

Shoichi Sasaki · Hitoshi Warita · Koji Abe · Makoto Iwata

## Slow component of axonal transport is impaired in the proximal axon of transgenic mice with a G93A mutant SOD1 gene

Received: 26 September 2003 / Revised: 14 January 2004 / Accepted: 14 January 2004 / Published online: 17 March 2004  
© Springer-Verlag 2004

**Abstract** The purpose of this study was to determine whether slow axonal transport of neurofilaments (NFs) is impaired in the spinal cord of G93A Cu/Zn superoxide dismutase (SOD1) mutant transgenic mice expressing a relatively low mutant protein (gene copy 10) and, if so, how the impairment occurs in this animal model. Transgenic mice were killed at the ages of 24, 28 and 32 weeks, and the cervical and lumbar spinal cords were examined under an electron microscope. Age-matched non-transgenic wild-type mice served as controls. At 24 weeks (early presymptomatic stage), anterior horn cells were well preserved. The earliest morphological changes were mild vacuolar changes in the neuronal processes, particularly in proximal axons. At 28 weeks (late presymptomatic stage), mild neuronal loss of anterior horn neurons was observed. Vacuolar changes were more prominent in the proximal axons, including swollen axons (spheroids) and neuropils of the anterior horns. Vacuoles in the axons were frequently large enough to occupy almost the entire axonal caliber. The anterior roots were degenerative, showing vacuolar changes and myelin ovoids. Lewy body-like inclusions (LIs) consisting of filaments thicker than NFs (about 1.5 times larger in diameter) were frequently demonstrated in the neuronal processes including swollen axons (spheroids) and occasionally in the somata. At 32 weeks (symptomatic stage), the anterior horns showed a moderate to severe neuronal loss accompanied by prominent astrogliosis. Cord-like swollen axons consisting of accumulated

NFs and many neurofilamentous accumulations were frequently observed in the anterior horn. Vacuolar changes were less prominent or disappeared in the neuropils of the anterior horns and the anterior roots, whereas LIs were frequently demonstrated within the neuronal processes including the cord-like swollen axons. In the anterior roots, degenerative changes such as marked fiber loss and frequent myelin ovoids were remarkable. No accumulation of NFs or mitochondrial vacuolation was detected in somata or proximal dendrites at any stage. These findings suggest that the slow component of axonal transport in the proximal axons is impaired at an early stage in this transgenic mouse model, and that the impairment is probably caused by a mechanical impediment of NFs, or by the accumulation of NFs in the proximal axon, as a result of the obstruction of the axonal flow that initially occurs by vacuolar changes, and is later exacerbated by accumulation of LIs.

**Keywords** Amyotrophic lateral sclerosis · SOD1 mutant · Neurofilament · Axonal transport · Vacuolation

### Introduction

Accumulations of neurofilaments (NFs) in the cell bodies and proximal axons of motor neurons are a common feature of sporadic amyotrophic lateral sclerosis (ALS) [8], familial ALS [9, 15], and transgenic (Tg) mice expressing the Cu/Zn superoxide dismutase (SOD1) mutation [10]. The aberrant accumulation of NF proteins, which has been implicated in the development of motor neuron disease (MND), may be an indication of abnormal transport of these large proteins [10]. Moreover, transgenes encoding mutant NF subunits could directly cause selective degeneration and death of motor neurons and the ensuing axonal disorganization [12]. Previous findings suggest that damage to NFs might be directly involved in ALS pathogenesis. However, how these accumulations of NFs are formed and how they contribute to the disease remain unknown. Using electron microscopy, we carried out a

S. Sasaki (✉) · M. Iwata  
Department of Neurology, Neurological Institute,  
Tokyo Women's Medical University,  
8-1 Kawada-cho, Shinjuku-ku, 162-8666 Tokyo, Japan  
Tel.: +81-3-33538111 ext 39232, Fax: +81-3-52697324,  
e-mail: ssasaki@nij.twmu.ac.jp

H. Warita  
Department of Neurology, Yonezawa National Hospital,  
Yonezawa, Japan

K. Abe  
Department of Neurology, Okayama University, Okayama, Japan

prospective longitudinal pathological study on the spinal cords of Tg mice of a relatively low Tg copy number with a G93A mutant SOD1 gene that were generated in our own laboratories, covering the presymptomatic to symptomatic stages, to determine whether the slow component of axonal transport is impaired in the proximal axons, and, if so, how the impairment occurs.

## Materials and methods

### Experimental animals and clinical assessment

Tg mice expressing the G93A mutant human SOD1 [7] were originally obtained from the Jackson Laboratory [B6SJL-TgN (SOD1-G93A) 1 Gur<sup>dl</sup>, Bar Harbor, Me., USA] and backcrossed to a C57BL/6 background strain by mating hemizygote males with inbred C57BL/6 female mice (C57BL/6CrSlc, Nihon SLC, Shizuoka, Japan) to produce Tg and non-Tg littermates. Backcrossing them onto the black 6 background entirely eliminated the SJL (dysferlin gene-associated FSH dystrophy) background from the colony used for these studies. The Tg progeny was identified by polymerase chain reaction (PCR) amplification of tail DNA with specific primers for exon 4 [14]. These G93A SOD1 mutant mice expressed a relatively low mutant protein (gene copy 10).

At around 32 weeks of age, the G93A Tg mice developed progressive muscle weakness and spasticity in one or more limbs, beginning with a posterior limb. After 1–2 weeks, they could not feed themselves due to severe paralysis expressed by the hyperextension of their hind limbs. The G93A Tg and non-Tg mice were examined simultaneously. Throughout the present study, the mice were treated in accordance with the Helsinki declaration and the guiding principles in the care and use of animals.

### Histopathological analysis

Tg mice were divided into three groups: early presymptomatic Tg (aged 24 weeks,  $n=2$ ), late presymptomatic Tg (aged 28 weeks,  $n=2$ ), and symptomatic Tg (aged 32 weeks,  $n=2$ ). Age-matched non-Tg mice served as controls in each group ( $n=7$ ). All mice were deeply anesthetized with ether and perfused intracardially with heparinized saline (pH 7.4), followed by perfusion with ice-cold 4% paraformaldehyde (Katayama Chemical, Osaka, Japan) in 0.1 M phosphate buffer (pH 7.4). The spinal cords were removed rapidly and post-fixed by immersion in the same fixative (5 days, 4°C). Cross-sections of the spinal cord were embedded in paraffin, sectioned (4  $\mu$ m), and stained with hematoxylin and eosin (HE), and with cresyl violet.

### Immunohistochemistry

Sections (6  $\mu$ m thick) of the paraffin-embedded spinal cords were deparaffinized, quenched with 3% H<sub>2</sub>O<sub>2</sub>, treated with nonimmune serum as the blocking reagent, and incubated overnight at 4°C with a mouse monoclonal antibody to rat phosphorylated neurofilament (SMI-31, Sternberger Monoclonals, 1:1,000). Antibody binding was visualized by the biotin-streptavidin peroxidase complex method. The final chromogen was 3,3'-diaminobenzidine tetrahydrochloride.

### Ultrastructural study

Six Tg and six non-Tg wild-type mice were killed at the ages of 24, 28, and 32 weeks ( $n=2$  for each group, respectively). All mice were deeply anesthetized with ether and perfused intracardially with heparinized saline (pH 7.4) followed by perfusion with ice-cold 4% paraformaldehyde (Katayama Chemical, Osaka, Japan) and 0.2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The

spinal cords were rapidly removed and post-fixed by immersion in the same fixative (5 days, 4°C). Tissues were incubated in 2% osmium tetroxide in 0.1 M cacodylate for 2 h, washed, dehydrated, and embedded in epoxy resin (Epon). Serial semithin sections (1  $\mu$ m) of the whole transversed spinal cords stained with toluidine blue were examined by light microscopy. Appropriate portions were cut into ultrathin sections and subsequently stained with lead citrate and uranyl acetate for electron microscopic study.

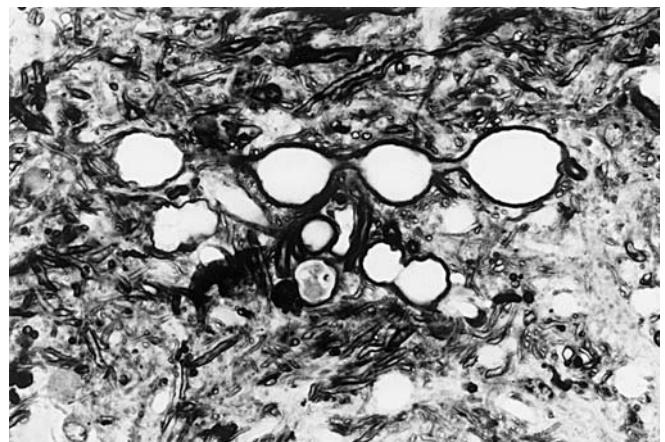
## Results

### Light microscopic findings

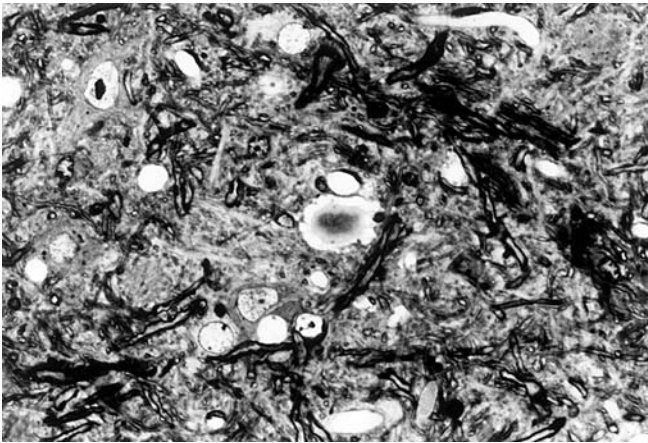
#### *Tg mice*

At the age of 24 weeks (early presymptomatic stage), HE and Klüver-Barrera stainings revealed no pathological change. However, in Epon-embedded plastic sections stained with toluidine blue, small vacuolar changes were observed in the neuropils of the ventral portion of the anterior horn, in the axons of the anterior horn, around the central canal, and in the axons of the anterior root exit zone of the anterior column in one of the two mice examined. White matter showed no abnormality in either of these mice.

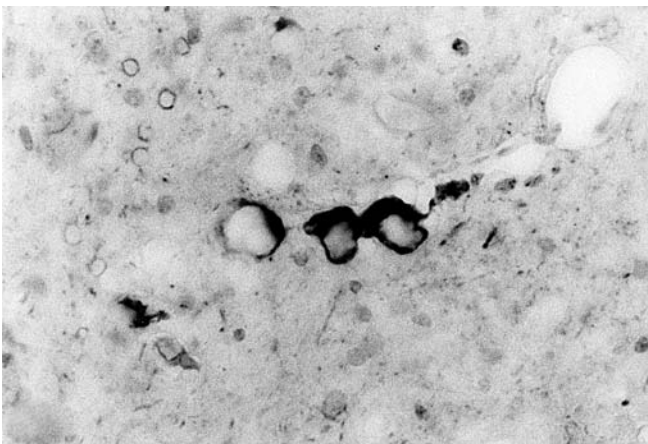
At the age of 28 weeks (late presymptomatic stage), slight neuronal loss of anterior horn cells was observed at the cervical and lumbar levels. Prominent vacuolar changes were recognized in the same regions showing changes in the early presymptomatic stage. Proximal swollen axons with prominent vacuolar changes shaped like a sausage or a string of beads were frequently found in a longitudinal section (Fig. 1). The anterior roots were degenerative showing vacuolar changes and myelin ovoids, whereas the posterior roots showed no abnormality. Lewy body-like inclusions (LIs) were frequently seen in the neuronal processes including cord-like swollen axons (Fig. 2). Some remaining anterior horn neurons also contained LIs, although they were only occasionally found in the posterior horn (Rexed's lamina V).



**Fig. 1** Proximal swollen axons with prominent vacuolar changes assuming the shape of a sausage or a string of beads in a longitudinal section (late presymptomatic stage). Plastic section, toluidine blue staining. Original magnification  $\times 714$



**Fig. 2** A Lewy body-like inclusion with a central core is observed in the neuronal process in the neuropils of the anterior horn (late presymptomatic stage). Plastic section, toluidine blue staining. Original magnification  $\times 714$



**Fig. 3** Proximal swollen axon with prominent vacuolar change shaped like a string of beads is immunostained for phosphorylated NF (SMI-31) (NF neurofilament). Original magnification  $\times 714$

At the age of 32 weeks (symptomatic stage), the anterior horns showed moderate to severe neuronal loss of anterior horn cells at all levels of the spinal cord. Most remaining anterior horn neurons showed types of degeneration such as central chromatolysis. Vacuolar changes were less prominent or had disappeared, although some persisted in the anterior root exit zone of the anterior column. On the other hand, LIs were frequently observed within the neuronal processes in the anterior horns, including the cord-like swollen axons. Proximal axonal swellings (spheroids) were occasionally found in the neuropils of anterior horns. Myelin ovoid formation was prominent in the white matter of the anterior and lateral columns, especially at the outer zones, and in that of the posterior column adjacent to posterior horns. Anterior roots showed marked degenerative changes such as marked fiber loss and myelin ovoids. Myelin ovoids were also found in the posterior roots, though to a lesser extent.

Immunohistochemically, spheroids or proximal swollen axons with prominent vacuolar changes were positively immunostained for phosphorylated neurofilament (SMI-31) (Fig. 3).

#### *Non-Tg littermates*

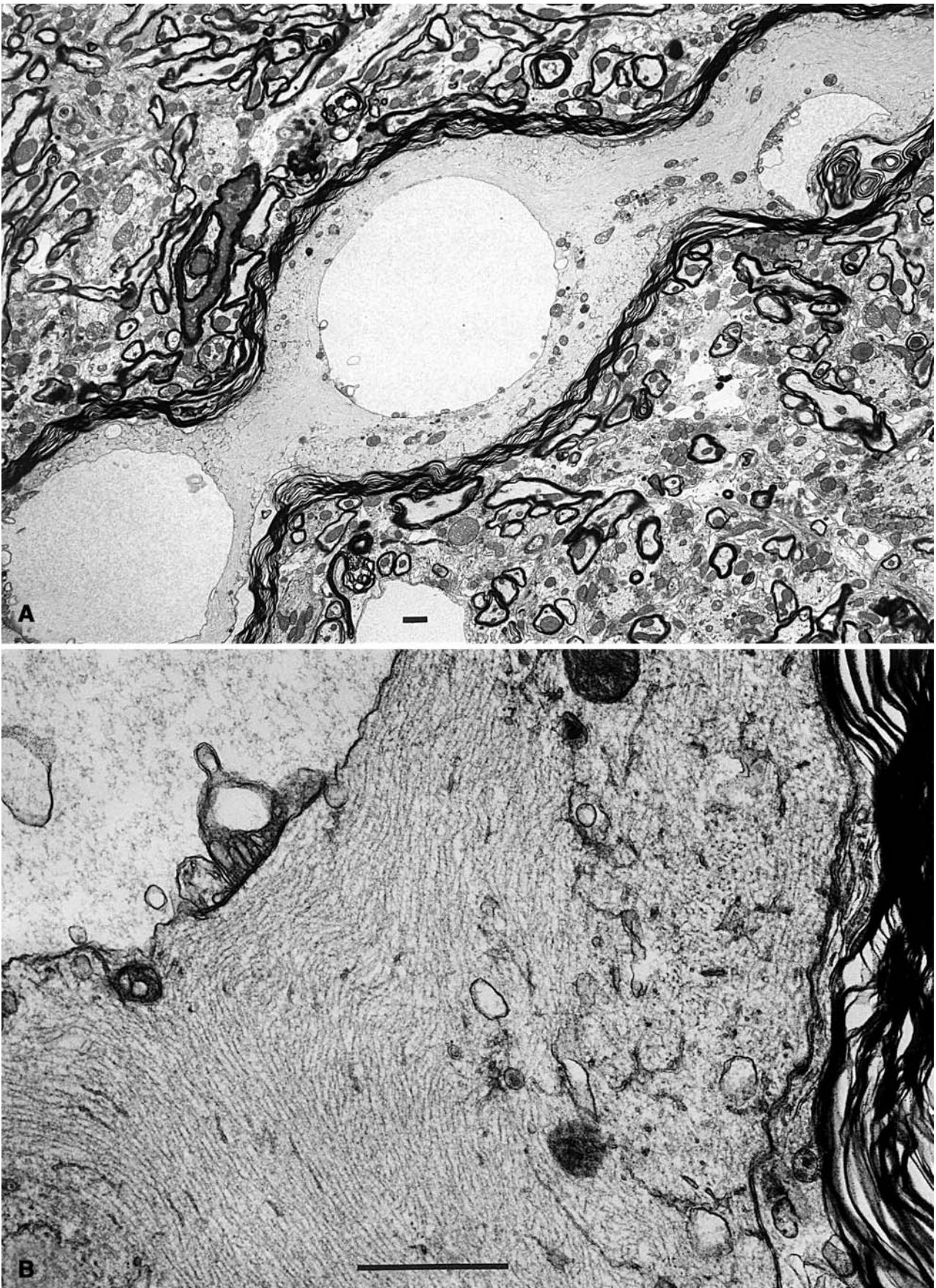
No vacuolar changes, LIs or swollen axons immunostained for phosphorylated NF were detected in the spinal cord at any age.

#### Ultrastructural findings

##### *Tg mice*

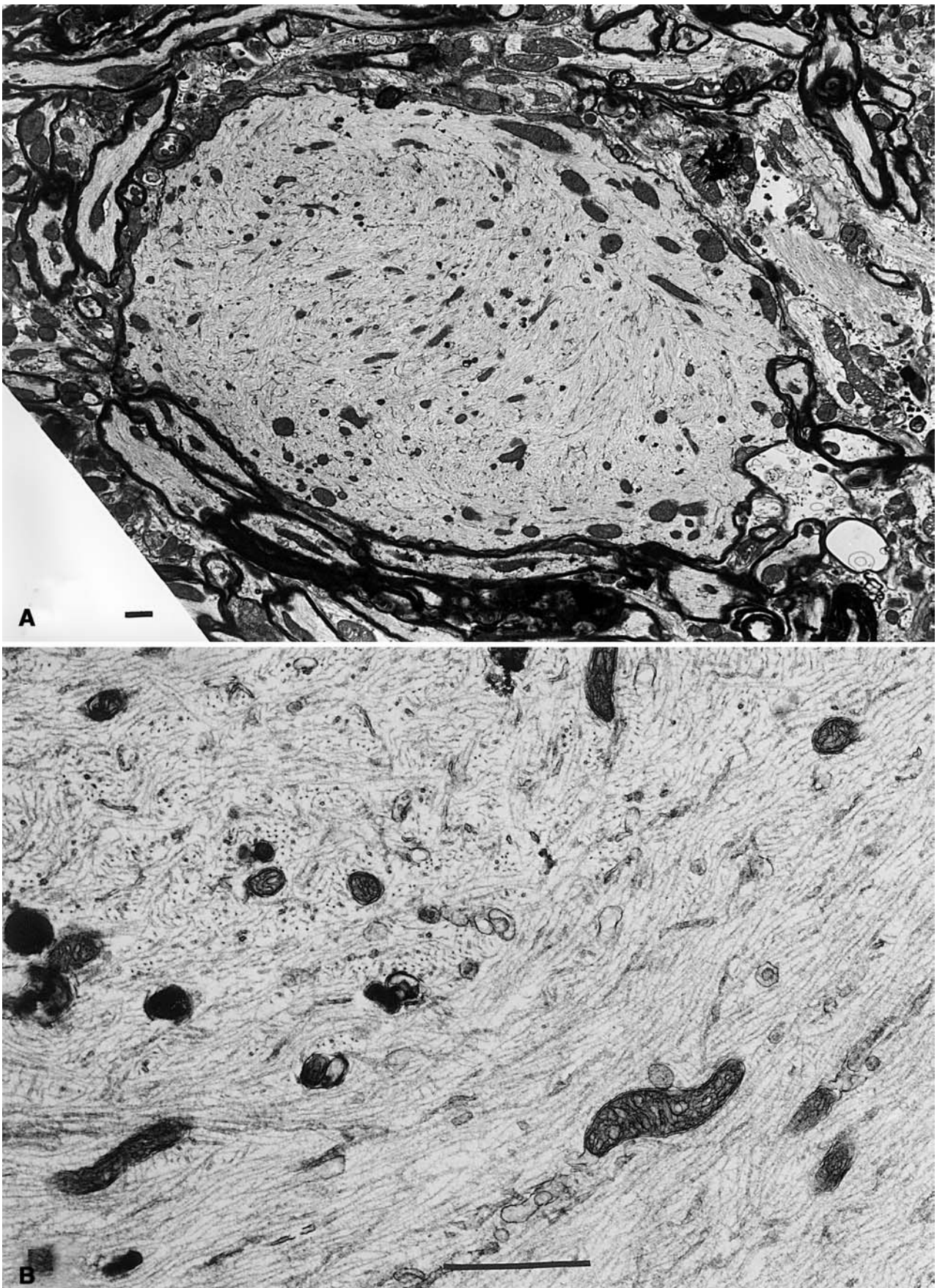
At 24 weeks (early presymptomatic stage), mitochondrial swelling and vacuolation of the inner compartment were observed predominantly in the proximal axons in the anterior root exit zone and anterior root, and in the neuropils of the ventral portion of the anterior horn. Most of these vacuoles were relatively small. In contrast, almost all of the mitochondria in the small myelinated axons in these regions had a normal appearance. Small filamentous aggregates were only occasionally observed in the neuronal processes including the axons in the anterior horns.

At 28 weeks (late presymptomatic stage), mitochondria were frequently swollen and vacuolated in the large myelinated axons of the same regions showing changes in the early presymptomatic stage. Vacuolar changes of various stages, from small focal vacuolar formation in the inner compartment to large vacuoles, were observed in these axons. The vacuoles tended to be larger than those of the early presymptomatic stage. The intermembrane space between the inner and outer membranes was also vacuolated, frequently with large vacuoles. In mitochondria with advanced vacuolation, the vacuolar space was filled with a granular or amorphous substance. Usually, giant vacuoles were accompanied by axonal swellings, and occupied almost the entire axonal caliber, thus blocking the axonal transport with accumulation of mitochondria and misdirected NFs (Fig. 4): accumulated NFs were seen in close proximity to vacuoles or in the axon between the large vacuoles in the longitudinal section of axons. Spheroids consisting of interwoven NFs were frequently observed in the anterior horn and the anterior column (Fig. 5). Filamentous aggregates were frequent, predominantly in the neuronal processes of the anterior horns including the proximal axons (Fig. 6), and, to a lesser extent, in the somata and the dendrites of the anterior horn neurons. The aggregates consisted basically of interwoven intermediate filaments thicker than NF (about 1.5 times larger in diameter). They were composed of filaments that were loosely or compactly packed in the advanced phase, and frequently contained electron-dense granular or amorphous cores in the center. Cord-like swollen axons with or without a myelin sheath consisted of accumulated NFs running parallel to the longitudinal axis, and frequently showed a

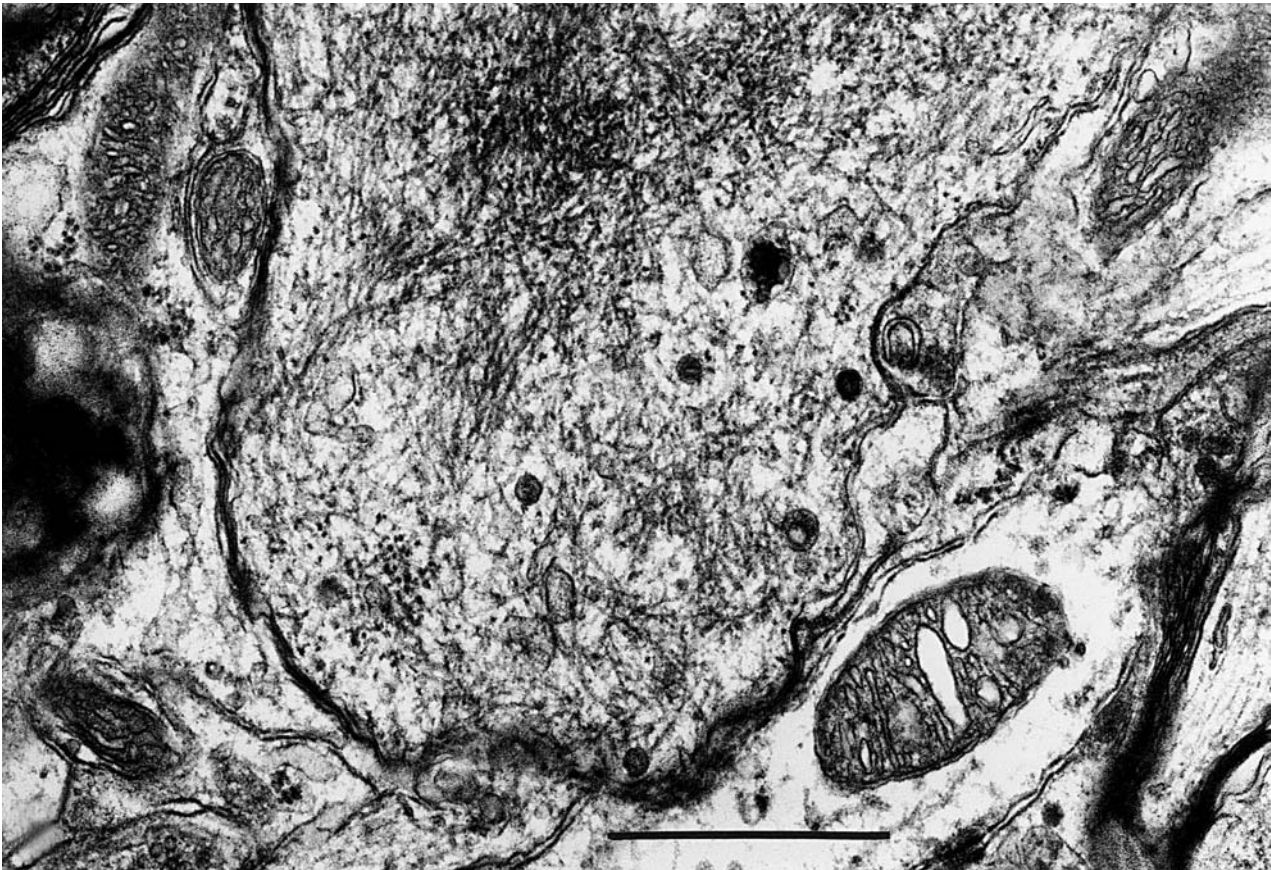


**Fig. 4** A Giant vacuoles accompanied by axonal swelling occupy almost the entire axonal caliber. Accumulation of mitochondria attached to the inner membrane of the vacuole is seen (late presymp-

tomatic stage). **B** Higher magnification of A. Accumulation of misdirected NFs is exhibited in close proximity to vacuoles or between large vacuoles. *Bar* 1  $\mu$ m



**Fig. 5** **A** A spheroid is observed in the anterior column (late presymptomatic stage). **B** Higher magnification of **A**. The spheroid is composed mainly of interwoven NFs with non-vacuolated round or sausage-shaped mitochondria. *Bar* 1  $\mu$ m



**Fig. 6** A filamentous aggregate is exhibited in the proximal axon. The aggregate consists basically of interwoven intermediate filaments slightly thicker than NFs (about 1.5 times larger in diameter) (late presymptomatic stage). Bar 1  $\mu\text{m}$

focal or massive misdirected array of NFs. They frequently contained vacuoles of various sizes, filamentous aggregates, LIs, and abundant non-vacuolated mitochondria.

At 32 weeks (symptomatic stage), mitochondrial vacuolation seen in the late presymptomatic stage persisted, although to a lesser extent. Accumulation of vacuolated or non-vacuolated mitochondria was frequently observed in accumulated NFs running parallel to the longitudinal section (Fig. 7). Mitochondria in axons were almost always round in shape, in contrast to the sausage-shaped, spindle-like, or slender rodlets seen in the controls. Many neurofilamentous accumulations of various sizes were scattered in the neuropils of the anterior horns (Fig. 8). Accumulation of filamentous aggregates tended to become much larger and frequently contained electron-dense cores in the center. On the other hand, mitochondrial vacuolation or abnormal accumulation of NFs was not observed in somata or proximal dendrites of anterior horn cells at any stage.

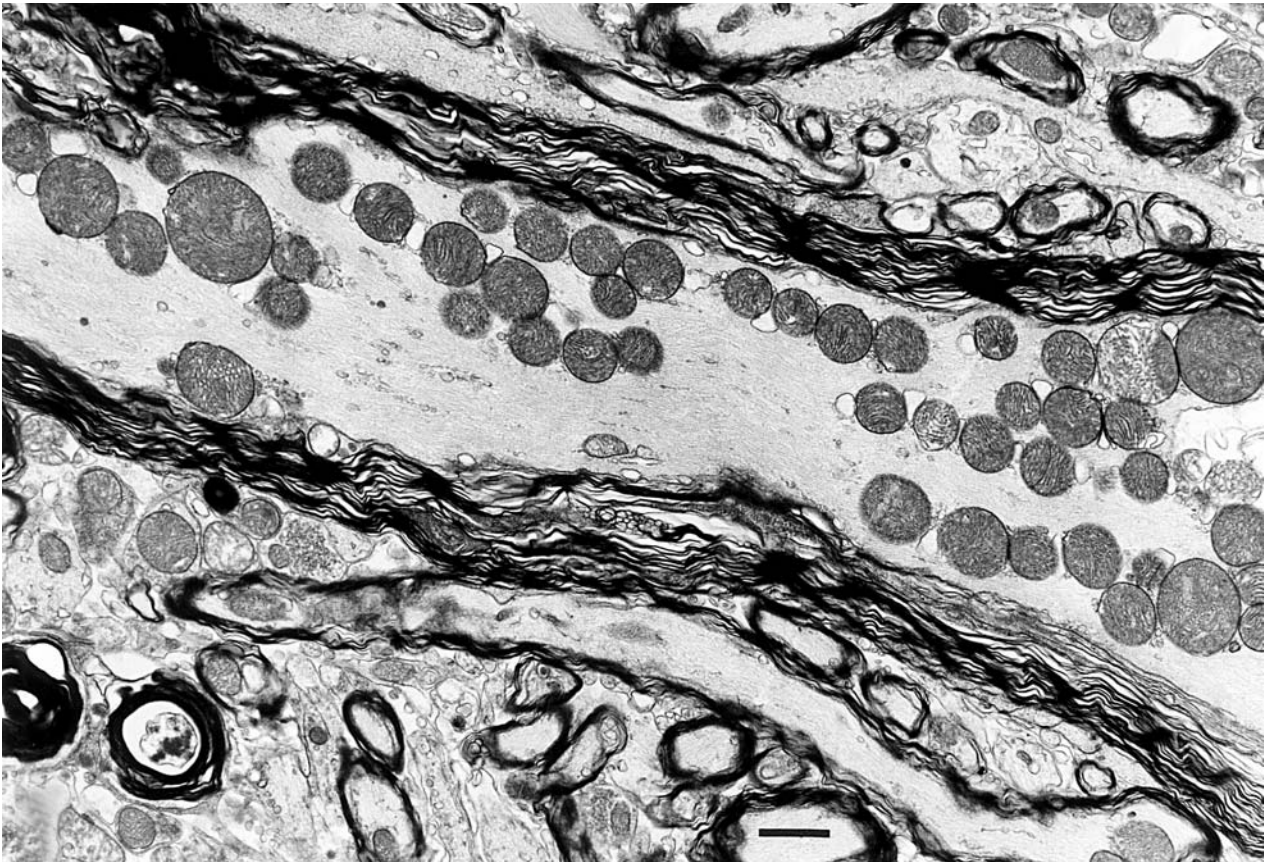
#### *Non-Tg littermates*

Occasionally vacuolar changes of mitochondria were seen in the axons of anterior horn neurons, but the vacuoles were almost always smaller in size and less common than

those observed in the Tg mice. No LIs or cord-like axonal swellings were observed in the spinal cord at any age.

#### **Discussion**

The G93A Tg mice used in this study were re-derived from the original line generated by Gurney et al. [7]. Mice expressing high copy numbers of human SOD1 carrying the G93A mutation (G1H/+ and G1L/+ lines) developed a disease with a relatively short course and with a pathology mainly characterized by severe vacuolar changes in the anterior horn neurons and their processes, LIs and swollen axons [3]. On the other hand, another line of G93A Tg mice with a low transgene copy number did not develop MND until 400 days of age and showed almost no vacuoles, although they had many more LIs in the motor neurons [5]. Thus, increasing levels of the mutated gene correlates with an earlier onset of MND, more abundant vacuoles, and less prominent LIs [4, 5, 16]. The expression level of the mutant transgene in our re-derived G93A mice (gene copy 10) is lower than that in the original G93A line, and the onset of MND was delayed by about 20 weeks compared to the time of onset in the original G93A line. Thus, from the clinical, pathological, and genetic aspects, the G93A Tg mice used in this study are unique and considered to be intermediate, between G93A Tg mice of a high transgene copy number and those of a low transgene copy number. The earliest change in this animal model is the appearance of mitochondrial vacuoles, subsequently



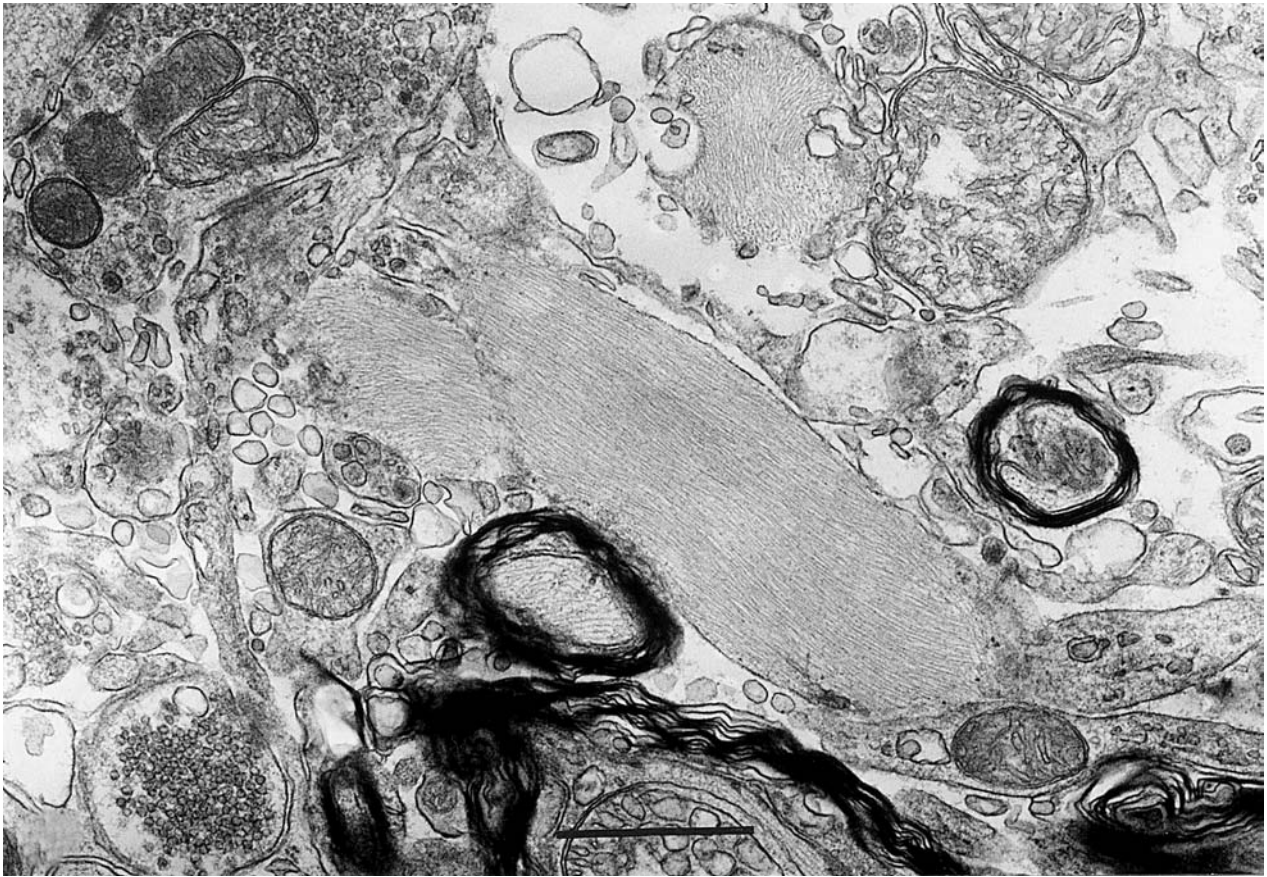
**Fig. 7** An accumulation of non-vacuolated mitochondria is observed in accumulated NFs running parallel to the longitudinal axis (symptomatic stage). Mitochondria in axons are almost always round in shape in contrast to the sausage-shaped or slender rodlets observed in the controls. Bar 1  $\mu$ m

followed by LIs and, later, accumulation of NFs. All these changes occurred already at the presymptomatic stage, predominantly in the neuronal processes, particularly in the proximal axons of the anterior horn cells; the somata of anterior horn neurons did not show mitochondrial vacuolation or accumulation of NFs at any stage, in contrast with previous reports of G93A Tg mice that showed vacuolation or accumulation of intermediate filaments in the somata [3, 4, 5]. These findings show that overproduction of NFs in the somata could not have led to the increase of NFs in the proximal axon. Thus, this animal model with a relatively low gene copy is fairly suitable for analyzing the pathomechanisms of impairment of slow axonal transport at the level of proximal axons.

Tg mice (SOD<sup>G37R</sup>, SOD<sup>G85R</sup>, SOD<sup>G93A</sup>) showing increased expression of wild-type NF subunits have been reported to develop decreased rates of slow axonal transport and prominent neurofilamentous accumulation near the end stage of disease [1, 3, 4, 13, 16, 18, 20, 21]. In Tg mice expressing the human SOD1<sup>G37R</sup> and SOD1<sup>G85R</sup> mutants, transport of NFs is markedly reduced before clinical disease onset or detectable pathology, and reduced transport of selective cargoes of slow transport, especially tubulin, arises months before neurodegeneration, demon-

strating that changes in slow axonal transport are an early event in the toxicity mediated by mutant SOD1 mutants [18]. Moreover, end-stage mice expressing the SOD1<sup>G93A</sup> mutation also demonstrate slowed axonal transport [21], although how early such changes occur has not been investigated. On the other hand, with regard to fast axonal transport, a reduced selective component of orthograde fast axonal transport in ventral root axons with the onset of motor weakness [21] and deficits in fast anterograde axonal transport in the peripheral nerves prior to disease onset [17] have been demonstrated in G93A Tg mice. Moreover, in G86R mutant mice, early up-regulation of a fast axonal transport component is seen in spinal cord motor neurons before the onset of disease [6]. However, in Tg mice expressing the human SOD1<sup>G37R</sup> and SOD1<sup>G85R</sup> mutants, no changes are detected in fast axonal transport [18].

In the present study, accumulations of NFs were observed in proximal axons but not in the cell bodies or proximal dendrites, and they became prominent with the progression of disease. This suggests that the impairment of slow axonal transport of NFs originates in the proximal axon before clinical onset, and that the progression of disease may be associated with impairment of axonal transport. The presence of abnormal NF aggregates in mice expressing mutant SOD1 raises the possibility that NFs may act as toxic intermediates in the disease. Vacuoles and LIs in the proximal axons, both of which are caused by SOD1 mutation, may physically block the slow axonal transport of NFs, secondarily leading to the accumulation of NFs in the proximal axons, then to disturbance of the transport of



**Fig. 8** Small accumulations of NFs are scattered in the neuropils of the anterior horn (symptomatic stage). Bar 1  $\mu$ m

vital organelles necessary to maintain the viability of neurons, and finally to axonal degeneration. Moreover, round swollen mitochondria and accumulations of round mitochondria in the proximal axon suggests that the fast component of axonal transport is also impaired in this region. Mitochondrial damage due to mutant SOD1 may interfere with oxidative phosphorylation and cause an energy shortage for the NF motor proteins via decreased ATP production. This could result in the failure of axonal transport, thereby causing accumulation of NFs and formation of large NF swellings in proximal axons.

Double Tg mice expressing human NF-H and SOD<sup>G37R</sup> proteins show that overexpression of NF-H, which raises perikaryal NF levels and lowers axonal levels, conferred remarkable protection against SOD1-mediated toxicity and extended the longevity of mutant SOD1 mice by 65% [2]. In the SOD1 mutant (G85R) with NF-L deletion, levels of the two remaining NF subunits, NF-M and NF-H, are markedly reduced in axons but are elevated in motor neuron cell bodies, and complete elimination of NFs, through disruption of the NF-L gene, delays onset of SOD1-mediated disease and extends life span by approximately 6 weeks (i.e., a ~15% extension of life span), showing that NFs are one determinant of the selectivity of motor neuron toxicity mediated by the SOD1 mutant [19]. Tg mice express-

ing SOD1 mutant G93A with overexpression of the mouse NF-H and NF-L subunit developed ALS later and survived longer than the G93A mice with a wild-type background (15% extension of life span) [11]. Whether the slowing of disease is a consequence of the depletion of NF in motor axons or of the accumulation of neurofilamentous proteins in the cell bodies of motor neurons remains to be shown. In this context, the accumulation of NFs in the proximal axons, without accumulation of NFs in the somata of anterior horn cells found in the present study, may be consistent with the selective motor neuron degeneration mediated by mutant SOD1.

**Acknowledgments** This work was supported by a Grant-in-Aid for General Scientific Research (C) from the Japanese Ministry of Education, Science and Culture, and by a grant from the Japan ALS Association.

## References

1. Bruijn LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG, Sisodia SS, Rothstein JD, Borchelt DR, Price DL, Cleveland DW (1997) ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* 18:327–338
2. Couillard-Despreés S, Zhu Q, Wong PC, Price DL, Cleveland DW, Julien J-P (1998) Protective effect of neurofilament heavy gene overexpression in motor neuron disease induced by mutant superoxide dismutase. *Proc Natl Acad Sci USA* 95:9626–9630



3. Dal Canto MC, Gurney ME (1994) Development of central nervous system pathology in a murine transgenic model of human amyotrophic lateral sclerosis. *Am J Pathol* 145:1271–1280
4. Dal Canto MC, Gurney ME (1995) Neuropathological changes in two lines of mice carrying a transgene for mutant human Cu, Zn SOD, and in mice overexpressing wild-type human SOD: a model of familial amyotrophic lateral sclerosis (FALS). *Brain Res* 676:25–40
5. Dal Canto MC, Gurney ME (1997) A low expressor line of transgenic mice carrying a mutant human Cu, Zn superoxide dismutase (SOD1) gene develops pathological changes that most closely resemble those in human amyotrophic lateral sclerosis. *Acta Neuropathol* 93:537–550
6. Dupuis L, Tapia M de, Rene F, Lutz-Bucher B, Gordon JW, Mercken L, Pradier L, Loeffler JP (1997) Differential screening of mutated SOD1 transgenic mice reveals early up-regulation of a fast axonal transport component in spinal cord motor neurons. *Neurobiol Dis* 7:274–285
7. Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, Caliendo J, Hentati A, Kwon YW, Deng H-X, et al (1994) Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. *Science* 264:1772–1775
8. Hirano A, Donnenfeld H, Sasaki S, Nakano I (1984) Fine structural observations of neurofilamentous changes in amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 43:461–470
9. Hirano A, Nakano I, Kurland LT, Mulder DW, Holley PW, Saccomanno G (1984) Fine structural study of neurofibrillary changes in a family with amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 43:471–480
10. Julien J-P (2001) Amyotrophic lateral sclerosis: unfolding the toxicity of the misfolded. *Cell* 104:581–591
11. Kong J, Xu Z (2000) Overexpression of neurofilament subunit NF-L and NF-H extends survival of a mouse model for amyotrophic lateral sclerosis. *Neurosci Lett* 281:72–74
12. Lee MK, Marszalek JR, Cleveland DW (1994) A mutant neurofilament subunit causes massive, selective motor neuron death: implications for the pathogenesis of human motor neuron disease. *Neuron* 13:975–988
13. Morrison BM, Janssen WG, Gordon JW, Morrison JH (1998) Time course of neuropathology in the spinal cord of G86R superoxide dismutase transgenic mice. *J Comp Neurol* 39:64–77
14. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng H-X, et al. (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362:59–62
15. Rouleau GA, Clarke AW, Rooke K, Pramatarova A, Krizus A, Suchowersky O, Julien J-P, Figlewicz DA (1996) SOD1 mutation is associated with accumulations of neurofilaments in amyotrophic lateral sclerosis. *Ann Neurol* 39:128–131
16. Tu P-H, Raju P, Robinson KA, Gurney ME, Trojanowski JQ, Lee VM-Y (1996) Transgenic mice carrying a human mutant superoxide dismutase transgene develop neuronal cytoskeletal pathology resembling human amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA* 93:3155–3160
17. Warita H, Itoyama Y, Abe K (1999) Selective impairment of fast anterograde axonal transport in the peripheral nerves of asymptomatic transgenic mice with a G93A mutant SOD1 gene. *Brain Res* 819:120–131
18. Williamson TL, Cleveland DW (1999) Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nat Neurosci* 2:50–56
19. Williamson TL, Bruijn LI, Zhu Q, Anderson KL, Anderson SD, Julien J-P, Cleveland DW (1998) Absence of neurofilaments reduces the selective vulnerability of motor neurons and slows disease caused by a familial amyotrophic lateral sclerosis-linked superoxide dismutase 1 mutant. *Proc Natl Acad Sci USA* 95:9631–9636
20. Wong PC, Pardo CA, Borchelt DR, Lee MK, Copeland NG, Jenkins NA, Sisodia SS, Cleveland DW, Price DL (1995) An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* 14:1105–1116
21. Zhang B, Tu P-H, Abtahian F, Trojanowski JQ, Lee VM-Y (1997) Neurofilaments and orthograde transport are reduced in ventral root axons of transgenic mice that express human SOD1 with a G93A mutation. *J Cell Biol* 139:1307–1315