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Progression of morphological changes within CNS in a transgenic rat model of familial amyotrophic lateral sclerosis

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Abstract

An analysis of the dynamics of histological and immunocytochemical changes in the CNS of a transgenic rat model of fALS in various periods of life was performed. Material was obtained from animals on the 60th day of age (4), 93rd day of age (3) and 120th presymptomatic day and from 3 animals in paretic stage of the disease. Formalin-fixed and paraffin-embedded slices were stained with HE and Klüver-Barrera method. Immunoreactions to GFAP, S-100, ferritin, neurofilament, ubiquitin, synaptophysin and tau protein were also performed.

Within the brain tissues patchy neuronal loss and dark or ischaemic neurons were dispersed in cortical layers, CA1, CA3 and CA4 hippocampal areas and structures of the hemispheres and brain stem.

In the spinal cord, numerous alpha motoneurons were dark or ischaemic. Vacuoles or small pale spots were visible in their cytoplasm. Microspongiosis surrounded some motoneurons, particularly cells subjected to neuronophagy. Neuronophagy, sporadically observed at the age of 60th day, was more extensive on the 93rd day of age, and at the age of 120 days already involved all interneurons of the anterior and posterior horns. In the immune reaction to neurofilament numerous fibres, often thick, fragmented or rosary-like, were observed. They were located within subcortical white matter, external and internal capsules, anterior horns of the spinal cord. Changes became more intensive with age.

Astrocytic reactivity was weak in animals on the 60th and 93rd day of life. Non-numerous cells were immunoreactive to GFAP and S-100, although an increase of astrocytic nuclei was observed. On the 120th day of age and in symptomatic stage astrocytic hypertrophy and proliferation were intensive.

But from the 60th day of age ubiquitin and tau protein immunopositive material was accumulated in the perinuclear area of astroglial cytoplasm. Immunoreaction of nerve cells to these proteins was negative.

Conclusions: 1) In the subclinical stage of the disease the pathological process within the CNS takes place already on the 60th day of age and its intensity increases with age. 2) Morphological changes are not limited to motor neuronal cells. Various structures of the CNS are damaged. 3) Weak astroglial reaction probably depends on pathological accumulation of ubiquitin and tau protein in cytoplasm. 4) Astroglial cells are probably also a "target" for pathogenic factors in the rat model of fALS.

Key words: *nerve cell degeneration, neurofilament pathology, tau protein accumulation.*

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Introduction

Amyotrophic Lateral Sclerosis (ALS) is a motor system disease. It clinically manifests as progressive motor system damage leading ultimately to tetraplegia, weakness of the diaphragm and intercostal muscles, and inevitable death usually within a few years after the disease onset.

The majority of ALS cases are sporadic, but some (5-10%) are familial (fALS). 20% fALS cases are associated with mutations of the SOD-1 gene. Transgenic expression of mutated human SOD-1 gene in mice and various other models [12] leads to symptomatology resembling ALS [6-8,14,22,38]. Transgenic rats [2,18,24] also developed similar symptomatology. One of these, transgenic Sprague-Dawley rats expressing human mutated SOD-1G93A gene created by Howland (the "Howland rat"), have been bred in our institution since 2003 (details, see [15]).

In the present paper we describe results of light-microscopic CNS study of the aforementioned transgenic "Howland " symptomatics. The aim of our research was to describe morphological changes occurring in these animals in the presymptomatic period and characterize their progression in relation to disease severity.

Materials and methods

The materials comprised 9 rat brains and 11 spinal cords. The animals were sacrificed in the early presymptomatic stage of the disease, i.e. at the age of 60 days (4 rats), in the late presymptomatic stage, i.e. at the age of 93 days (3 rats) and 120 days (1 rat), and in the symptomatic stage, i.e. at the age of 122-135 days (3 rats). The animals in the symptomatic stage of the disease demonstrated paresis of hind limbs or tetraparesis.

Morphological examination was performed on formalin-fixed and paraffin-embedded tissues. Routine histological stains (hematoxylin-eosin and Klüver-Barrera method) and immunocytochemical reactions were applied. In immune reactions antibodies against neurofilament (Immunotech, ready-to-use), synaptophysin (DAKO, 1:50), ubiquitin (DAKO, 1:350), tau protein (DAKO 1:100), and astroglial markers Glial Fibrillary Acidic Protein (GFAP) (DAKO, 1:1000) and S-100 (DAKO, 1:1000) were applied. The immune reactions were performed on formalin-fixed and paraffin-embedded tissue samples using peroxidase-antiperoxidase method.

After slide deparafinization, endogenous peroxidase was blocked by incubation in 0.5% hydrogen peroxide for 10 minutes. Next, the slides were incubated overnight at room temperature with primary antibodies. After rinsing in PBS, they were incubated for 1 hour with secondary antibodies from DAKO LSAB+HRP 2 system and then with 3,3'-diaminobenzidine (DAB) used as a chromogen.

Results

Spinal cord pathology

In the examined material the most pronounced pathological changes were observed within the spinal cord. In early (aged 60 days) and late presymptomatic rats (aged 93 days) some motoneurons were dark and shrunken (ischaemic-like dark neurons) (Fig. 1). In some of these cells vacuoles (Fig. 2) or pale spots within their cytoplasm were visible. Some motoneurons were surrounded by parenchymal microspongiosis (Figs. 2, 3) which was also observed in anterior horn tissue. In rats at this age, only a small number of motoneurons were subjected to neuronophagy (Fig. 3, Table I).

In rats at the age 93 days (Table I) numerous ischaemic-like motoneurons were darker and more shrunken, and neuronophagy was more frequent. Sometimes empty beds in anterior horn parenchyma were surrounded by astroglial and/or microglial cells (Fig. 4). Occasionally, in the cytoplasm of pale cells vacuoles were observed (Fig. 5). Loss of motoneurons was already visible.

On the 120th day of life and in paretic stage (Table I) in anterior horns only a few hyperchromatic shrunken motoneurons were observed. Small interneurons (Fig. 6) and nerve cells of posterior horns were subjected to neuronophagy. At this age, empty beds (Fig. 7) and microspongiosis (Figs. 6, 7) of the grey matter was evident. It also involved white matter of the spinal cord, visible as tissue vanishing in Klüver-Barrera stain.

Immunocytochemical investigations revealed pathology of neurofilaments found already in rats at the 60th day of life. Within anterior horns clusters of fragmented, frequently thick, rosary-like neurofilaments were noted (Fig. 8). With age these changes became more intensive. In anterior horns with evident loss of motoneurons, weaker reactivity to synaptophysin was noted (Fig. 9). Immune reaction to synaptophysin revealed

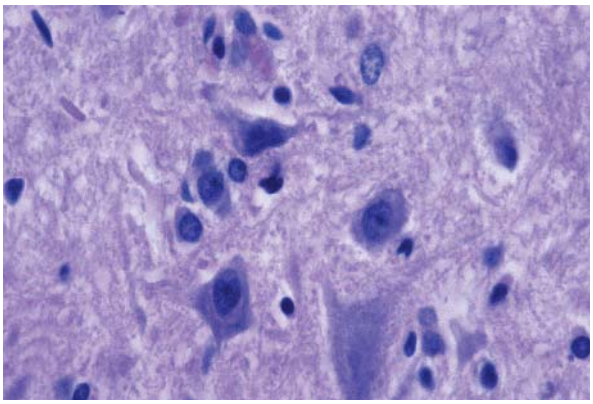

Fig. 1. Ischaemic-like dark motoneurons in anterior horn of the cervical spinal cord. Rat 60 days old, H&E, x 200

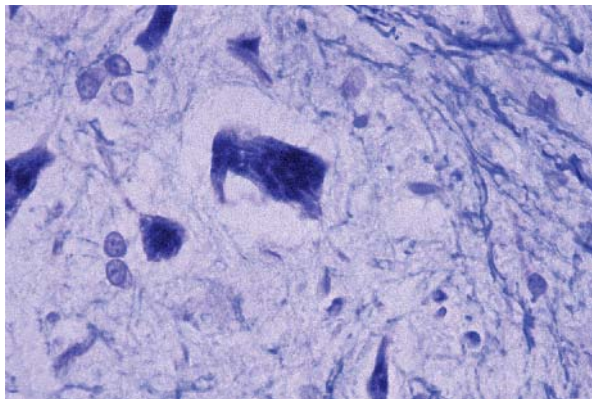

Fig. 2. Intracytoplasmic vacuoles in motoneurons of the lumbar spinal cord. Rat 93 days old, H&E, x 200

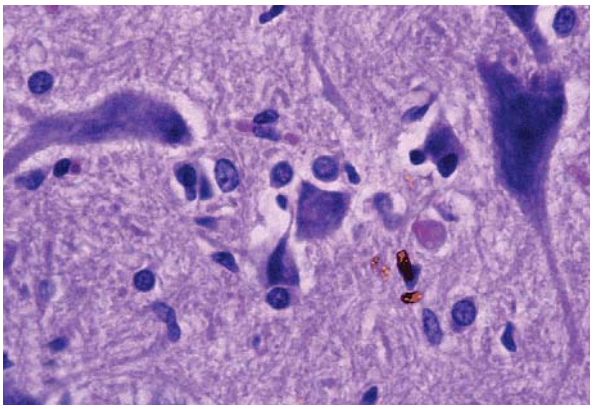

Fig. 3. Motoneurons surrounded by parenchymal microspongiosis and subjected to neuronophagy. Numerous visible „naked nuclei". Rat 93 days old, lumbar spinal cord, H&E, x 200

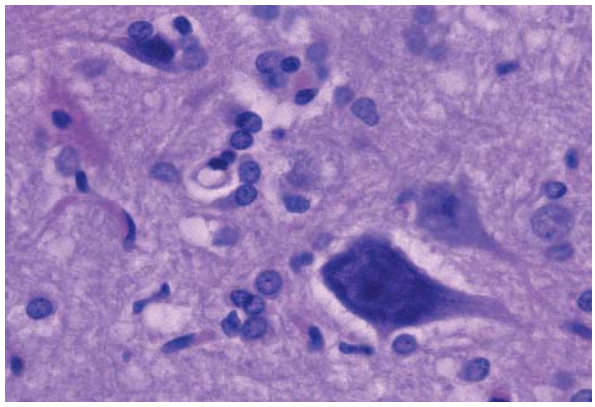

Fig. 4. Empty beds in anterior horn parenchyma surrounded by glial cells. Rat 93 days old, lumbar spinal cord, H&E, x 200

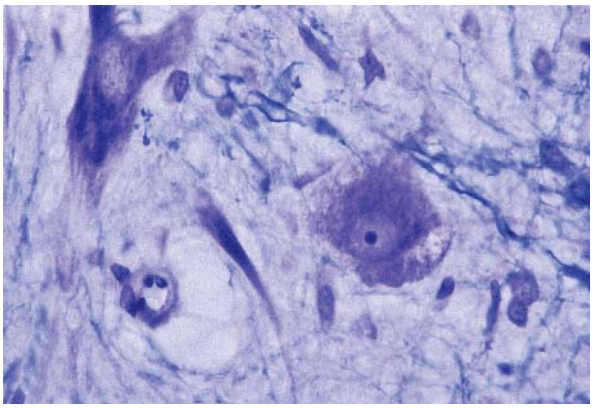

Fig. 5. Vacuoles in cytoplasm of pale motoneurons in the lumbar spinal cord. Rat 93 days old, Klüver-Barrera, x 200

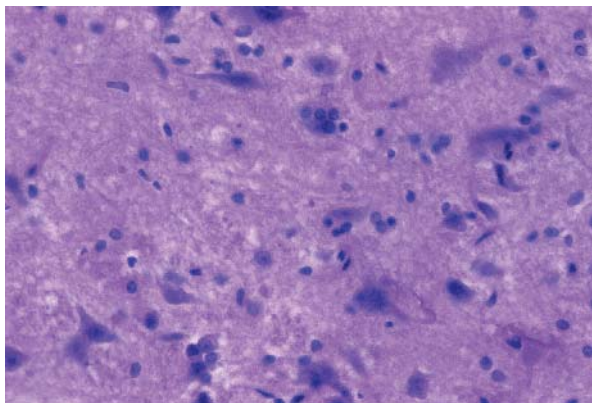

Fig. 6. Small interneurons subjected to neuronophagy in lumbar posterior horn. Rat 120 days old, H&E, x 100

Table I. Types of morphological changes in rat spinal cord

Types of changes	Presymptomatic stage								Paretic stage
	day 60 no. 82/03	day 60 no.83/03	day 60 no. 627	day 60 no. 628	day 93 no. 30/04	day 93 no. 31/04	day 93 no. 599	day 120 no. 696	3 rats
loss of	/-/	/-/	/-/	?	+/-	+	++	+++	+++
ischaemic-like cells	+	+	+	+	++	++	++	+	+
vacuolization of motoneurons	+	+	+	+/-	+	+	+	–	–
neuronophagy	+	+	+/-	+/-	+/-	+	++	+++	+++
neurofilament pathology	+	+/-	++	+/-	+	++	++	+++	+++
ubiquitin immunoreactivity				/-/					mainly neuron body, axons sporadically
tau immunoreactivity				/-/					part of neurons

+++ very severe intensity of morphological changes
++ severe intensity of morphological changes
+ mild intensity of morphological changes
+/- poor intensity of morphological changes
/-/ lack of morphological changes or immunoreactivity

irregular distribution of synapses on motoneuron bodies (Fig. 10). In the presymptomatic stage motoneuron reactivity to tau protein was negative.

Brain pathology

Pathological changes were also evident in the brain (Table II). Patchy neuronal loss (Fig. 11) and dark, ischaemic-like neurons were seen in the cortex, particularly within the 3rd and the 5th cortical layer (Fig. 12), deep structures of the cerebral hemispheres and brain stem. Similar changes were observed in CA1 (Fig.13), CA3 and CA4 hipocampal areas and dentate gyrus (Fig. 14). In the immune reaction to neurofilament, numerous, often thick, fragmented and rosary-like fibres were also observed. They were located within the subcortical

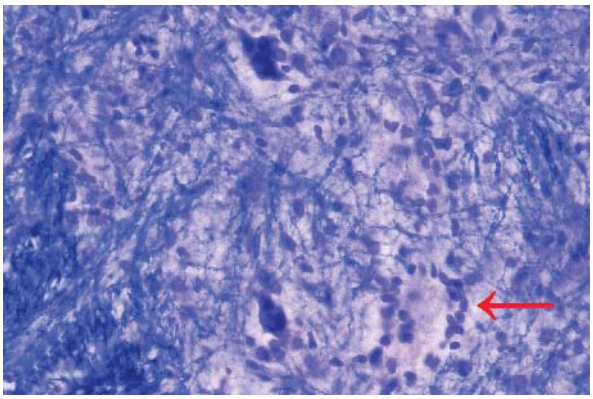

Fig. 7. Empty bed (arrow) and microspongiosis of the lumbar spinal cord grey matter. Rat 120 days old, Klüver-Barrera, x 100

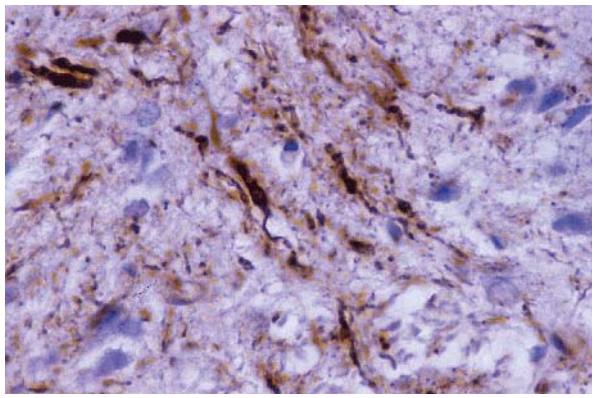

Fig. 8. Clusters of fragmented, thick, rosary-like neurofilaments within anterior horn of the lumbar spinal cord. Rat 60 days old, anti-neurofilament, x 200

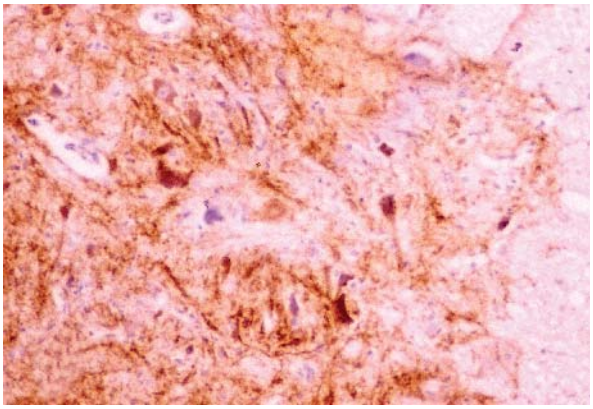

Fig. 9. Weak reactivity to synaptophysin and loss of motoneurons in anterior horn of the lumbar spinal cord. Rat 93 days old, anti-synaptophysin, x 50

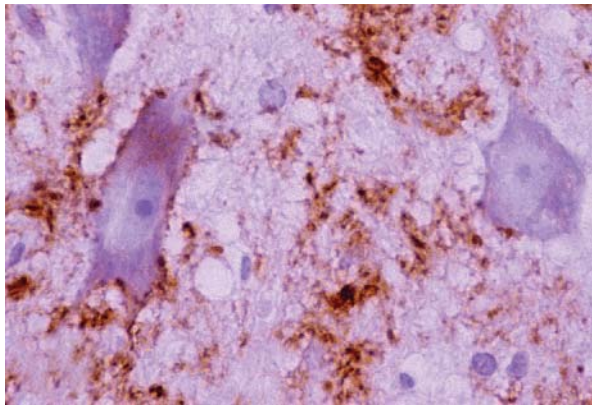

Fig. 10. Irregular distribution of synapses on motoneuron bodies in the cervical spinal cord. On motoneuron with cytoplasmic vacuoles visible nearly total lack of synapses. Rat 93 days old, anti-synaptophysin, x 200

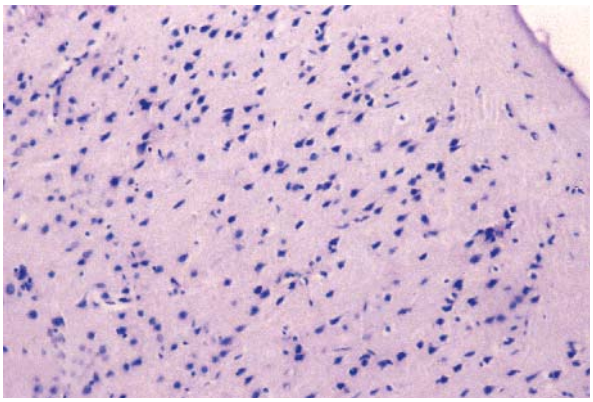

Fig. 11. Patchy neuronal loss in frontal cortex. Rat 60 days old, H&E, x 50

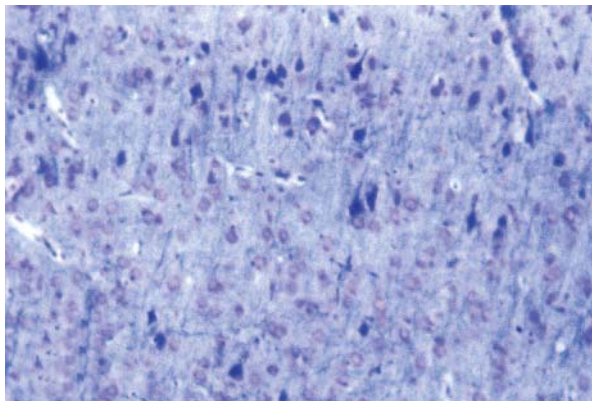

Fig. 12. Dark, ischaemic-like neurons in the 3rd and 5th layer of the frontal cortex. Rat 60 days old, Klüver-Barrera, x 50

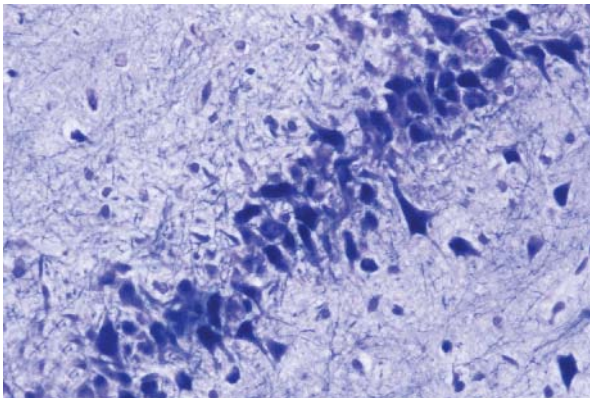

Fig. 13. Ischaemic-like neurons in CA1 hippocampal area. Rat 60 days old, H&E, x 100

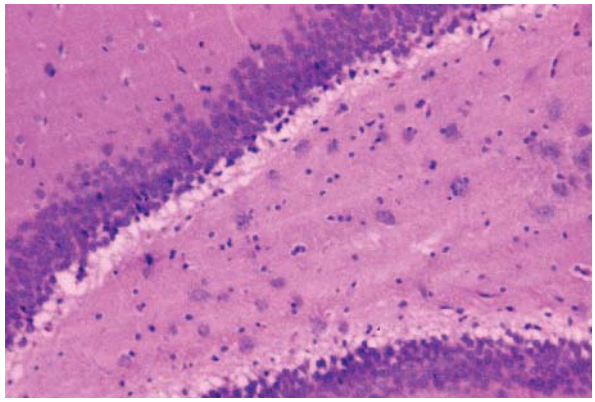

Fig. 14. Ischaemic-like neurons in dentate gyrus. Rat 93 days old, H&E, x 50

Table II. Localization and types of morphological changes in rat brains

	Presymptomatic stage					Paretic stage		
Localization of ischaemic like neurons	day 60 no. 82/03	day 60 no. 83/03	day 60 no. 627	day 60 no. 628	day 93 no. 30/04	day 93 no.31/04	no.45/03,	no. 46/03	no.47/03
cortex layers	III, V	III, V	III c	III	III	III		III	
CA1	+	+	/-/	/-/	+	++		+	
CA3, CA4	/-/	/-/	/-/	+/-	+	++		+	
depth structures	+/-	+/-	+/-	+/-	+	+		+	

++ very numerous ischaemic-like neurons
+ numerous ischaemic-like neurons
+/- a small number of ischaemic-like neurons
/-/ lack of ischaemic-like neurons

types of morphological changes

white matter spongiosis	+	+	+	+	+	+		+	
neurofilament pathology in the white matter	+	+	+	+	++	++		+++	
tau protein immunoreactivity		/-/	/-/	/-/	/-/	sporadically in neurons		part of neurons	

+++ very severe intensity of morphological changes
++ severe intensity of morphological changes
+ mild intensity of morphological changes
/-/ lack of morphological changes or immunoreactivity

white matter (Fig. 15), and external (Fig. 6) and internal capsule. Intensity of these changes also increased with age.

On the 60th and 93rd day of life, astrocytic reaction (Table III) in spinal cord and brain was very weak. In anterior horns (Table IV) increased numbers of astrocytic nuclei and astroglial "naked nuclei" were noted but in the immune reaction to GFAP only non-numerous cells were immunolabelled (Fig. 17). Somewhat more pronounced glial reactivity was

Table III. Astroglial reaction and immunoreactivity in presymptomatic and symptomatic stage of fALS

	Protein accumulation				
	hypertrophy	proliferation	GFAP	tau	ubiquitin
60 days	+/-	+/-	+/-	+	+
93 days	+/-	+	+/-	+	+
120 days	++	++	+	++	++
symptomatic stage	++	++	++	+++	++

+++ very severe intensity of morphological changes
++ severe intensity of morphological changes
+ mild intensity of morphological changes
+/- poor intensity of morphological changes

Table IV. Astroglial immunoreactivity in rat spinal cord

	60 days				93 days		
GFAP			/-/		+/-	+/-	+/++
S-100	+/-	+/-	+/-	+/-	+/-	+	++
ubiquitin	+	+	+	+	+	++	++
tau	+	++	++	++	++	++	++

+++ very severe intensity of morphological changes
++ severe intensity of morphological changes
+ mild intensity of morphological changes
+/- poor intensity of morphological changes

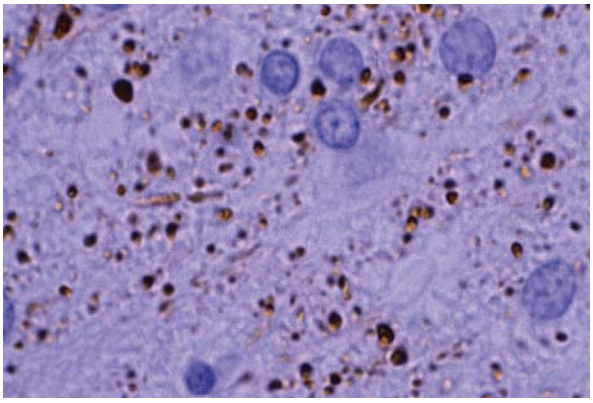

Fig. 15. Numerous thick, fragmented, rosary-like fibres immunoreactive to neurofilament within subcortical white matter. Rat 93 days old, anti-neurofilament, x 500

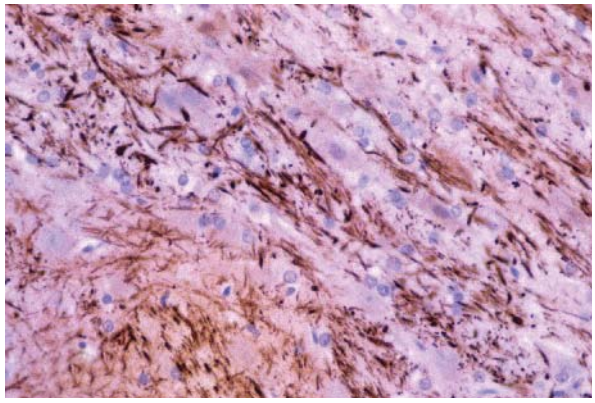

Fig. 16. Fragmented, rosary-like fibres immunoreactive to neurofilament in external capsule. Rat 60 days old, anti-neurofilament, x 100

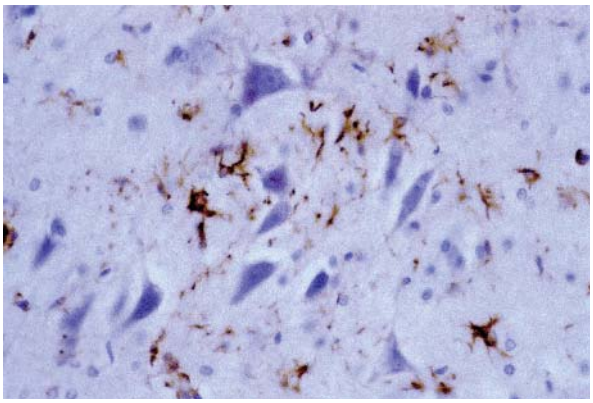

Fig. 17. Poor astroglial immune reaction to GFAP in anterior horn of the cervical spinal cord. Visible increased number of astrocytic nuclei and astroglial "naked nuclei". Rat 93 days old, anti-GFAP, x 100

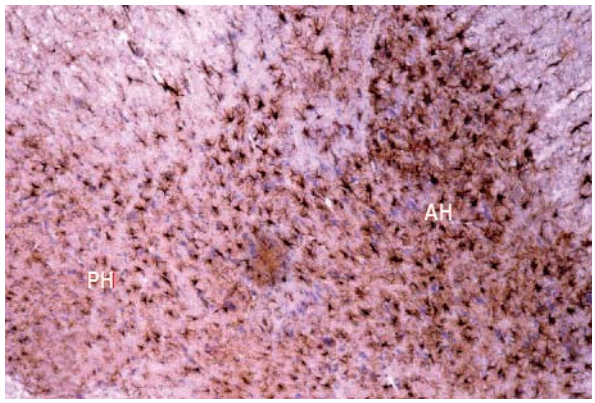

Fig. 18. Very intensive immunoreactivity to GFAP with astrocyte hypertrophy and proliferation in anterior horn of the lumbar spinal cord. Rat 120 days old, anti-GFAP, x 25

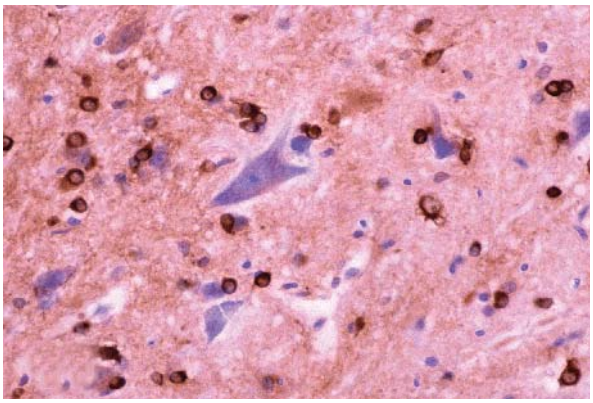

Fig. 19. Numerous astrocytes with tau immunopositive ring surrounding cell nucleus in cervical spinal cord. Rat 93 days old, anti-tau, x 100

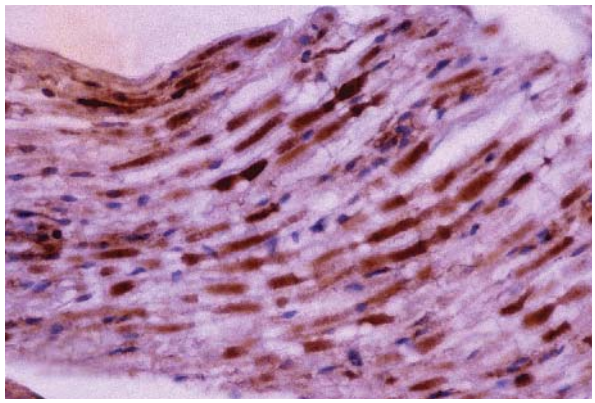

Fig. 20. Numerous fibres within anterior lumbar spinal root immunoreactive to tau protein. Rat 93 days old, anti-tau, x 100

observed in the immune reaction with antibodies against S-100 protein. In the brain, mild immunoreactivity to GFAP and S-100 was noted within the internal and external capsule. Very intensive astrogliosis with astrocyte hypertrophy and proliferation and evident immunoreactivity to glial markers was observed only in the spinal cord of the rat on the 120th day of life (Fig. 18), and in the symptomatic stage (Table III).

From the 60th day of life, in some astrocytes tau (Fig. 19) and ubiquitin immunopositive ring surrounding cell nuclei were observed. With increasing age, accumulation of these proteins was more intense and involved more numerous astrocytes. In some animals, fibres within anterior (Fig. 20) and posterior spinal roots were also immunolabelled for tau protein.

Within the white matter of hippocampal fimbria, a discrepancy between immunoreactivity to glial markers (GFAP and S-100) and ubiquitin and tau protein was visible (Table V, Fig. 21).

Within the brain nerve cells were tau positive only sporadically.

Discussion

Under the light microscope, two major types of spinal cord motoneurons, dark and pale, can be identified. Cytoplasm of the dark motoneurons is abundant in Nissl bodies resulting in dark appearance of cells. The pale motoneurons are bigger, their cytoplasmic Nissl bodies are less numerous and the cells have larger euchromatic nuclei. These two types of motoneurons can also be distinguished ultrastructurally and functionally. Dark motoneurons are characterized by expanded sarcoplasmatic reticulum, nuclei abundant in chromatin, and numerous mitochondria; these cells connect with slow (type I) muscle fibres. Fewer mitochondria, more delicate endoplasmic reticulum and large euchromatic nuclei are characteristic of motoneuronal cells innervating fast muscle fibres; these cells connect to the fast (type II) muscle fibres [33].

In many pathological conditions nerve cells demonstrate features typical for chronic ischaemia. Such neurons are triangular-shaped, shrunken and darker than normal. Their nucleus is homogeneously dark-grey. Nissl bodies are not visible, or only their particles dispersed within the cytoplasm are seen. Under a light microscope these dark, degenerating neurons may look somewhat similar to the normal dark spinal cord type I motoneurons. To avoid misunderstanding, such spinal cord motoneuron pathology will be named ischaemic-like dark neuron.

In our material, pathological changes were found in all examined rats at the level of both brain and spinal cord, although the latter structure was definitely more affected. Degenerative process involved motoneuron bodies as well as axons. Ischaemic-like dark neurons and neurofilament pathology constituted the essence of the pathologic

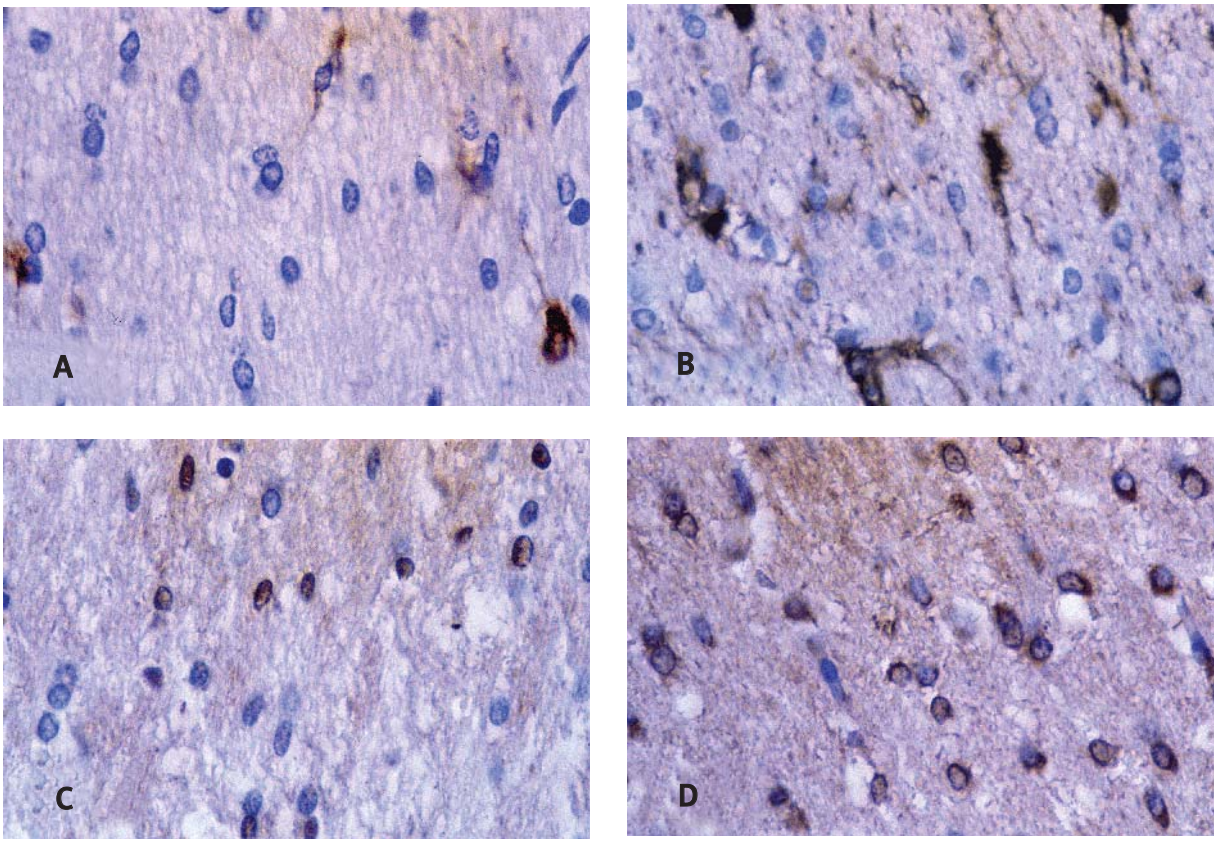

Fig. 21. A. Solitary astrocytes immunoreactive to GFAP in the white matter of the hippocampal fimbria. Rat 60 days old, anti-GFAP, x 200; **B.** Non-numerous astroglial cells immunoreactive to S-100 in the white matter of the hippocampal fimbria. Rat 60 days old, anti-S-100, x 200; **C.** Immunoreactive to ubiquitin ring surrounding cell nucleus visible in some astrocytes within the hippocampal fimbria. Rat 60 days old, anti-ubiquitin, x 200; **D.** Numerous astrocytes with tau immunopositive ring around cell nucleus in hippocampal fimbria. Rat 60 days old, anti-tau, x 200

process. In astroglial cells, immunoreactivity to glial markers was weak but immune reactions to ubiquitin and tau protein were evident.

Our present observations stimulated us to briefly discuss the following issues:

1). Are mouse and rat models of fALS similar?
2). What is the main (primary) target for pathogenic factor(s) in the "Howland rat"?
3). What is the role of astrocytic reaction and immunoreactivity?
4). Does "selective motoneuron death" occur in the Howland rat?

Table V. Astroglial immunoreactivity in hippocampal fimbria. Rat, 60th day of life

GFAP	S-100	Ubiquitin	Tau
/-/	+/-	+	++

+++ very strong immunoreactivity in all astrocytes
++ strong immunoreactivity in numerous astrocytes
+ mild immunoreactivity in a small number of astrocytes
+/- poor immunoreactivity in a few astrocytes
/-/ very weak or total lack of immunoreactivity

Comparison of mouse and rat model of fALS

We found pathological changes both in spinal cord and brain in all examined rats. In spinal cord, numerous alpha-motoneurons were dark and resembled ischaemic ones. Vacuoles or small pale spots were visible in their cytoplasm already from the 60th day of rat life. In anterior horn and anterior and lateral columns microspongiosis was also observed.

Similar changes were observed in mouse model of fALS, where in the early stage of the disease the most prominent morphological change was microvacuolization of the anterior horn neurons and their processes [6]. At the ultrastructural level, the most affected structures were spinal cord axons. Axonal mitochondria were swollen and their cristae destroyed [16,32]. However, vacuolar degeneration of mitochondria was also observed in dendrites [41]. In axons, accumulation of neurofilaments was also seen.

In the mouse model, in the early presymptomatic phase of the disease, loss of motoneurons was inconsiderable and their degenerative changes were also discrete. These axonal changes caused impaired slow [32] and fast [39] axonal flow. Before the paretic stage, the number of motor units in the distal hind limb declined and motor unit size increased [36].

Our morphological examination revealed that the majority of motoneurons and other nerve cells, particularly pyramidal cells, displayed signs of both ischaemic-like and chronic degenerative. Ischaemic-like dark neurons were observed throughout the presymptomatic period. It seems that the spinal cord degeneration process manifested by a presence of ischaemic-like dark neurons involved preferentially type I motoneurons. Degeneration of type II motoneurons resembled changes observed in a cell after cutting off its axon near the cell body. Intracytoplasmic vacuoles or pale spots visible in rats from the 60th day of life probably were abnormal swelling mitochondria, which were found ultrastructurally in the same period. In the early presymptomatic stage of the disease, the main ultrastructural changes involved mitochondria, and in the late presymptomatic stage giant swollen mitochondria devoid of cristae occupied almost the entire axonal caliber; at axon periphery, accumulation of neurofilaments was also visible [11]. Axonal damage was manifested by abnormal immunoreaction to neurofilament in which numerous immunoreactive fibres within the anterior horn were fragmented, thick and rosary-like. All these pathological changes increased with animal age. In rats at the age of 93 days, loss of motoneurons with neuronophagy was evident. At the 120th day of life still in asymptomatic phase of the disease, only single ischaemic-like dark neurons were observed. In anterior horn parenchyma, numerous clusters of pathological neurofilaments and sponginess were present.

In our material pathological changes were not limited to the spinal cord. In the brain, ischaemic-like dark neurons were disseminated in new and old brain cortex. In immune reaction to neurofilament clusters and fragmentation of filaments were seen. Such accumulation of neurofilaments was observed in transgenic mouse models leading to degeneration of motoneurons of the spinal cord [23], and also in human ALS [30].

In summary, our data confirm the essential similarity between transgenic mouse fALS models and the “Howland rat”.

Primary target for pathological factor in fALS model

The presence of axonal abnormality both in the mouse model of fALS and our material poses the question whether the primary target for pathological factors in this model is the motoneuron body or its axon.

Astrocytes are thought to be involved in the pathological process both in human ALS [27,29] and in transgenic fALS models [1,4]. Astroglial cells are the main glutamate transporter to synapses located on dendritic spines and neuron body. Failure of astrocytic glutamate reuptake is the basis of excitotoxicity [31] and may lead to motor neuron degeneration. In the rodent fALS models focal loss of astroglial glutamate transporters EAAT2 [18] and GLT-1 [10], and qualitative changes in glutamate/aspartate transporter GLAST [10] were found in the spinal cord. The above data and experiment in vitro [21] confirm the hypothesis that astroglia can participate in glutamate-dependent neuron degeneration.

A hypothesis that reactive astrocytes secreting nerve growth factor actually induce apoptosis of motoneurons and in this way participate in the degenerative process was put forward by Pehar et al. [26]. Although data about apoptosis are controversial [37] apoptosis or apoptotic-like process

being initiated in the cell nucleus is also being postulated in ALS as well as in rodent fALS models [13], and tissue culture [20]. This would imply cell bodies as a primary target for pathological factor(s). Biochemical examination of spinal cord myelin indicated a gradual decrease of lipids, cholesterol, phospholipids and proteolipid protein concentration in the "Howland rat" [25]; these changes were qualitatively similar to those described by others in Wallerian degeneration of the optic nerve. However, concomitant ultrastructural studies seem to indicate that in the "Howland rat" in the presymptomatic stage mitochondrial pathology occurs preferentially in axons, not in cell bodies [11]. Because at the level of axonal change its cell body is not present, the severity of this cell pathology and degree of parallelism of axonal and cellular damage are not known.

Astrocytic reaction and immunoreactivity

Estimation of astroglial reactivity in presymptomatic and terminal phases of the rat fALS model delivered very interesting data. Contrary to some previous reports [18,24] in which evident gliosis in the presymptomatic period was found, in our rats at the age of 60 and 93 days, the astrocytic reaction in anterior horns was very weak. Although increased numbers of astrocytic nuclei and "naked" nuclei (Alzheimer type II cells) were seen, GFAP and S-100 immunoreactivity was weak and limited to a small number of astrocytes. Astroglial proliferation and hypertrophy was evident only in rats in the late presymptomatic stage (120 day of life) and in the symptomatic and terminal phase. In the early presymptomatic period, instead of the expected intensive immune reaction to glial markers, in some astrocytes we found accumulation of tau protein and ubiqutin around the nuclei. With increase of animal age, this tau-positive ring became more distinct and appeared in larger number of astrocytes. Tau protein pathology has been found in neurons in many human degenerative disorders [3,19] and also in some old animals [34]. Neuronal and glial tau positive inclusions were observed in human ALS [41], in other degenerative diseases with or without accompanying dementias of various types, and in elderly individuals [17, 35]. Diseases with pathological accumulation of tau protein are involved in a group of disorders called tauopathies [9].

Somewhat unexpectedly, in our material immunoreactivity of nerve cells to tau protein was negative but tau positive astrocytes were observed already in the early presymptomatic stage. This observation provides confirmation of the hypothesis that astroglial cells may also be a target for pathogenic factors in the rat model of fALS. The accumulation of tau protein is probably a cause of weak reaction and immunoreactivity of astrocytes. Pathological accumulation of tau protein may disturb synthesis of skeletal or cytoplasmic proteins such as GFAP or S-100. Increased expression of ubiquitin around astroglial nuclei may be associated with a process of tau protein removal by the ubiquitin-proteasome system. Progressive disturbances in ubiquitin-proteasome mechanisms shown in a murine transgenic fALS model [5] confirm this hypothesis. Intensive astroglial reactivity observed in the advanced stage of the disease probably involved a young astrocytic population in which tau protein did not have time for accumulation.

„Selective motoneuron death" in the rat fALS model

Is degeneration of motoneurons within spinal cord selective? Some investigations indicated that the intensity and frequency of pathological changes depend on the degree of mutant protein accumulation [40]. Our observations showed that, although spinal motoneurons are the most affected, many other structures in the brain and spinal cord are also involved in the Howland's rat model of fALS. Although motoneurons appear to be more vulnerable than the other CNS cell types, they are not the only cells degenerating in this fALS model, as is also in the case of human ALS [28].

Conclusions

1. In the subclinical stage of the disease, the pathological process within the CNS takes place as soon as the 60th day of age and its intensity increases with age.
2. Morphological changes are not limited to motor neuronal cells. Various structures of the CNS are damaged.

3. Weak astroglial reaction probably depends on pathologic accumulation of ubiquitin and tau protein in cytoplasm.
4. Astroglial cells are probably also a "target" for pathogenic factors in the rat model of fALS.

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