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Morphological Characteristics of the Hippocampus in OXYS and Wistar Rats during the Aging Process

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Light and electron microscopy studies were performed to evaluate structural changes in the hippocampus in prematurely aging rats of the OXYS strain ($n = 20$) and Wistar rats ($n = 20$) as they aged. Light microscopy showed neurons with signs of chromatolysis in fields CA1 and CA3 and the dentate gyrus of the hippocampus, along with hyperchromic neurons, providing evidence that OXYS rats had signs of degeneration as early as age four months. By 18 months of age, structural changes developed in all areas of the hippocampus in OXYS rats. Ultramicroscopy studies at age four months identified that these animals showed signs of destruction of mitochondria and accumulation of lipofuscin granules; these changes progressed with age, such that animals aged 18 months were characterized by significant organelle destruction. The data obtained here provide evidence of more extensive age-related changes to neurons in OXYS rats than in Wistar rats.

Keywords: hippocampus, aging, OXYS rats, ultrastructure.

Increases in longevity and population aging have led to an increase in the proportion of people with structural-functional changes in the brain accompanied by psychological and behavioral disorders. The cerebral cortex and limbic system are the most affected brain areas. Degeneration has significant effects on hippocampal field CA1 and CA3 and the dentate gyrus [9].

A model for studying age-related neurodegenerative damage is provided by OXYS rats, created in the 1970s at the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, produced by selection and inbreeding of Wistar rats sensitive to the cataractogenic action of galactose. The decreased sensitivity of OXYS rats to oxidative stress leads to the development of cataracts, age-related chorioretinal degeneration, early involution of the thymus [4], and osteoporosis. Acceleration of brain aging in OXYS rats is apparent as the development of behavioral and cognitive disorders by as early as age three months [11]. With age, the brain in OXYS rats shows faster accumulation of oxidative proteins than seen in Wistar rats [2]. Studies of the brain vessels and measures of brain blood flow using MRI scans in 12-month-old OXYS rats identified structural and functional changes, including decreased vascular reactivity [5]. The content of altered neurons provides an objective measure of brain aging in OXYS rats. Thus, the aim of the present work was to study structural changes in pyramidal neurons in the hippocampus during aging in OXYS and Wistar rats.

Materials and Methods

Experiments were performed on 20 prematurely aging OXYS rats and 20 Wistar rats at the Genofondy Laboratory Animals Collaborative Resource Center, Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences (Novosibirsk) in compliance with the "Regulations for Studies Using Experimental Animals." All animals were divided into four groups. Groups 1 and 2 included OXYS rats aged four and 18 months; groups 3 and 4 consisted of Wistar rats of the same ages.

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Animals were anesthetized by CO_2 inhalation and underwent transcardiac perfusion with 10% formalin in phosphate buffer pH 7.4. Brains were extracted and further fixed in the same solution for one day. After fixation, specimens were dehydrated in ascending ethanol concentrations, embedded in paraffin by a standard method, and sagittal brain sections of thickness 5 μm were cut. Chromatophilic substance was detected in hippocampal neuron perikarya by staining sections with 0.1% cresyl violet by the Nissl method. Neurons with focal and total chromatolysis, hyperchromic shrunken neurons, and hyperchromic neurons without shrinkage were counted in 10 microscope fields (objective ×100, ocular ×10) in hippocampal field CA1 and CA3 and the dentate gyrus in sections from each animal.

For electron microscopy, brains were fixed by transcardiac perfusion with solution containing a mixture of paraformaldehyde (4%) and glutaraldehyde (0.5%) in 0.2 M cacodylate buffer pH 7.4. Specimens were additionally fixed in 2% osmium tetroxide solution for 3 h in the cold, dehydrated in ascending ethanol concentrations, acetone, and propylene oxide, and embedded in Epon 812. Semithin and ultrathin sections were cut on a Leica EM UC7 ultratome (Leicamicrosystems, Austria). Semithin sections were stained with toluidine blue. Ultrathin silver and pale gold sections were mounted on grid supports, contrasted with uranyl acetate and lead citrate, and examined and photographed under a JEM-7A electron microscope (Jeol, Japan). An ocular grid was used at a magnification of ×10000 to measure the specific areas of organelles in pyramidal neurons (% of total area of cytoplasm).

Results were analyzed statistically using Statistica 6.0 for Windows. Data were analyzed by descriptive statistical methods with calculation of the median (Me) and interquartile interval (Q_1–Q_3). Differences were evaluated using the nonparametric Mann–Whitney test. Differences between values in study groups were regarded as significant at $p < 0.05$.

Results

Light microscopy studies of the hippocampus of four-month-old animals showed that field CA1 in OXYS rats had significantly larger proportions of neurons with signs of total chromatolysis and hyperchromic neurons without shrinkage than Wistar rats, while field CA3 and the dental gyrus contained a greater proportion of hyperchromic neurons without shrinkage. At age 18 months, these changes were greater in animals of both strains, but more so in OXYS rats. Thus, the proportions of neurons with signs of total (Fig. 1, *a*) and focal (Fig. 1, *b*) chromatolysis and hyperchromic shrunken neurons in field CA1 and the proportions of hyperchromic neurons without shrinkage (Fig. 1, *c*) and neurons with signs of total chromatolysis in field CA3 were significantly greater in OXYS rats than in Wistar rats. Analysis of neurons in the dentate gyrus revealed a significant increase in hyperchromic shrunken and hyperchromic neurons without shrinkage in OXYS rats over the levels seen in Wistar rats (Table 1).

Electron microscope studies of pyramidal neurons in hippocampal field CA1 in OXYS rats aged four months demonstrated swelling of mitochondria with lightening of the matrix and partial fragmentation of cristae (see Fig. 1, *d*). Rough endoplasmic reticulum cisterns lost their typical straight, parallel orientation and were curved and fragmented; some cisterns were severely dilated. In some neurons, the cytoplasm was almost devoid of these organelles, as well as free individual ribosomes and polysomes, creating the typical picture of total chromatolysis. Dilated perinuclear spaces and increases in the number of nuclear pores were encountered. The cytoplasm contained lipofuscin granules (see Fig. 1, *e*).

Prematurely aging OXYS rats aged 18 months had greater destruction of and damage to neurons than seen in the previous age group (see Fig. 1, *f*). Mitochondrial size was generally smaller than in Wistar rats, and cristae showed signs of destruction; the matrix was electron-dense and homogenized, and the outer and inner membranes had uneven outlines. The Golgi complex showed dilation and fragmentation of cisterns. Lysosomes were of different sizes and were intensely and homogeneously stained with osmium tetroxide. The cytoplasm had vacuoles of different sizes and configurations. Some neurons showed nucleolar hypertrophy, with increases in their granular component, and increases in the number of nuclear pores. Morphometric analysis of pyramidal neurons at age four months demonstrated that OXYS rats had lower specific densities of mitochondria and rough endoplasmic reticulum than Wistar rats, as well as significantly greater specific areas of vacuoles and lysosomes, including lipid inclusions. The specific area of Golgi complexes was similar in the two strains studied. At age 18 months, OXYS rats showed increases in the specific areas of lysosomes and vacuoles in the perikarya of neurons as compared with the previous age and with Wistar rats, though there were decreases in the specific areas of mitochondria and rough endoplasmic reticulum. The specific areas of Golgi complexes were not significantly different between the two groups (Table 2).

Discussion

The hippocampus, which is involved in the mechanisms of learning and memory consolidation, is one of the first brain structures impaired by the aging process. Our study of neurons in the pyramidal layer of the hippocampus in rats reflects this age-dependent relationship between the increase in the number of altered neurons in aged animals. Age-related changes in the brain were found to be morphologically due to an increase in the proportion of altered neurons, their death, and their replacement by glial elements [7].

Comparison of Wistar and OXYS rats revealed significant differences in the severity of destructive changes to the structural components of the hippocampus developing with age, providing evidence of accelerated aging in OXYS rats.

Thus, during the period of accelerated brain aging in OXYS rats (at age four months), altered cells are dominated by hyperchromic neurons without shrinkage, which is inter-

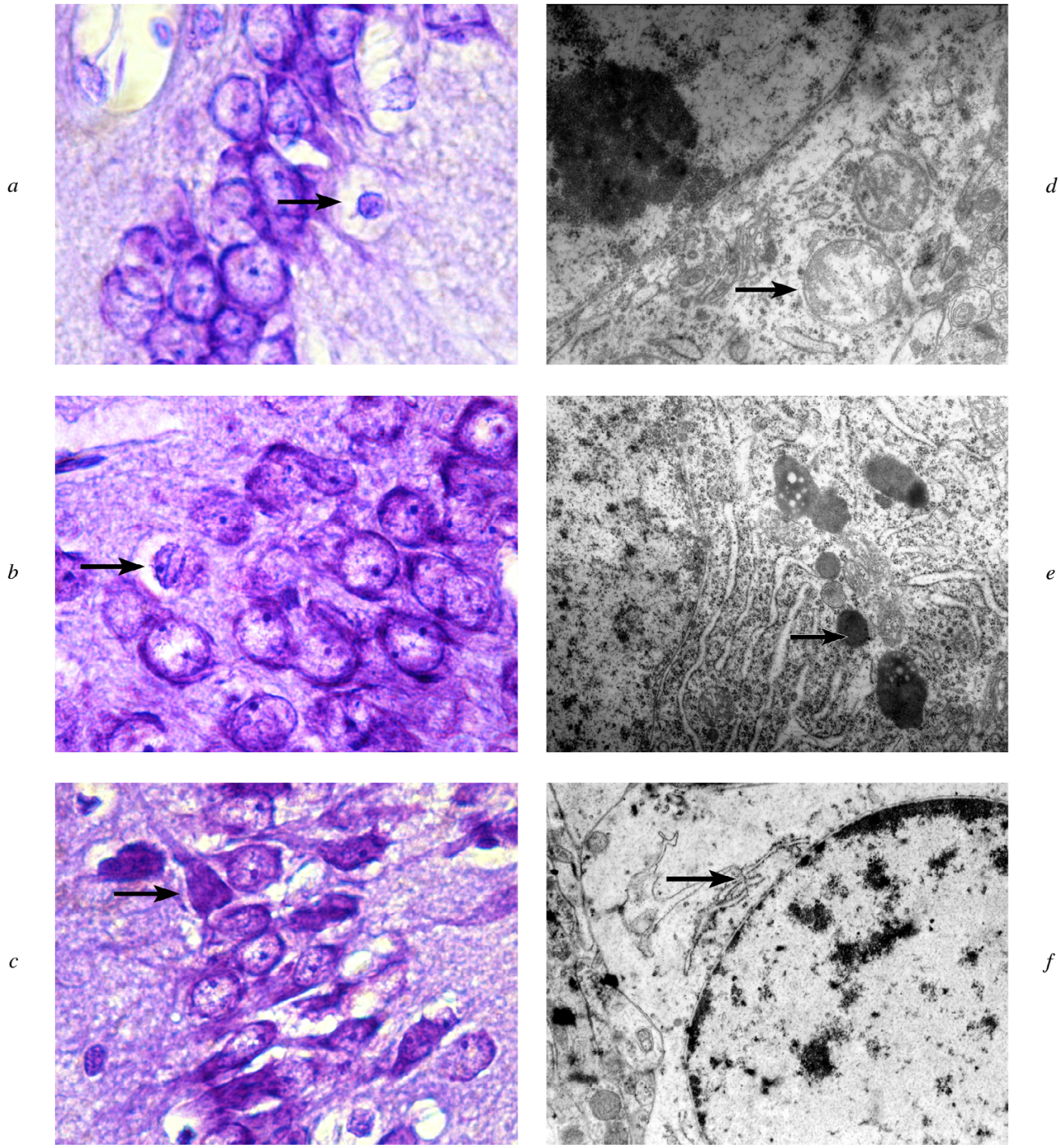

Fig. 1. Pyramidal neurons in the hippocampus of OXYS rats aged four months (*d*, *e*) and 18 months (*a–c*, *f*). *a*) A neuron with total chromatolysis (arrow) in field CA1; *b*) a neuron with focal chromatolysis (arrow) in field CA1; *c*) a hyperchromic pyknomorphic neuron (arrow) in field CA3; *d*) mitochondria with partial fragmentation of the cristae and swelling (arrow), dilation of endoplasmic reticulum cisterns in a neuron in field CA1; *e*) lipofuscin granules (arrow), dilation of rough endoplasmic reticulum cisterns in a neuron in field CA1; *f*) decreased endoplasmic reticulum and ribosome contents in a neuron in field CA1. *a–c*) Stained with cresyl violet, Nissl method; *d–f*) electron photomicrographs. *a–c*) Objective ×100, ocular ×10; *d–f*) magnification ×10000.

preted functionally as the neuron being in a state of reactive inhibition. At this age, the most susceptible area of the hippocampus was field CA1, as evidenced by an increase in the number of neurons with focal chromatolysis in this structure, along with hyperchromia. This phenomenon probably results from prolonged stimulation of and functional tension on neurons, with subsequent exhaustion and possible conversion to ghost cells [3]. The differences in the extents of

TABLE 1. Relative Contents of Pyramidal Neurons with Different Morphological Changes in the Hippocampus of OXYS and Wistar Rats of Different Ages [Me (Q_1–Q_3), %]

Hippocampal area	Neurons studied	Age of Wistar rats, months		Age of OXYS rats, months	
		4	18	4	18
Field CA1	Unaltered	93.75 (93.65–95.46)	78.95* (78.36–79.95)	85.74* (85.58–88.84)	65.05*+ (64.98–65.72)
	With total chromatolysis	0.19 (0.17–0.26)	6.43* (5.41–6.78)	2.21*# (1.25–2.43)	11.92*#+ (11.39–12.55)
	With focal chromatolysis	3.68 (2.18–4.76)	5.45* (5.16–5.98)	4.69 (4.01–5.99)	11.61*#+ (10.38–12.35)
	Hyperchromic shrunken	0.26 (0.17–0.39)	3.47* (3.09–4.28)	0.41# (0.33–0.53)	3.74*+ (3.71–4.49)
	Hyperchromic without shrinkage	1.39 (1.07–2.63)	4.87* (4.33–5.01)	5.63* (5.24–8.36)	7.55*# (7.28–8.76)
Field CA3	Unaltered	93.81 (92.82–95.59)	79.71* (79.39–83.95)	90.57 (88.81–90.84)	70.53*+ (69.84–72.26)
	With total chromatolysis	0.66 (0.31–0.71)	5.91* (4.46–6.08)	0.81*# (0.77–0.81)	10.08*#+ (9.21–10.97)
	With focal chromatolysis	2.72 (1.85–2.59)	3.38* (2.77–5.06)	2.83 (2.77–3.08)	5.61*#+ (5.24–5.64)
	Hyperchromic shrunken	0.33 (0.31–0.35)	2.81* (2.77–3.09)	0.67# (0.38–0.81)	3.81*+ (3.17–4.07)
	Hyperchromic without shrinkage	2.12 (1.32–3.71)	6.79* (6.75–7.91)	4.74* (4.09–6.94)	9.66*#+ (9.24–11.42)
Dentate gyrus	Unaltered	96.94 (96.87–97.09)	82.84* (80.45–83.19)	96.34 (95.99–96.36)	72.08*+ (71.84–76.47)
	With total chromatolysis	0.26 (0.25–0.27)	4.79* (4.73–4.94)	0.25# (0.24–0.26)	5.37*+ (4.92–5.54)
	With focal chromatolysis	1.39 (1.3–1.42)	4.64* (4.53–6.01)	1.25# (1.03–1.44)	4.16*+ (4.01–6.78)
	Hyperchromic shrunken	0.27 (0.26–0.41)	2.15* (2.07–2.47)	0.68# (0.62–0.87)	0.64*#+ (4.48–5.84)
	Hyperchromic without shrinkage	0.97 (0.82–1.01)	4.84* (4.44–6.66)	1.37*# (1.25–1.76)	11.96*#+ (9.73–12.64)

Here and Table 2: *significant differences compared with values in Wistar rights aged 4 months, $p < 0.05$; #significant differences compared with values in Wistar rats aged 18 months, $p < 0.05$; +significant differences compared with values in OXYS rats aged 4 months, $p < 0.05$.

TABLE 2. Specific Areas of Organelles in the Cytoplasm of Pyramidal Neurons in Hippocampal Field CA1 in OXYS and Wistar Rats ($\bar{x} \pm s_{\bar{x}}$, %)

Strain	Age, months	Mitochondria	Lysosomes	Golgi complex	Rough endoplasmic reticulum	Vacuoles
Wistar	4	12.5 ± 1.5	8.4 ± 1.1	1.8 ± 0.7	38 ± 5	2.7 ± 0.9
	18	10.2 ± 0.8*	8.2 ± 1.5	1.0 ± 0.4	27 ± 4*	3.9 ± 2.9
OXYS	4	10.9 ± 1.8*	10.9 ± 1.5*	2.0 ± 0.7	27 ± 6*	4.8 ± 1.2*
	18	3.2 ± 0.7*#+	11.8 ± 2.7*#	1.2 ± 0.4	11.1 ± 2.7*#+	8 ± 3*#+

changes to hippocampal pyramidal neurons between strains may be evidence supporting differences in the sensitivity of rats of these strains to oxidative stress. Increased levels of oxidative damage to protein and lipids are seen in the brains of OXYS rats later than the appearance of the phenotypic signs of accelerated aging in their brains [2]. Hypoxia has the potential to initiate the early development of neurodegenerative processes in the brains of OXYS rats. As shown previously, development of the brain on the background of hypoxia results in delay to the formation of the microcirculatory bed. By the end of the first month of life, changes in energy metabolism typical of adaptation to hypoxia are seen, i.e., increased accumulation of phosphocreatine and its consumption for ATP synthesis [10]. Adaptive resources are depleted with age, and MRI studies in one-year-old OXYS rats demonstrated signs of chronic hypoxia typical of ischemia due to changes in cerebral blood flow [1]. Thus, chronic ischemia unavoidably facilitates progression of neurodegenerative changes.

Structural-functional changes in the mitochondria of OXYS rats, which increase with age, are regarded as one of the key factors in their premature aging. At age three months, the livers of these rats showed changes in the ratio of cytochromes in the inner mitochondrial membrane, with decreases in the activity of F_1F_0-ATP synthetase, respiratory control, and the rate of phosphorylation [6]. Structural changes in mitochondria consisting of destruction of cristae, lysis of the matrix, and decreases in the volume and surface density of mitochondria have previously been seen in retinal, hepatic, and muscle cells [5].

Electron microscopy studies of hippocampal pyramidal neurons showed that initial signs of mitochondrial destruction and the appearance of lipofuscin granules were noted in four-month-old OXYS rats. Animals aged 18

months displayed greater destruction of mitochondria and the rough endoplasmic reticulum, higher levels of lipofuscin accumulation, and greater deformation of the nuclear outline. Furthermore, there were significant changes in the form of lipofuscin accumulation in the neuron cytoplasm, demonstrating irreversible cell damage [8]. Increases in the relative content of lysosomes in OXYS rats can be regarded as an expression of elevated autophagy directed to removing damaged membranes and organelles. Along with destructive changes, neurons showed clear adaptive and compensatory processes: increases in nuclear envelope folding and the number of nuclear pores, nucleolar hypertrophy, and displacement of nucleoli to the nuclear periphery.

Thus, the hippocampal changes seen here, increasing intensely with age in OXYS rats, demonstrate the suitability of these animals as a model for seeking new approaches to the treatment and prophylaxis of premature aging.

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