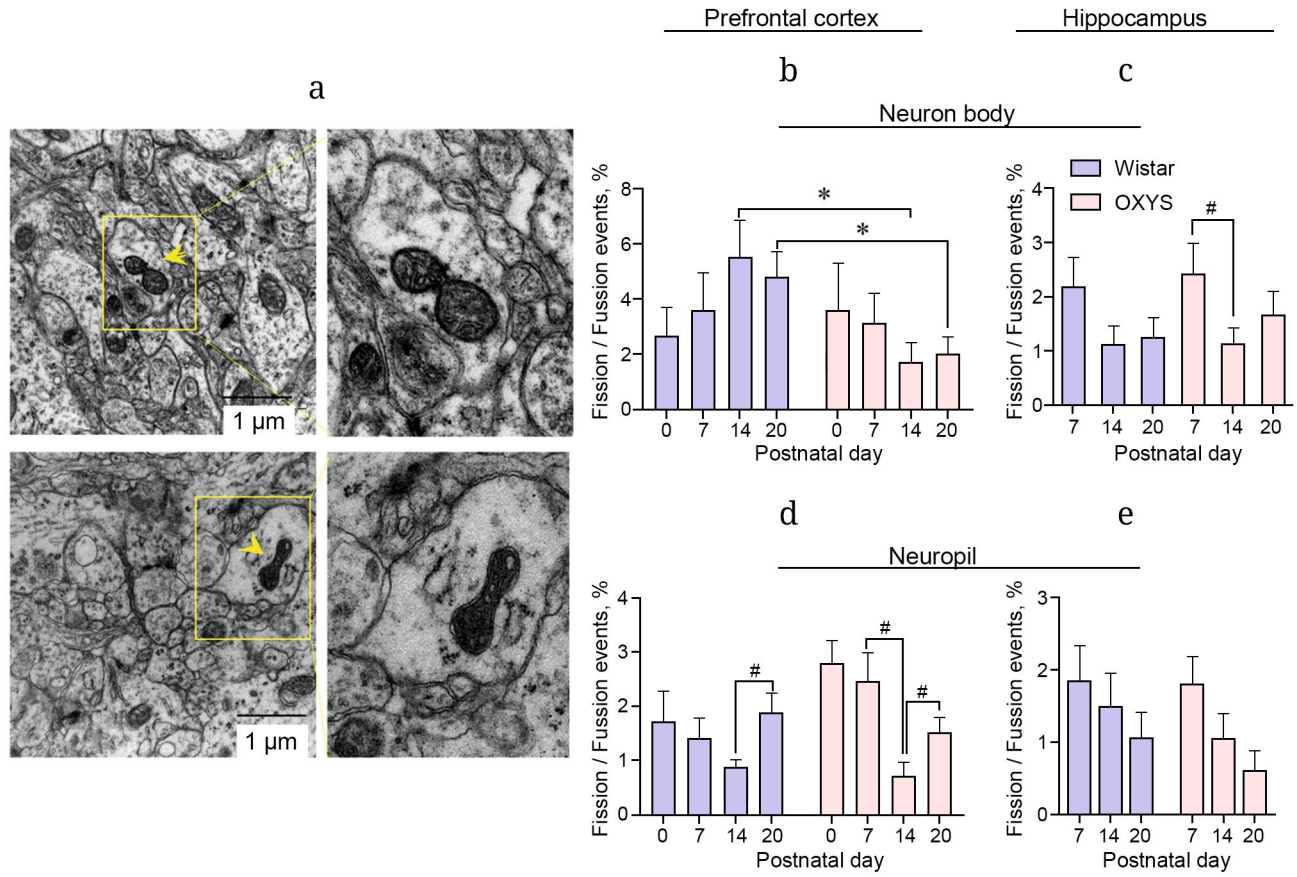

Fig. 3. Representative EM images of the neuropil; axonal processes contain mitochondria undergoing fission/fusion (yellow arrows). The right panel shows mitochondria at higher magnification (a). Specific content of mitochondria undergoing fission/fusion, relative to the total number of mitochondria in neuronal bodies (b, c) and neuropil (d, e) of the prefrontal cortex and hippocampus. Data are presented as M ± S.E.M. * Significant differences compared to the age-matched Wistar rats; # compared to the previous age. Differences were considered statistically significant at $p < 0.05$.

With age, the content of Mfn2 changed in both rat strains ($F_{3,40} = 4.3$; $p < 0.010$), but its dynamics differed. In Wistar rats, the level of Mfn2 decreased by 7 days of age ($p < 0.008$) and increased by 20 days of age ($p < 0.001$). In OXYS rats, the level of Mfn2 increased from 7 to 14 days of age ($p < 0.001$) and decreased by 20 days of age ($p < 0.001$).

ANOVA analysis showed that the content of Drp1 in the prefrontal cortex depended on genotype ($F_{1,40} = 51.5$; $p < 0.0001$): at 7 and 20 days of age, its level was higher in OXYS rats ($p < 0.001$ and $p < 0.005$, respectively), which may indicate enhancement of mitochondrial fission processes. This is also supported by the lower Mfn1/Drp1 ($F_{1,40} = 21.9$; $p < 0.0001$) and Mfn2/Drp1 ($F_{1,4040} = 91.2$; $p < 0.0001$) indices in OXYS rats compared to Wistar rats, reflecting the state of the balance between fusion and fission processes.

In the hippocampus (Fig. 4b), the content of Mfn1 and Mfn2 also changed unidirectionally with age in both rat strains ($F_{3,4040} = 11.1$; $p < 0.001$ and $F_{3,40} = 5.0$; $p < 0.005$, respectively), and their levels at birth were lower in OXYS rats ($p < 0.01$ and $p < 0.034$, respectively). The content of Drp1 in the hippocampus of rats depended on age ($F_{3,440} = 15.5$; $p < 0.001$): in Wistar rats, its level increased by two weeks of age ($p < 0.001$), while in the OXYS rats, it increased by the end of the first week of life ($p < 0.02$) and decreased by 20 days of age ($p < 0.015$). As a result, the content of Drp1 in OXYS rats was higher at 7 days of age and lower at 20 days of age compared to Wistar rats ($p < 0.002$ and $p < 0.015$, respectively).

Assessment of the balance between fusion/fission processes (Mfn1/Drp1 and Mfn2/Drp1 indices) showed its change during the early postnatal period in both rat strains ($F_{3,4040} = 5.3$; $p < 0.003$ and $F_{3,40} = 5.1$; $p < 0.005$, respectively). In OXYS rats, the Mfn1/Drp1 ratio increased by 14 and 20 days of age ($p < 0.015$ and $p < 0.05$, respectively), and the Mfn2/Drp1 ratio increased by 20 days of age ($p < 0.017$). In Wistar rats, the Mfn2/Drp1 ratio decreased by two weeks of age ($p < 0.050$). Meanwhile, in OXYS rats, the decrease in Mfn1/Drp1 and Mfn2/Drp1 during the first week

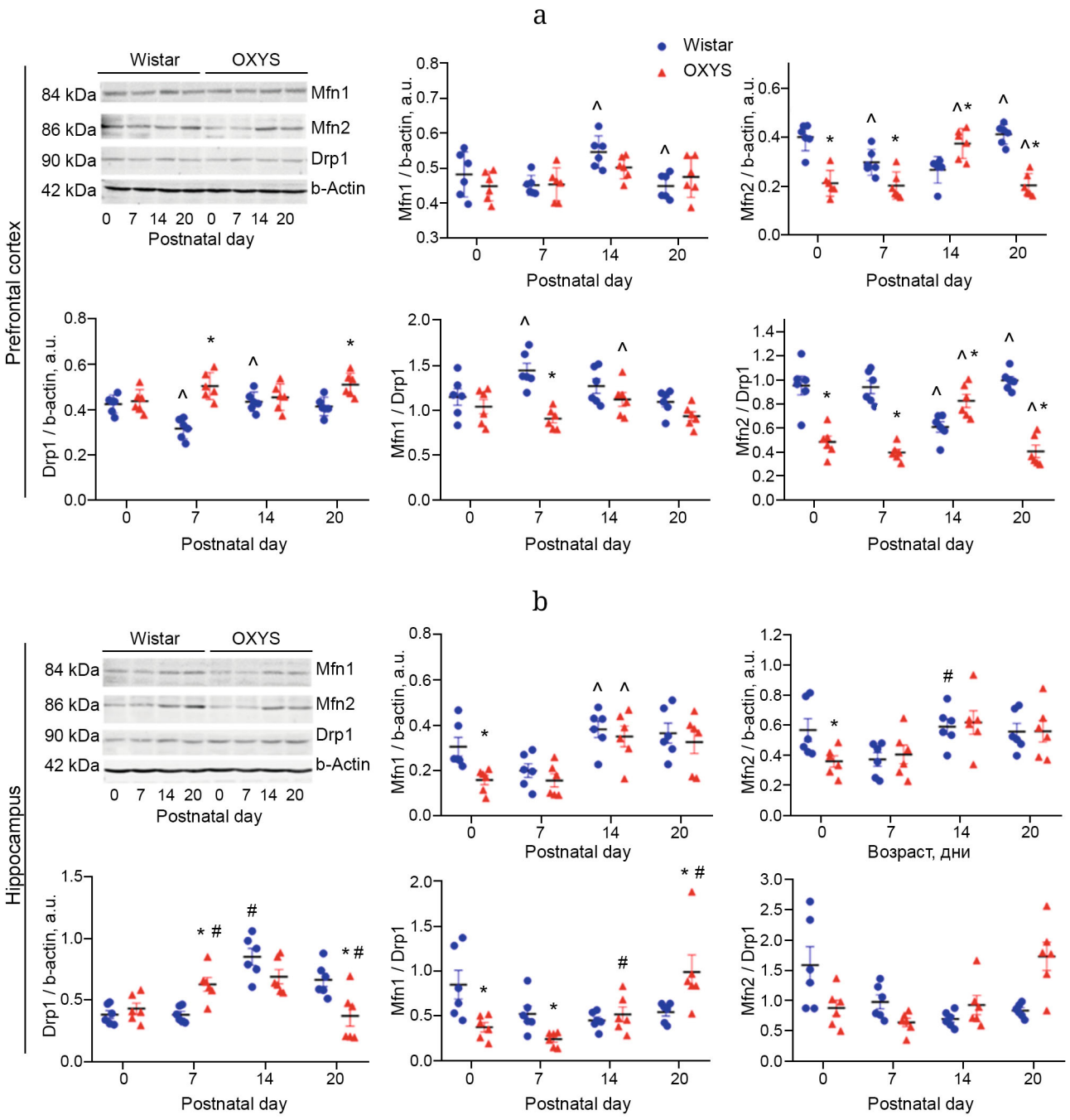

Fig. 4. Changes in the content of Mfn1, Mfn2, and Drp1 in the prefrontal cortex (a) and hippocampus (b) of Wistar and OXYS rats during the early postnatal period according to Western blot analysis. Representative blot images and relative amounts of Mfn1/2, Drp1, Mfn1/Drp1, and Mfn2/Drp1, normalized to beta-actin. Data are presented as M ± S.E.M. (n = 6). * Significant differences compared to the age-matched Wistar rats; # compared to the previous age. Differences were considered statistically significant at $p < 0.05$.

of life ($p < 0.05$) indicates enhancement of fission processes, while the increase in their levels at 20 days of age indicates mitochondrial fusion.

Mitochondrial trafficking in the early postnatal period. To assess the state of mitochondrial trafficking in neurons, Western blot analysis was used to determine the level of adaptor proteins of the mitochondrial transport system – GTPases Miro1 and Miro2, which ensure binding of mitochondria to the transport proteins – dyneins and kinesins [25] – in the prefrontal cortex and hippocampus of OXYS and Wistar rats in the early postnatal period. The content of transport proteins – kinesins TRAK1 and TRAK2 [26] – was also studied.

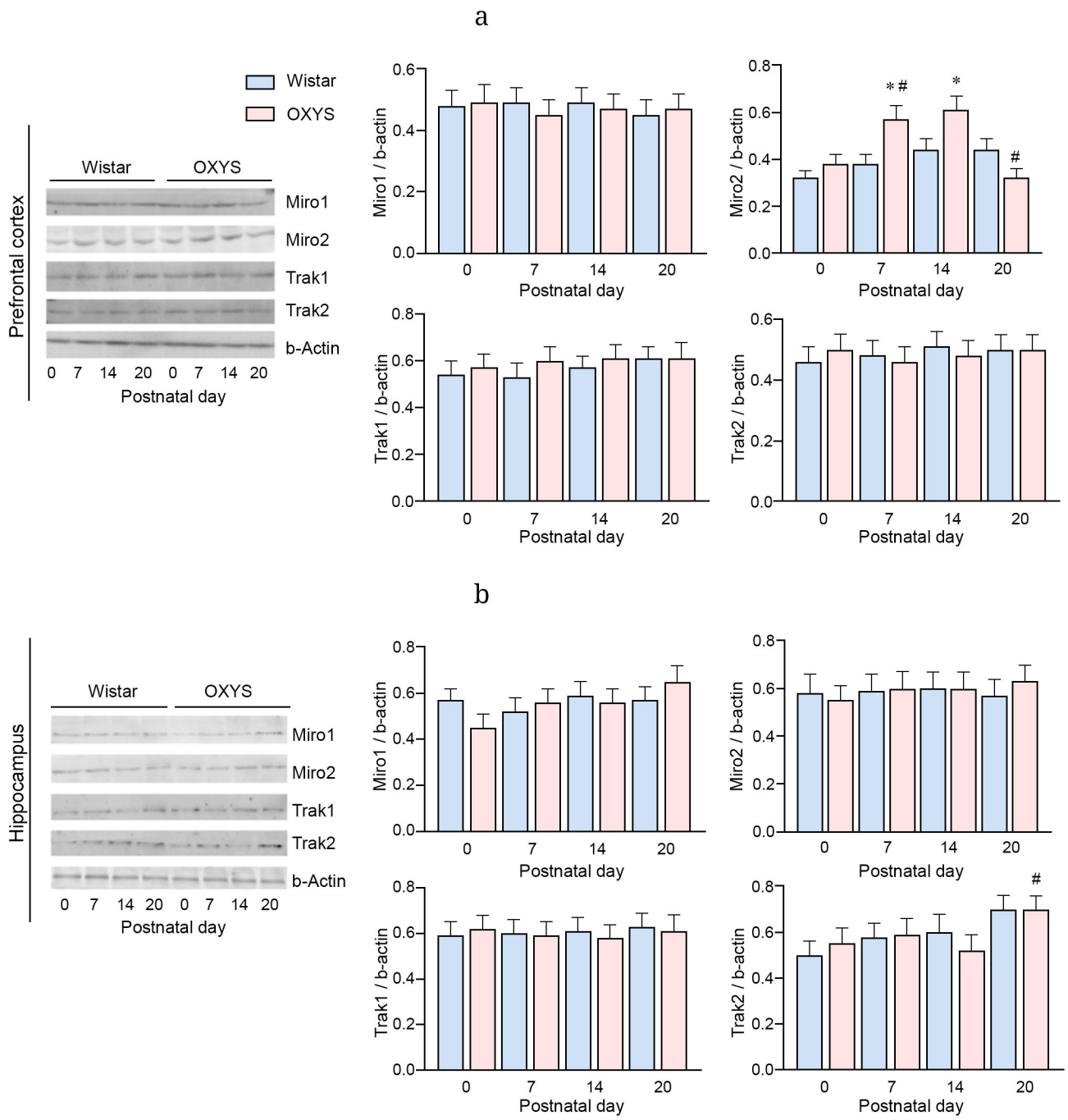

Fig. 5. Changes in the content of Miro1/2, Trak1/2 in the prefrontal cortex (a) and hippocampus (b) of Wistar and OXYS rats in the early postnatal period according to the Western blot analysis (n = 5). Representative blot images and relative amounts of Miro1/2, Trak1/2, normalized to beta-actin. Data are presented as M ± S.E.M. * Significant differences compared to the age-matched Wistar rats; # compared to the previous age. Differences were considered statistically significant at $p < 0.05$.

According to the results of ANOVA analysis, in the prefrontal cortex (Fig. 5a) of Wistar and OXYS rats, the content of Miro1, Trak1, and Trak2 did not depend on genotype and did not change with age ($p > 0.05$). The content of Miro2 was higher in OXYS rats at 7 and 14 days of age ($F_{1,40} = 4.57$; $p < 0.038$), and by 20 days of age, interline differences were leveled off against the background of its decrease in OXYS rats ($p < 0.01$).

In the hippocampus of Wistar and OXYS rats, the content of Miro1/2, Trak1/2 (Fig. 5b) did not depend on genotype of the animals and did not change with age ($p > 0.05$). It should be noted that from 14 to 20 days of age, the content of Trak2 in OXYS rats

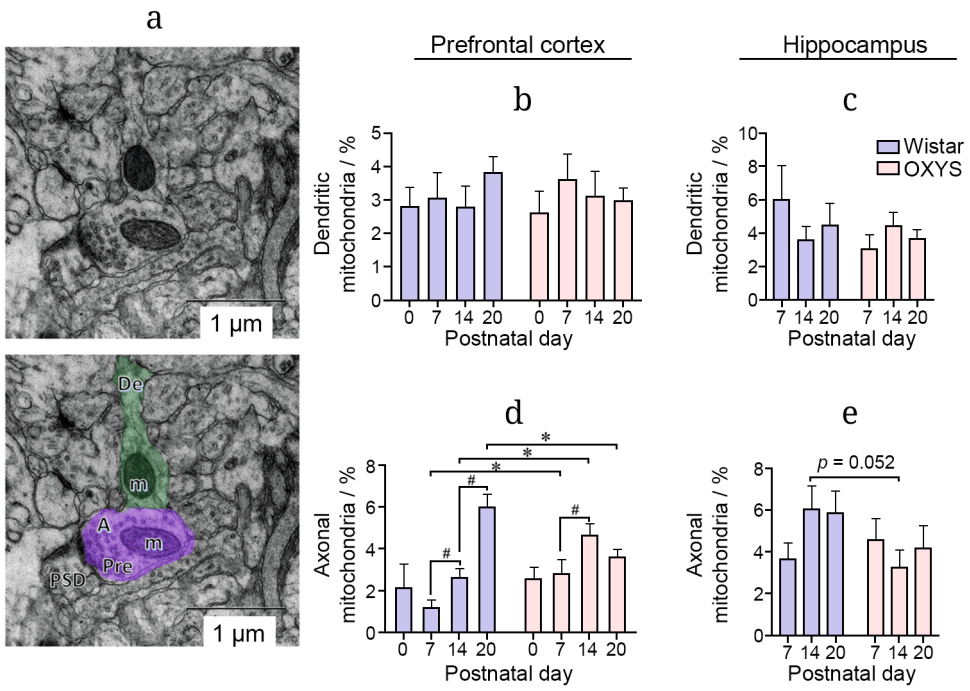

Fig. 6. Representative electron microscopy (EM) image of a neuropil. Axonal (A, pseudo-colored purple, lower panel) and dendritic (De, pseudo-colored green, lower panel) processes contain mitochondria (m). Pre- (Pre) and postsynaptic (PSD) compartments of the synapse (a). Specific content of mitochondria in dendritic (b, c) and axonal (d, e) processes of neurons in the prefrontal cortex and hippocampus. Data are presented as M ± S.E.M. * Significant differences compared to the age-matched Wistar rats; # compared to the previous age. Differences were considered statistically significant at $p < 0.05$.

increased ($p < 0.02$), but was not significantly higher than in Wistar rats.

Additionally, assessment of the specific content of mitochondria in the dendritic processes of neurons in the prefrontal cortex and hippocampus of Wistar and OXYS rats did not reveal interline differences or age-related changes (Fig. 6, a-c). Regarding the axonal mitochondria, in the hippocampus of OXYS rats at 14 days of age, their specific content was lower than in Wistar rats ($p = 0.052$; Fig. 6e). In the prefrontal cortex, this parameter was lower at 7 and 14 days of age ($p < 0.05$) and higher at 20 days of age ($p < 0.01$; Fig. 6d), which is indicative of the changes in neuronal mitochondrial trafficking.

DISCUSSION

It is assumed that anatomical (number and connectivity of neurons) and functional (ability to engage alternative neural networks) parameters of the adult brain formed in the early period of life could influence its vulnerability to the development of AD and the brain's ability to realize its compensatory reserves [27]. Successful differentiation and maturation of neurons, formation of neural networks, and completion of brain formation as a whole is optimal when mitochondrial biogenesis in the developing brain corresponds to the demand for energy resources [23]. In the early postnatal period, the rate of metabolism, energy consumption, and blood flow in the brain are significantly higher than in the adult organism (in mice – 3-7 times higher in the first 2-3 weeks of postnatal life, in humans it is elevated until the age of 5) [28]. It is obvious that the process of completing brain maturation in the early postnatal period could potentially be influenced by disruptions in any mitochondrial functions: calcium homeostasis, generation of reactive oxygen species, regulation of apoptosis, mitochondrial biogenesis, their dynamics, mitophagy, and trafficking – movement of mitochondria toward sites with increased energy consumption in response to growth factors. In this study, we compared the features of mitochondrial apparatus formation in the cortex and hippocampus of Wistar and the senescence-accelerated OXYS rats – a model of sporadic AD – from birth to 20 days of age, the period of brain formation completion.

Mitochondria play a key role in neurogenesis, regulating transition of the neural stem cells into neural progenitor cells and, ultimately, into neurons [29-31]. Neurogenesis in the rat hippocampus occurs in waves: it begins prenatally and continues into the postnatal period [32, 33]. The peak of neurogenic activity in rats occurs at 7 days of age [34], which corresponds to the development of the fetal brain in the third trimester

of gestation in humans – a period particularly critical for human nervous system development. We found that by the end of the first week of life, the number of mitochondria in the neuronal bodies of OXYS rats is lower in the hippocampus, while proportion of the organelles with pronounced structural alterations is higher compared to Wistar rats.

Simultaneously with neurogenesis and migration, the newly formed neurons begin to establish contacts with each other, which later develop into synapses. During migration of immature neurons to their functional sites, selective apoptotic selection occurs, resulting in the neuroblasts of a specific phenotype. This is followed by the second wave of selective selection associated with integration of the newly formed neurons into the neural network [35]. The axons and dendrites of the newly formed neurons continue to grow and mature during the first 2-3 weeks of postnatal development, reaching mature morphology by the end of the first month of life. Increased expression of the primary regulator of mitochondrial biogenesis, PGC1α, leads to the enhanced growth of neuronal processes and increase in the number of mitochondria within them (without reduction in their number in the neuronal body) [23]. We found that brain maturation in OXYS rats during the early postnatal period occurs against the background of reduced mitochondrial biogenesis, as indicated by the lower PGC1α levels in the prefrontal cortex and hippocampus compared to Wistar rats. Earlier, we identified the signs of impaired differentiation of neural stem cells, a delayed peak of neurogenesis in the dentate gyrus [12], a delay in the pre- and postnatal waves of apoptosis [11], and reduction in the density of neuronal processes and synapses [36] in the hippocampus and prefrontal cortex of OXYS rats during the early postnatal period. It must be emphasized that all these signs of delayed neuronal maturation in the brains of OXYS rats are manifested under conditions of insufficient astrocytic and microglial support [14].

Changes in the mitochondrial morphology are characteristic of both differentiated and pluripotent stages of the neuronal and glial cells in embryonic and adult neurogenesis [31, 37]. Retrograde signaling from mitochondria to the nucleus regulates transcription of the genes responsible for differentiation and determines changes in the mitochondrial dynamics during differentiation of the neural stem cells [38]. Drp1 regulates mitochondrial remodeling cycles, inducing their division to stimulate glycolytic metabolism, while Mfn1/2 plays a key role in mitochondrial fusion to promote oxidative phosphorylation [24, 31]. Analysis of our results indicates reduction in the activity of fusion processes (assessed by Mfn1/2 levels) and enhancement of mitochondrial fission (increased Drp1 levels) in the brains of OXYS rats during the first week of life. This is further supported by the Mfn1/Drp1 and Mfn2/Drp1 indices, which reflect the balance between fusion and fission processes.

By the end of the second week of life, the number of mitochondria in the neuronal bodies and neuropil of the hippocampus of OXYS rats becomes higher than in Wistar rats. By this age (14 days), the number of mitochondria in the neuronal bodies and neuropil of the prefrontal cortex increases in both rat strains, and proportion of the organelles with intact ultrastructure reaches its maximum. However, OXYS rats exhibit reduced mitochondrial dynamics activity: proportion of the organelles undergoing fusion/fission in the neuronal bodies is significantly lower than in Wistar rats.

During the early postnatal maturation of the brain, mitochondrial trafficking is essential not only for supplying ATP to the neuronal processes but also for their proper formation, and energy deficits during this period lead to the impaired neuronal plasticity [39-43]. We did not detect obvious disruptions in the mitochondrial trafficking in the brains of OXYS rats during the early postnatal period. However, signs of altered neuronal mitochondrial trafficking in the axonal processes were noted: in the hippocampus of OXYS rats, the number of organelles was slightly lower than in Wistar rats (p = 0.052) at 14 days of age, and in the prefrontal cortex, it was lower during the first two weeks but increased at 20 days of age.

No interline differences were found in the mitochondrial content of the neuronal bodies in the prefrontal cortex during completion of brain maturation (20 days). However, the reduced proportion of organelles undergoing fusion/fission in OXYS rats indicates decreased mitochondrial dynamics in these animals. As in the first week of life, fusion processes (Mfn2) are reduced in the cerebral cortex of OXYS rats, while fission (Drp1 and Mfn2/Drp1) is enhanced. In the cortical neuropil, the number of mitochondria was higher due to their content in the axonal processes. In the hippocampus of OXYS rats during this period, the number of mitochondria in the neuronal bodies and neuropil was higher than in Wistar rats, and according to the assessment of mitochondrial dynamics activity, the fusion/fission balance was shifted toward mitochondrial fusion. As a sign of disrupted mitochondrial dynamics, we consider the previously identified [44] increase in the number of mitochondria with an unusual phenotype – “mitochondria-on-a-string” (MOAS) – in the processes of cortical neurons in OXYS rats. Such mitochondria have been found in the brain neurons of the AD patients and AD mouse models and are believed to be associated with incomplete fission [45]. Importantly, the specific quantity of MOAS in the neuropil of OXYS rats is most significantly increased – 8 times – at 20 days

of age, during completion of the brain maturation, when effective axonal mitochondrial trafficking is a necessary condition for formation of the neuronal networks.

It is only logically to assume that the identified structural and functional changes in the mitochondria of OXYS rats are underpinned by the changes in the expression of associated genes. Earlier, we analyzed changes in the transcriptomes of the cerebral cortex and hippocampus of Wistar and OXYS rats (RNA-seq data) from the early postnatal period to the age of AD progression (P3, P10, P20 (P – postnatal day), 5 and 18 months), identifying metabolic pathways and processes whose alterations precede and accompany the disease development in OXYS rats. Notably, the most significant and comparable differences in the gene expression and associated processes are observed during the early postnatal period and at the stage of pronounced neurodegenerative changes, when they are comparable between OXYS rats and the AD patients [16, 18]. Expression of the mitochondria-associated genes is already altered in OXYS rats of 3 and 10 days of age [18] and at 20 days, during the "preclinical" period of AD development, and remains altered during manifestation and progression of the disease [19].

CONCLUSION

We previously showed that manifestation and progression of all key signs of AD in OXYS rats – destructive changes and neuronal death, synaptic insufficiency, hyperphosphorylation of tau protein, enhanced accumulation of Aβ1-42 (Aβ – amyloid beta), and formation of amyloid plaques in the brain, as well as memory impairment and learning ability – occur against the background of increasing dysfunction and significant decrease in the specific number of mitochondria in the neurons of the hippocampus and cerebral cortex [15, 16]. Overall, the results obtained in this and previous studies showed that as early as the early postnatal period (from birth to 20 days of age), OXYS rats exhibit structural and functional changes in the mitochondria in neurons of the hippocampus and cerebral cortex, which subsequently increase during manifestation of AD signs (3-5 months of age) and their progression (24 months of age). The causes leading to the accelerated brain aging in OXYS rats and the development of AD signs in them remain unclear, but we believe that deviations in formation of the mitochondrial apparatus identified in this study in the early postnatal period could contribute to the delayed brain maturation in OXYS rats, promote mitochondrial dysfunction, reduce synaptic density, and ultimately lead to the neuronal death and development of the early neurodegenerative changes. Thus, we have shown that mitochondrial dysfunction mediates and/or possibly even initiates the pathological molecular cascades of AD development in OXYS rats and could be considered a predictor of the early development of the sporadic form of this disease in humans.

Abbreviations

AD Alzheimer's disease

Contributions

N. A. Stefanova and N. G. Kolosova – concept and supervision of the work; N. A. Muraleva, D. V. Sityaeva, M. A. Tyumentsev, and N. A. Stefanova – conducting experiments; N. A. Stefanova and N. G. Kolosova – discussion of the research results; N. A. Stefanova and N. G. Kolosova – writing the text; N. G. Kolosova – editing the article text.

Funding

This work was supported by the federal budget project FWNR-2022-0016.

Ethics approval and consent to participate

The study was conducted on OXYS and Wistar rats (control strain) at the Shared Research Facility "Vivarium of Conventional Animals" of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences. Maintenance of animals (including appropriate facilities, qualified personnel, and necessary documentation) and all experiments with animals were carried out in accordance with the position on the ethics of using animals in research supported by the Russian Science Foundation, as well as in accordance with Directive 2010/63/EU of the European Parliament and the Council of the European Union of September 22, 2010, and approved by the Bioethics Commission of ICG SB RAS (no. 85/1 dated 18.06.2021).

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Prince, M., Ali, G., Guerchet, M., Prina, A., Albanese, E., and Wu, Y. T. (2016) Recent global trends in the prevalence and incidence of dementia, and survival with dementia, *Alzheimer's Res. Ther.*, **8**, 23, https://doi.org/10.1186/s13195-016-0188-8.
2. Fu, W., and Ip, N. (2023) The role of genetic risk factors of Alzheimer's disease in synaptic dysfunction, *Semin. Cell Dev. Biol.*, **139**, 3-12, https://doi.org/10.1016/j.semcdb.2022.07.011.
3. Kovacs, G., Adle-Biassette, H., Milenkovic, I., Cipriani, S., van Scheppingen, J., and Aronica, E. (2014) Linking pathways in the developing and aging brain with neuro-degeneration, *Neuroscience*, **269**, 152-172, https://doi.org/10.1016/j.neuroscience.2014.03.045.
4. Heinonen, K., Eriksson, J., Lahti, J., Kajantie, E., Pesonen, A. K., Tuovinen, S., Osmond, C., and Raikkonen, K. (2015) Late preterm birth and neurocognitive performance in late adulthood: a birth cohort study, *Pediatrics*, **135**, e818-e825, https://doi.org/10.1542/peds.2014-3556.
5. Barchitta, M., Maugeri, A., La Rosa, M., Magnano San Lio, R., Favara, G., Panella, M., Cianci, A., and Agodi, A. (2018) Single nucleotide polymorphisms in vitamin D receptor gene affect birth weight and the risk of preterm birth: results from the "Mamma & Bambino" cohort and a meta-analysis, *Nutrients*, **10**, 1172, https://doi.org/10.3390/nu10091172.
6. Donley, G., Lönnroos, E., Tuomainen, T., and Kauhanen, J. (2018) Association of childhood stress with late-life dementia and Alzheimer's disease: the KIHD study, *Eur. J. Public. Health*, **28**, 1069-1073, https://doi.org/10.1093/eurpub/cky134.
7. Lemche, E. (2018) Early life stress and epigenetics in late-onset Alzheimer's dementia: a systematic review, *Curr. Genomics.*, **19**, 522-602, https://doi.org/10.2174/1389202919666171229145156.
8. Lesuis, S., Hoeijmakers, L., Korosi, A., de Rooij, S., Swaab, D., Kessels, H., Lucassen, P., and Krugers, H. (2018) Vulnerability and resilience to Alzheimer's disease: early life conditions modulate neuropathology and determine cognitive reserve, *Alzheimer's Res. Ther.*, **10**, 95, https://doi.org/10.1186/s13195-018-0422-7.
9. Gauvrit, T., Benderradji, H., Buée, L., Blum, D., and Vieau, D. (2022) Early-life environment influence on late-onset Alzheimer's disease, *Front. Cell Dev. Biol.*, **10**, 834661, https://doi.org/10.3389/fcell.2022.834661.
10. Leitzel, O., Francis-Oliveira, J., Khedr, S., Ariste, L., Robel, S., Kano, S. I., Arrant, A., and Niwa, M. (2024) Adolescent stress accelerates postpartum novelty recognition impairment in 5xFAD mice, *Front. Neurosci.*, **18**, 1366199, https://doi.org/10.3389/fnins.2024.1366199.
11. Rudnitskaya, E., Kozlova, T., Burnyasheva, A., Kolosova, N. G., and Stefanova, N. A. (2019) Alterations of hippocampal neurogenesis during development of Alzheimer's disease-like pathology in OXYS rats, *Exp. Gerontol.*, **115**, 32-45, https://doi.org/10.1016/j.exger.2018.11.008.
12. Rudnitskaya, E., Kozlova, T., Burnyasheva, A., Tarasova, A., Pankova, T., Starostina, M., Stefanova, N., and Kolosova, N. (2020) Features of postnatal hippocampal development in a rat model of sporadic Alzheimer's disease, *Front. Neurosci.*, **14**, 533, https://doi.org/10.3389/fnins.2020.00533.
13. Kozlova, T., Rudnitskaya, E., Burnyasheva, A., Stefanova, N., Peunov, D., and Kolosova, N. (2022) Delayed formation of neonatal reflexes and of locomotor skills is associated with poor maternal behavior in OXYS rats prone to Alzheimer's disease-like pathology, *Biomedicines*, **10**, 2910, https://doi.org/10.3390/biomedicines10112910.
14. Rudnitskaya, E., Kozlova, T., Burnyasheva, A., Stefanova, N., and Kolosova, N. (2021) Glia not neurons: uncovering brain dysmaturation in a rat model of Alzheimer's disease, *Biomedicines*, **9**, 823, https://doi.org/10.3390/biomedicines9070823.
15. Tyumentsev, M., Stefanova, N., Muraleva, N., Rumyantseva, Y., Kiseleva, E., Vavilin, V., and Kolosova, N. (2018) Mitochondrial dysfunction as a predictor and driver of Alzheimer's disease-like pathology in OXYS rats, *J. Alzheimer's Dis.*, **63**, 1075-1088, https://doi.org/10.3233/JAD-180065.
16. Stefanova, N., Muraleva, N., Korbolina, E., Kiseleva, E., Maksimova, K., and Kolosova, N. G. (2015) Amyloid accumulation is a late event in sporadic Alzheimer's disease-like pathology in nontransgenic rats, *Oncotarget*, **6**, 1396-1413, https://doi.org/10.18632/oncotarget.2751.
17. Stefanova, N., Ershov, N., Maksimova, K., Muraleva, N., Tyumentsev, M., and Kolosova, N. (2019) The rat prefrontal-cortex transcriptome: effects of aging and Sporadic Alzheimer's disease-like pathology, *J. Gerontol. A. Biol. Sci. Med. Sci.*, **74**, 33-43, https://doi.org/10.1093/gerona/gly198.
18. Stefanova, N., and Kolosova, N. (2023) The rat brain transcriptome: from infancy to aging and sporadic Alzheimer's disease-like pathology, *Int. J. Mol. Sci.*, **24**, 1462, https://doi.org/10.3390/ijms24021462.
19. Swerdlow, R. H., and Khan, S. M. (2004) A "mitochondrial cascade hypothesis" for sporadic Alzheimer's disease, *Med. Hypotheses*, **63**, 8-20, https://doi.org/10.1016/j.mehy.2003.12.045.

20. Swerdlow, R. H. (2023) The Alzheimer's disease mitochondrial cascade hypothesis: a current overview, *J. Alzheimer's Dis.*, **92**, 751-768, https://doi.org/10.3233/JAD-221286.
21. Paxinos, G., and Watson, C. (2007) *The Rat Brain in Stereotaxic Coordinates*, 6th Edn., Academic Press, Cambridge, MA, USA.
22. Gu, Y. Y., Zhao, X. R., Zhang, N., Yang, Y., Yi, Y., Shao, Q. H., Liu, M. X., and Zhang, X. L. (2024) Mitochondrial dysfunction as a therapeutic strategy for neurodegenerative diseases: current insights and future directions, *Ageing Res. Rev.*, **102**, 102577, https://doi.org/10.1016/j.arr.2024.102577.
23. Vaarmann, A., Mandel, M., Zeb, A., Wareski, P., Liiv, J., Kuum, M., Antsov, E., Liiv, M., Cagalinec, M., Choubey, V., and Kaasik, A. (2016) Mitochondrial biogenesis is required for axonal growth, *Development*, **143**, 1981-1992, https://doi.org/10.1242/dev.128926.
24. Tilokani, L., Nagashima, S., Paupe, V., and Prudent, J. (2018) Mitochondrial dynamics: overview of molecular mechanisms, *Essays Biochem.*, **62**, 341-360, https://doi.org/10.1042/EBC20170104.
25. Eberhardt, E. L., Ludlam, A. V., Tan, Z., and Cianfrocco, M. A. (2020) Miro: a molecular switch at the center of mitochondrial regulation, *Protein Sci.*, **29**, 1269-1284, https://doi.org/10.1002/pro.3839.
26. Henrichs, V., Grycova, L., Barinka, C., Nahacka, Z., Neuzil, J., Diez, S., Rohlena, J., Braun, M., and Lansky, Z. (2020) Mitochondria-adaptor TRAK1 promotes kinesin-1 driven transport in crowded environments, *Nat. Commun.*, **11**, 3123, https://doi.org/10.1038/s41467-020-16972-5.
27. Seifan, A., Schelke, M., Obeng-Aduasare, Y., and Isaacson, R. (2015) Early life epidemiology of Alzheimer's disease – a critical review, *Neuroepidemiology*, **45**, 237-254, https://doi.org/10.1159/000439568.
28. Hagberg, H., Mallard, C., Rousset, C. I., and Thornton, C. (2014) Mitochondria: hub of injury responses in the developing brain, *Lancet Neurol.*, **13**, 217-232, https://doi.org/10.1016/S1474-4422(13)70261-8.
29. Arrázola, M. S., Andraini, T., Szelechowski, M., Mouledous, L., Arnauné-Pelloquin, L., Davezac, N., Belenguer, P., Rampon, C., and Miquel, M. C. (2019) Mitochondria in developmental and adult neurogenesis, *Neurotox. Res.*, **36**, 257-267, https://doi.org/10.1007/s12640-018-9942-y.
30. Brunetti, D., Dykstra, W., Le, S., Zink, A., and Prigione, A. (2021) Mitochondria in neurogenesis: implications for mitochondrial diseases, *Stem Cells*, **39**, 1289-1297, https://doi.org/10.1002/stem.3425.
31. Garone, C., De Giorgio, F., and Carli, S. (2024) Mitochondrial metabolism in neural stem cells and implications for neurodevelopmental and neurodegenerative diseases, *J. Transl. Med.*, **22**, 238, https://doi.org/10.1186/s12967-024-05041-w.
32. Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., and Noble-Haeusslein, L. J. (2013) Brain development in rodents and humans: identifying benchmarks of maturation and vulnerability to injury across species, *Prog. Neurobiol.*, **106-107**, 1-16, https://doi.org/10.1016/j.pneurobio.2013.04.001.
33. Huszár, R., Zhang, Y., Blockus, H., and Buzsáki, G. (2022) Preconfigured dynamics in the hippocampus are guided by embryonic birthdate and rate of neurogenesis, *Nat. Neurosci.*, **25**, 1201-1212, https://doi.org/10.1038/s41593-022-01138-x.
34. Erecinska, M., Cherian, S., and Silver, I. A. (2004) Energy metabolism in mammalian brain during development, *Prog. Neurobiol.*, **73**, 397-445, https://doi.org/10.1016/j.pneurobio.2004.06.003.
35. Encinas, J. M., Michurina, T. V., Peunova, N., Park, J. H., Tordo, J., Peterson, D. A., Fishell, G., Koulakov, A., and Enikolopov, G. (2011) Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus, *Cell Stem Cell*, **8**, 566-579, https://doi.org/10.1016/j.stem.2011.03.010.
36. Stefanova, N. A., Maksimova, K. Y., Tyumentsev, M. A., Telegina, D. V., Rudnitskaya, E. A., and Kolosova, N. G. (2025) The early postnatal synapse assembly and expression profiles of synapse-related genes in a sporadic Alzheimer's disease-like pathology, *J. Alzheimer's Disease*, https://doi.org/10.1177/13872877251396932.
37. Cicali, K. A., and Tapia-Rojas, C. (2024) Synaptic mitochondria: a crucial factor in the aged hippocampus, *Ageing Res. Rev.*, **101**, 102524, https://doi.org/10.1016/j.arr.2024.102524.
38. Khacho, M., Harris, R., and Slack, R. S. (2019) Mitochondria as central regulators of neural stem cell fate and cognitive function, *Nat. Rev. Neurosci.*, **20**, 34-48, https://doi.org/10.1038/s41583-018-0091-3.
39. Li, Z., Okamoto, K.-I., Hayashi, Y., and Sheng, M. (2004) The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses, *Cell*, **119**, 873-887, https://doi.org/10.1016/j.cell.2004.11.003.
40. Quiroz, J. A., Gray, N. A., Kato, T., and Manji, H. K. (2008) Mitochondrially mediated plasticity in the pathophysiology and treatment of bipolar disorder, *Neuropsychopharmacology*, **33**, 2551-2565, https://doi.org/10.1038/sj.npp.1301671.
41. Ruthel, G., and Hollenbeck, P. J. (2003) Response of mitochondrial traffic to axon determination and differential branch growth, *J. Neurosci.*, **23**, 8618-8624, https://doi.org/10.1523/JNEUROSCI.23-24-08618.2003.
42. Spillane, M., Ketschek, A., Merianda, T. T., Twiss, J. L., and Gallo, G. (2013) Mitochondria coordinate sites of axon branching through localized intra-axonal protein synthesis, *Cell Rep.*, **5**, 1564-1575, https://doi.org/10.1016/j.celrep.2013.11.022.
43. Sheng, Z. H. (2017) The interplay of axonal energy homeostasis and mitochondrial trafficking and

anchoring, *Trends Cell Biol.*, **27**, 403-416, https://doi.org/10.1016/j.tcb.2017.01.005.
44. Tyumentsev, M. A., Stefanova, N. A., Kiseleva, E. V., and Kolosova, N. G. (2018) Mitochondria with morphology characteristic for Alzheimer's disease patients are found in the brain of OXYS rats, *Biochemistry (Moscow)*, **83**, 1083-1088, https://doi.org/10.1134/S0006297918090109.
45. Zhang, L., Trushin, S., Christensen, T., Bachmeier, B., Gateno, B., Schroeder, A., Yao, J., Itoh, K., Sesaki, H., Poon, W., Gylys, K., Patterson, E., Parisi, J., Diaz Brinton, R., Salisbury, J., and Trushina, E. (2016) Altered brain energetics induces mitochondrial fission arrest in Alzheimer's disease, *Sci. Rep.*, **6**, 18725, https://doi.org/10.1038/srep18725.