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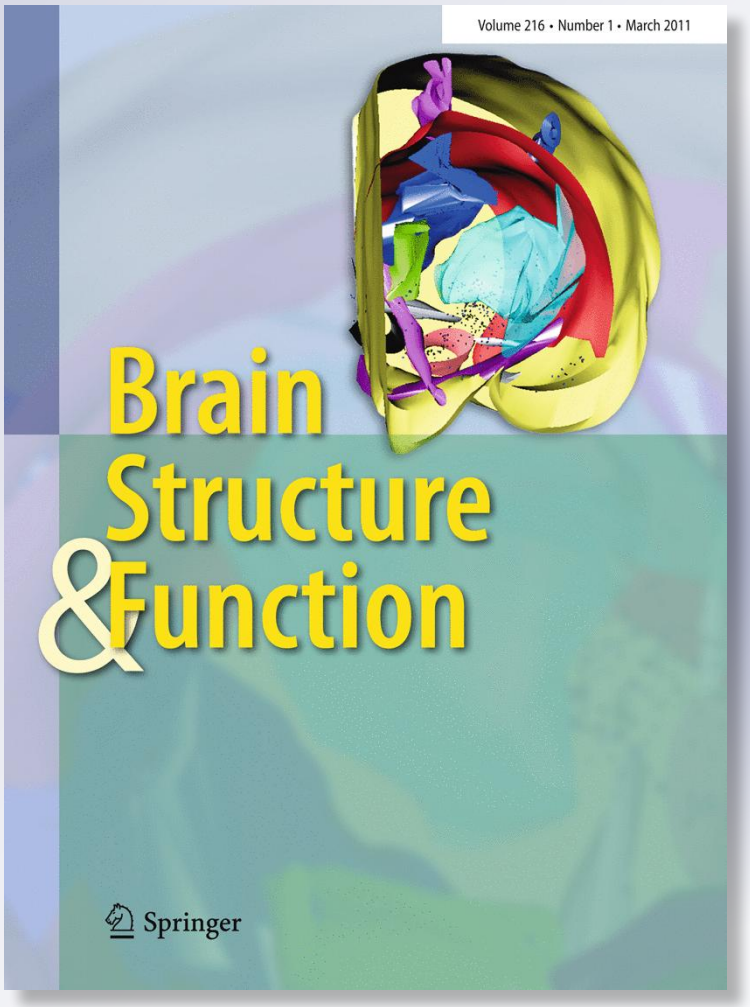

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ORIGINAL ARTICLE

Structural abnormalities in the cortex of the rTg4510 mouse model of tauopathy: a light and electron microscopy study

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Abstract rTg4510 transgenic (TG) mice overexpress mutant (P301L) human tau protein. We have compared the dorsal premotor cortex of TG mice versus non-transgenic (NT) mice at 4, 9, and 13 months of age, using light (LM) and electron microscopy (EM). LM assessment shows that cortical thickness in TG mice is reduced by almost 50% from 4 to 13 months of age, while at the same time layer I thickness is reduced by 80%, with most of the cortical thinning occurring between 4 and 9 months. In TG mice, spherical, empty vacuoles, up to 60 μm in diameter, become increasingly abundant with age and by 9 months, pyramidal and non-pyramidal neurons with large intracellular tangles of tau protein are common throughout the cortex. These tangles occur in the perikarya; we have not observed them entering into cellular processes, nor have we observed ghost tangles in the intercellular matrix. In TG mice, nerve fiber pathology is widespread by 13 months, and split myelin sheaths, ballooned sheaths, and swollen axons containing mitochondrial aggregations are all common. Astrocytes become increasingly filled with glial filaments as TG mice age, and microglial cells almost always contain phagocytic inclusions. However, no glial cells are seen to contain tau in their cytoplasm. These observations add to the base of knowledge available on this commonly employed model of tauopathy.

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Introduction

Tau protein is responsible for stabilizing microtubules and promoting their assembly from tubulin dimers (for review see Ashe and Zahs 2010; Weingarten et al. 1975). Malfunction of this protein is believed to be the primary cause of pathology in a wide range of neurodegenerative disorders referred to as tauopathies. These diseases include Alzheimer's disease, Pick's disease, and frontotemporal dementia and Parkinsonism linked to mutations in chromosome 17 (FTDP-17) (Iqbal et al. 2009; Janus 2008). In these conditions, the appearance of tau aggregated into neurofibrillary tangles (NFTs) in the somata of neurons is accompanied by widespread, progressive neuronal dysfunction and death, producing the severe cognitive and memory impairments characteristic of such disorders. Alzheimer's disease is the most common of the age-related dementias, and is characterized by the presence of extracellular deposits of amyloid-beta (Aβ) in addition to intracellular aggregates of tau (Ashe and Zahs 2010).

The initial cause of tau fibrillization to an aggregated form remains unclear; hyperphosphorylation is an early pathogenic event, and evidence that ties the activity of caspase to NFT formation and cellular pathology implies that tau protein must first be cleaved to become toxic and ultimately insoluble (de Calignon et al. 2010; Gamblin et al. 2003; Rissman et al. 2004; Rohn and Head 2008), but the specific biochemical process is still poorly understood. One hypothesis is that soluble tau species accumulate progressively in a neuron until apoptotic pathways are initiated, leading to cleavage of tau by caspase. The

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resulting fragments then either bring about the death of the neuron by triggering apoptosis, or they fibrillize into NFTs, allowing the neuron to continue functioning despite the presence of insoluble tau. If this is the case, neurons are faced with an initial apoptotic "attack", and subsequently either survive to form NFTs or undergo apoptosis and die (de Calignon et al. 2010; Ramalho et al. 2008).

In an attempt to replicate the symptoms and progression of tauopathy, several mutant mouse lines have been developed that overexpress wild-type or mutated human tau. Many of the early models of tauopathy mimic only some features of human disease, such as tau phosphorylation and pretangle formation, but fail to reproduce other important characteristics of tauopathy in humans, such as neuronal loss in the cortex and hippocampus and severe memory deficits (Duff and Suleman 2004). However, mutant mouse lines that more closely approximate human disease states have been developed in recent years, and one such mutant is the rTg4510 model (Janus 2008; Santacruz et al. 2005; Spires et al. 2006). Mice in this lineage overexpress mutant (P301L) human tau, which has been linked to hereditary tauopathy (Bird et al. 1999; Mirra et al. 1999; Nasreddine et al. 1999; Ramsden et al. 2005), and produce only one of the six possible isoforms of tau, designated 4R0N. Afflicted mice rapidly develop intraneuronal aggregates of fibrillar tau, experience massive cortical and hippocampal neuronal loss, and show symptoms of memory loss similar to human patients. Importantly, mutant tau expression is repressible in the rTg4510 line by adding doxycycline to the mouse diet, which prevents a transactivator from binding to and activating the promoter driving human tau expression (Janus 2008; Santacruz et al. 2005). This makes it possible to observe the effects of suppression of tau pathology at any age.

Because the rTg4510 mutant has been relatively recently developed (Janus 2008; Santacruz et al. 2005), the amount of cortical ultrastructural data available is limited, since most of the previous studies on this mutant have focused on the hippocampus (Santacruz et al. 2005; Spires et al. 2006). Ultrastructural studies have been undertaken on other tau mutant mice such as the JNPL3 mutants (Lin et al. 2003b; Lin et al. 2005), but not on the rTg4510 mutant, in which tau expression is restricted to the forebrain. This is in contrast to JNPL3 mutants, in which the expression of tau pathology develops primarily in brainstem and spinal cord (Janus 2008; Lin et al. 2005). Despite the difference in expression pattern, JNPL3 tau pathology is similar to rTg4510 in many respects, including swollen axons, degenerating myelin, NFTs, and active microglial cells (Lin et al. 2003b; Lin et al. 2005). However, rTg4510 mice do not show filamentous tau in neuroglial cells, as JNPL3 mice do, and the swollen neurons with sparsely scattered tau seen in JNPL3 mice are not present in rTg4510 (Lin et al. 2003a, 2003b). Strains that have been specifically developed to express tau in oligodendrocytes have yielded insight into the role of oligodendroglial degeneration in tauopathy, but such models are intended only to investigate this specific disease process and do not recreate the wider spectrum of human symptoms accurately (Higuchi et al. 2005).

The present study aims to present a thorough examination of structural abnormalities in the cerebral cortex of rTg4510 TG mice, both on the light (LM) and electron microscopic (EM) levels. This additional information will increase the depth of knowledge available on rTg4510 mice, and help guide future studies on this mutant strain.

Materials and methods

Experimental subjects

A total of six rTg(tau_{P301L})4510 experimental mice (TG) and five non-transgenic mice (NT) were used in this project. At perfusion, the ages of the mice were 4 months (*n*: 1 TG; 1 NT), approximately 9 months (range: 8 months 28 days–9 months 16 days; *n*: 2 TG, 1 NT) and approximately 13 months (range: 12 months 15 days–12 months 21 days; *n*: 3 TG, 4 NT). Using PCR analysis of tail DNA, all mice were screened for the tetracycline-controlled transactivator (tTA) transgene downstream of a Ca^{2+}-calmodulin kinase II promoter, and for responder human P301L tau downstream of a tetracycline operon (tetO) response element (TRE). Mice were given unlimited access to food and water. Animal care and experimentation was carried out in accordance with guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals, and was approved by the Institutional Animal Care and Use Committee (IACUC) at Boston University.

Slice preparation

Mice were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutol). A fixative solution of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2–7.4 and 37°C was perfused transcardially, and a tail clipping was removed for the genotypic analysis described above. Perfused mice were immediately decapitated and their calvaria removed; heads were submerged in a solution of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2–7.4, and stored at 4°C for at least 12 h before the brains were removed. Coronal frontal slices, approximately 1 mm thick, were obtained from the dorsal premotor cortex at

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Table 1 EM assessment of proportion of neurons containing NFTs

Age group (months)	Number of perikarya examined	Proportion of neurons containing NFTs		
		Overall (%)	Upper half (%)	Lower half (%)
4	150	0	0	0
9	150	37	51	28
13	290	31	32	28

the anteroposterior level between the anterior commissure and the hippocampus. The ventral portion of the slice was removed, and the dorsal cortex was divided into two or three pieces for embedding.

Embedding procedure for electron microscopy

For each experimental and control subject 3–4 blocks of tissue were examined. The pieces of dorsal premotor cortex were rinsed three times in 0.1 M sodium cacodylate buffer (pH 7.2–7.4) and osmicated using 1% osmium tetroxide in cacodylate buffer. Following osmication, pieces of cortex were dehydrated using ascending ethyl alcohol concentration steps, followed by two rinses in propylene oxide. Infiltration of the embedding medium was performed by immersing the pieces in a 1:1 mixture of propylene oxide and Araldite 502 plastic (Ernest F. Fullam, Inc.) overnight on a rotator and allowing the propylene oxide to evaporate. Cortical pieces were then rotated in pure Araldite for at least 6 hours, transferred to Beem capsules, and hardened at 60°C.

Section preparation

Sections were prepared for LM and EM using a RMC MT6000-XL ultramicrotome. One-micron sections were cut using a glass knife, mounted on glass slides and stained at 60°C for 2 minutes with 1% toluidine blue. Thin sections were cut and mounted on copper grids. Sections were then stained using uranyl acetate and lead citrate. Thin sections were examined and photographed using a JEOL 100S electron microscope (JEOL, USA, Peabody MA). Photographic negatives were scanned at 800 dpi using an Epson Perfection V700 photo scanner. Contrast adjustment of images and measurements of tau filament diameters were performed in Adobe Photoshop.

Neurons containing tau

To directly assess the proportion of neurons that contained fibrillar tau, vertical thin sections from 4, 9 and 13-month-old mice were examined by EM. The thin sections, which extended through the depth of the dorsal premotor cortex, were mounted on copper grids. Every perikaryal profile in a section was individually examined for the presence or absence of visible tau filaments. If dendrites were observed extending from the perikaryon they were also examined for the presence of tau. In both the 4 and 9-month-old mice, 150 neuronal profiles were examined, and in the 13-month-old mouse 290 neuronal profiles were assessed. The proportion of perikaryal profiles that contained tau filaments was determined for both the upper and the lower half of the cortical thickness (Table 1).

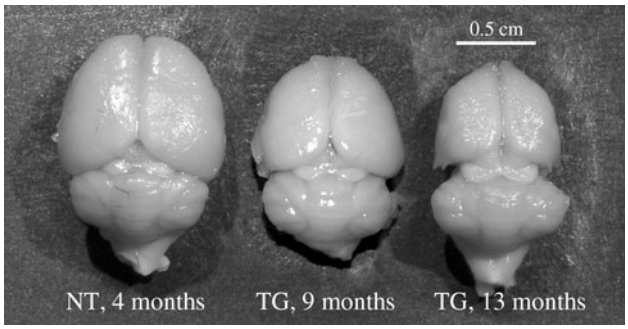

Fig. 1 Reduction in the size of the forebrain with age in TG mice. While 4-month-old NT (*left*) and TG brains did not differ in size, the cerebral hemispheres of 9 (*middle*) and 13-month-old TG (*right*) brains were markedly reduced in size. Note that atrophy is only apparent in forebrain structures

Results

Cortical atrophy

It is evident that the cortex of the tau mutant becomes thinner with age. Atrophy is apparent on gross examination of rTg4510 mouse brains, which have dramatically smaller cerebral hemispheres than age-matched controls (Fig. 1). At 4 months of age, the thickness of the premotor cortex of TG and NT mice is similar (see Fig. 2), but between 4 and 13 months the thickness of the premotor cortex in TG animals decreases dramatically (Fig. 2a), while remaining unchanged in NT mice. Because of this reduction in cortical thickness and an accompanying loss of neurons, it becomes increasingly difficult in older TG mice to identify cortical layers. As the overall thickness of the cortex is reduced in mutant mice, layer I, which contains the apical tufts of pyramidal cells, becomes very thin and is reduced by about 80% (Fig. 2a).

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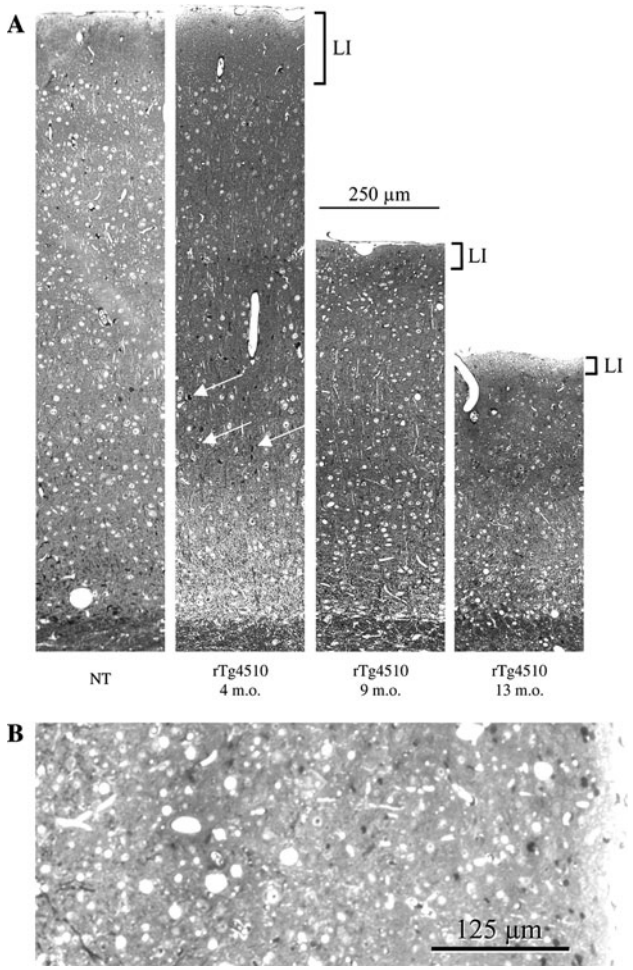

Fig. 2 Vertical sections through the cerebral cortex. All sections were stained with 1% toluidine blue. **a** NT and 4-month-old rTg4510 mice have similar cortical thicknesses, but in 9 and 13-month-old mice, cortical thicknesses are severely reduced in TG as compared to age-matched NT mice. Note the progressive atrophy of layer I (LI) in rTg4510 animals. Dark layer V neurons are visible in the image of 4-month-old cortex (*arrows*). **b** Enlarged image of 13-month-old rTg4510 cortex, illustrating the spongiform appearance of the cortex. The pial surface is to the right

Neurons

In TG mice, NFTs can be observed in some neurons. In the ages of mice that we examined, such tangles are seen at 9 months of age (Fig. 3) but not at 4 months of age. Tangles occur in the perikarya of both pyramidal (Figs. 3, 5) and non-pyramidal neurons (Fig. 6), and neurons containing NFTs are present in all layers of the cortex. Tau filaments are 16–20 nm in diameter and are separated from one another by 20–25 nm. In contrast to the profiles of microtubules, which appear hollow when cross-sectioned, tau filaments appear as discrete dots (Fig. 4). Their longitudinal profiles are regular in outline and they show no signs of twisting or periodicity. In NFTs, the tau filaments are usually arranged in either parallel or herringbone patterns (Figs. 3, 4, 5) and only rarely are tau filaments found in more diffuse, disorganized collections. In neurons that contain tau, the bundles of filaments are separated from one another by narrow corridors of cytoplasm, and the NFTs are most frequently located around the periphery of the cell body, displacing the cell's organelles towards the nucleus. Even when neurons contain only a small amount of tau the filaments are still organized into bundles (Fig. 6). Interestingly, in no neurons with a soma containing tau filaments have we observed tau filaments to pass from the soma into dendrites or axons (Figs. 3, 5), although some tau filaments are occasionally visible in the cytoplasm of cross-sections of neuronal processes within the neuropil.

Degenerating neurons are plentiful, but we have only encountered one degenerating neuron that has large NFTs. This NFT-containing neuron, in a 13-month-old TG mouse, has a dark cytoplasm, an irregular outline, and swollen astrocytic processes surrounding it (Fig. 7b).

No NFTs were observed at the EM level in 4-month-old mutants. In 9-month-old mutants, however, our EM analysis shows that overall some 37% of neuronal profiles that were examined (Table 1) contain NFTs, and by 13 months this proportion declines slightly to 31%. Between 9 and 13 months of age, the proportion of NFT-positive cells in deeper layers of cortex remains at about 28%, while the proportion of NFT-positive cells in superficial layers of cortex decreases from 51 to 32% (Table 1). Both our own assessments and the findings of other studies indicate that neurons are progressively lost with age in rTg4510 mutants (de Calignon et al. 2009; Ramsden et al. 2005; Santacruz et al. 2005; Spires et al. 2006), which combined with the fact that the proportion of NFT-positive cells decreases or remains the same during this time, means that tau-filled neurons are being lost as rTg4510 mice age.

In the TG mice that have been examined, those at 4 months of age have some shrunken pyramidal neurons with dark cytoplasm in cortical layer V, but such dark neurons have no visible tau filaments within their cytoplasm (Fig. 7a). These dark layer V neurons have axosomatic synapses, are often surrounded by swollen astrocytic processes, and are adjacent to apparently normal neurons. Neurons with dark cytoplasm are also present in older TG mice, though in these mice such neurons are present in all cortical layers, and it is assumed that the axonal and dendritic profiles with dark cytoplasm that are encountered in the neuropil originate from such darkened neurons, which are most likely dying.

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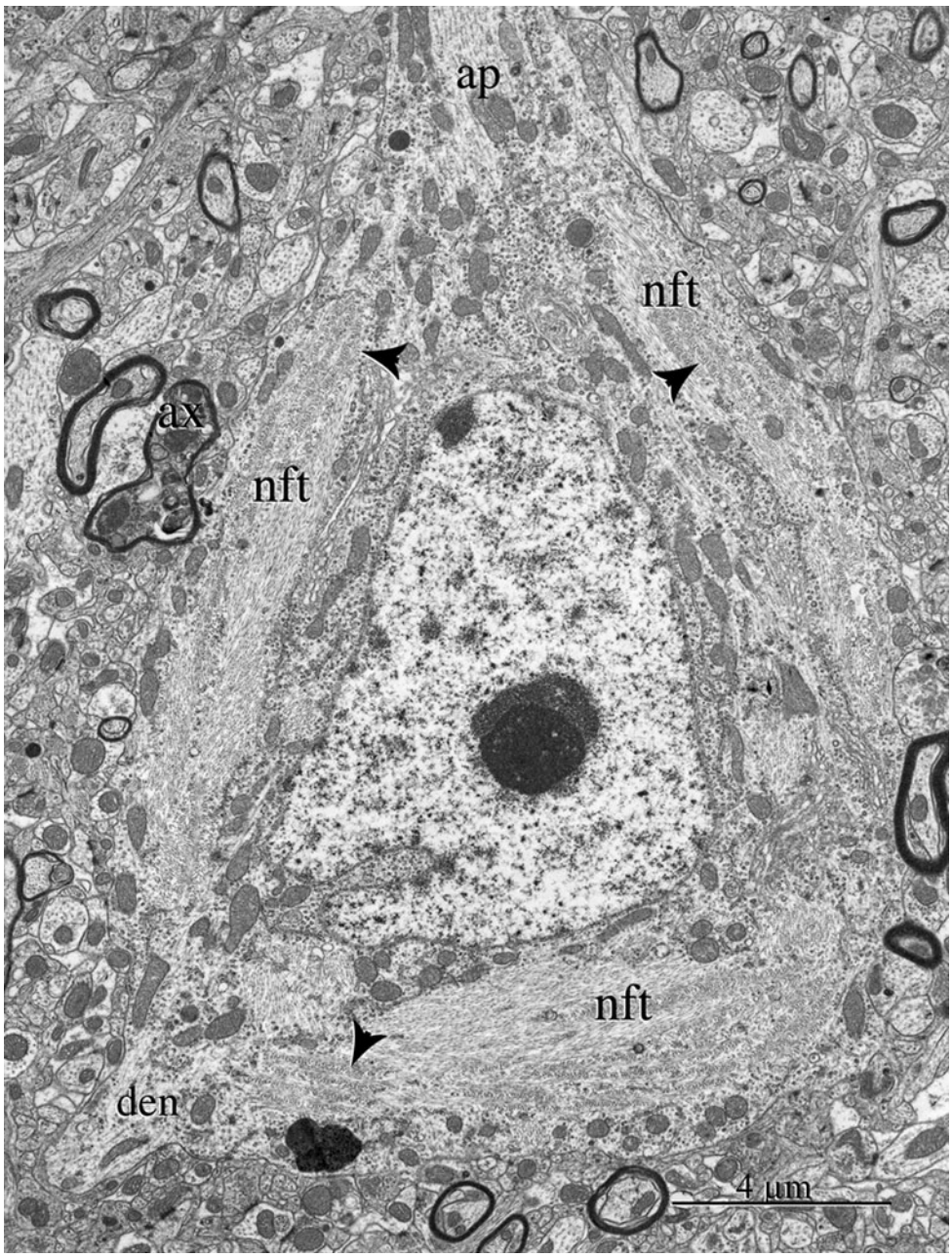

Fig. 3 Layer V pyramidal neuron with large neurofibrillary tangles (*nft*), some of which show herringbone patterns (*arrowheads*). The pial surface is towards the top. Tangles are arranged around the periphery of the cell, displacing organelles towards the nucleus. Note that NFTs do not enter either the basal dendrite visible at lower left (*den*) or the apical dendrite (*ap*). The degenerating axon (*ax*) of a myelinated nerve fiber is visible at left. 9-month-old rTg4510 mouse cortex

Myelinated nerve fibers

Myelin sheath abnormalities are a characteristic feature of TG cortical tissue at 13 months of age. Up to 9 months of age, most of the myelinated nerve fibers have a normal appearance, but by 13 months, myelin disruption is widespread. In some sheaths, the myelin lamellae have become separated from one another to form loose spirals, and in other nerve fibers the sheaths show extensive splitting; in some fibers the inner lamellae of the sheath are separated from the outer lamellae by a wide space. In yet other examples, large bulges, which may be empty or filled with degenerating cytoplasm, extend from the sheaths (Fig. 8).

In addition to the changes in their sheaths, some of the axons in myelinated nerve fibers are dark and degenerating, while other axons are swollen and filled with mitochondria. In yet other nerve fibers, the axon has been lost, leaving an empty, usually thin, myelin sheath. It should also be mentioned that a small number of myelinated and unmyelinated axons have an unusually high density of microtubules in their cytoplasm, though the origins of such axons could not be ascertained.

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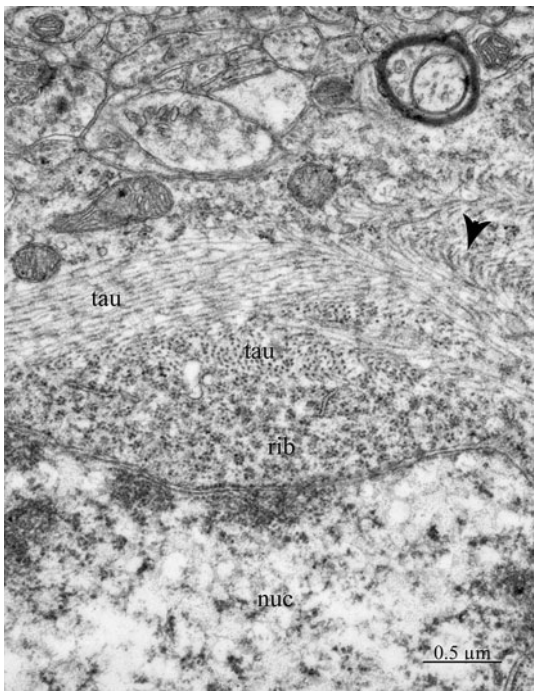

Fig. 4 Both herringbone (*arrowhead*) and parallel arrays of tau are visible in the cytoplasm of this neuron, the nucleus of which (*nuc*) is at the bottom of the image. Ribosomes (*rib*) cluster near the nuclear envelope, beyond which are tau filaments in cross-section and longitudinal section (*tau*). Filaments are regularly spaced, separated from each other by an interval of 20–25 nm, and are about 16–20 nm in diameter. Filaments show no sign of periodicity or twisting. 9-month-old rTg4510 mouse cortex

Vacuoles

The presence of numerous holes (or "vacuoles") within the neuropil and throughout the depth of the cortex, up to 60 μm in diameter, is a hallmark of the aging TG cortex. Though a few vacuoles are present at 9 months, they are abundant by 13 months (see Fig. 2b). Each one is bounded by a plasma membrane and although most are empty, some contain flocculent (Fig. 10) or membranous debris (Fig. 9). Because the profiles of the vacuoles are always circular, it is evident that the vacuoles must be spherical. Interestingly, some vacuoles indent neurons very deeply, so that processes of the neuronal soma sometimes completely surround the vacuoles (Fig. 9). The distribution of the vacuoles appears to be random, and the neuropil surrounding vacuoles appears to be normal, so that despite an extensive search, the origins of the vacuoles could not be determined.

Neuroglia

No tau filaments have been observed in any of the neuroglial cell types in the cortex. Oligodendrocytes do not appear to alter much with age in these mutant mice, despite that fact that there is degeneration of some nerve fibers. On the other hand, microglia which are distinguished from the oligodendrocytes by their long cisternae of endoplasmic reticulum and their irregular shapes, invariably contain phagocytic inclusions in the cortices of the 9 and 13-month-old mice. Also in the 9 and 13-month-old mice, the processes of astrocytes become thick and extremely fibrous, being filled with glial filaments that are organized in definite bundles, in which they are packed more tightly than tau filaments in NFTs (Fig. 10). As pointed out, swollen astrocytic processes, largely devoid of glial filaments, surround darkened neurons, although such processes do not surround tau-filled neurons.

Discussion

The obvious reduction in cortical thickness in rTg4510 mice has been noted by others (de Calignon et al. 2009). A reduction in cortical thickness has also been reported in human sufferers of tauopathy-related diseases such as Alzheimer's disease and frontotemporal dementia (Baloyannis 2005; Du et al. 2007; Im et al. 2008; Richards et al. 2009), although decreasing cortical thickness is not always correlated to declining mental function (Perl 2010). In the rTg4510 mice used in the present study, premotor cortical gross anatomical changes were most dramatic between 4 and 9 months, suggesting that during this time period a buildup of mutant tau leads to widespread cell death. Additional cortical atrophy occurs between 9 and 13 months of age and neurons continue to be lost, but at an apparently slower rate than at younger ages.

Layer I degenerates at a much greater rate than deeper cortical layers, and by 13 months it is reduced to only 20% of its original thickness. Since there are few neurons in layer I, neuronal loss cannot account for the reduction in thickness. However, because layer I largely consists of the apical tufts of pyramidal cells, the reduction in thickness of this layer must be due to both the loss of apical dendrites from neurons that have degenerated and a reduction in the apical dendritic complexity of extant neurons. This conclusion is supported by the recently published results of Rocher et al. (2009), who have shown a marked reduction in apical dendritic length and complexity in rTg4510 layer III pyramidal neurons of 8.5-month-old mice.

The fact that most neurons contain either large NFTs or no filaments at all suggests that the process of fibrillization is rapid (de Calignon et al. 2010). One of the most notable findings in the rTg4510 mouse is that NFT formation proceeds even after suppression of high levels soluble human tau (Santacruz et al. 2005), indicating that insoluble tau might itself be capable of catalyzing the transformation

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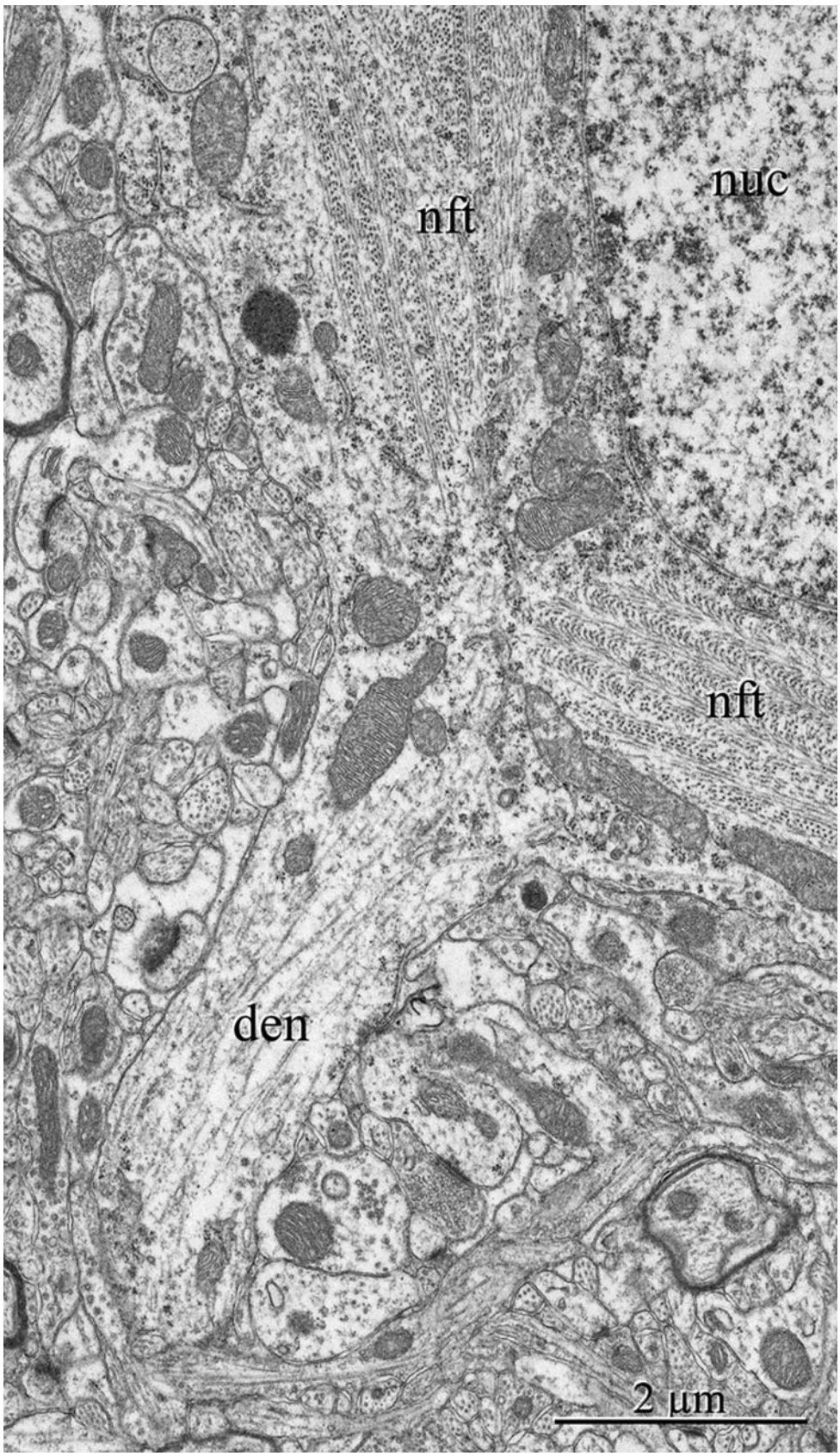

Fig. 5 Tau filaments are not observed to pass into dendrites or axons from the NFTs in the somata of neurons. Here, intraneuronal NFTs (*nft*) are seen terminating at the base of a basal dendrite (*den*), which extends towards *lower left*. The nucleus of the neuron (*nuc*) is visible at *upper right*. 9-month-old rTg4510 mouse cortex

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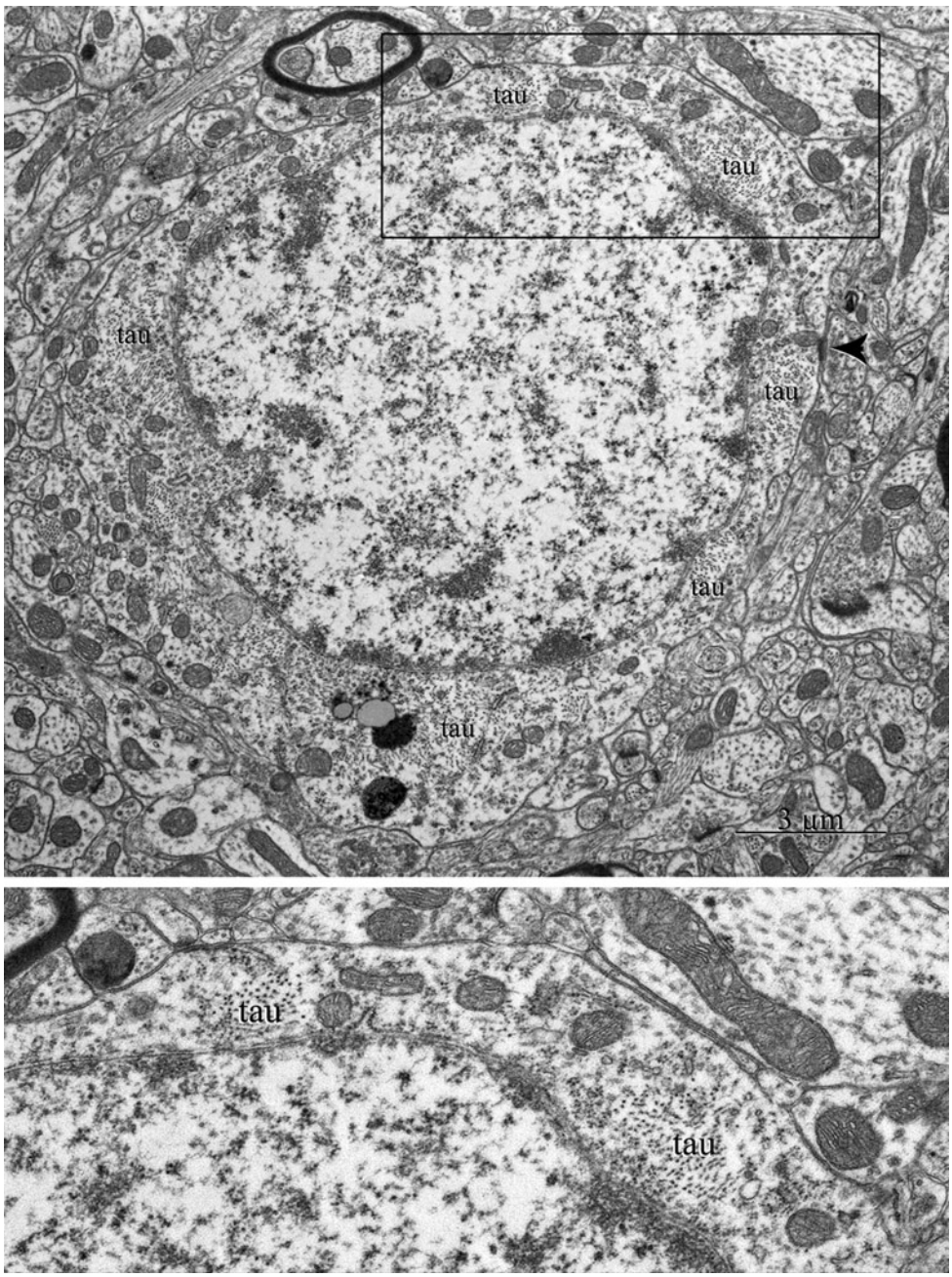

Fig. 6 While most tau-positive neurons are completely filled with tangles, some neurons have small amounts of filaments. However, in these neurons, filaments are still arranged into discrete bundles. This layer III neuron shows several small collections of tau filaments (*tau*) throughout its perikaryal cytoplasm. An asymmetric synapse on the soma of the neuron (*arrowhead*) identifies it as a non-pyramidal cell. The boxed region is enlarged below the image to show two small tau bundles in detail. 9-month-old rTg4510 mouse cortex

from the soluble to the insoluble form. Interestingly, we have not observed tau filaments to pass from the NFTs in the soma into either dendrites or axons, although neuronal processes containing some tau filaments have occasionally been encountered in the neuropil. However, the origins of such processes could not be ascertained. The mechanism by which tau is segregated so completely into the somatic compartment has not yet been elucidated, but it is possible that only the soma has the biochemical mechanisms to induce tau to fibrillize, which then drives the formation of large tangles.

An unresolved question in rTg4510 mice is why there is a lack of ghost tangles, the term for aggregates of tau that remain intact after cell death. Neuronal loss occurs in rTg4510 mice with advancing age (Santacruz et al. 2005; Spires et al. 2006), and we have shown that the overall proportion of neurons with fibrillar tau in their perikarya decreases or remains constant from 9 to 13 months of age. Since there is a concomitant overall loss of neurons, the implication is that neurons with NFTs are being lost with age. However, no ghost tangles have observed in this study, and neither has extracellular tau been seen, which is

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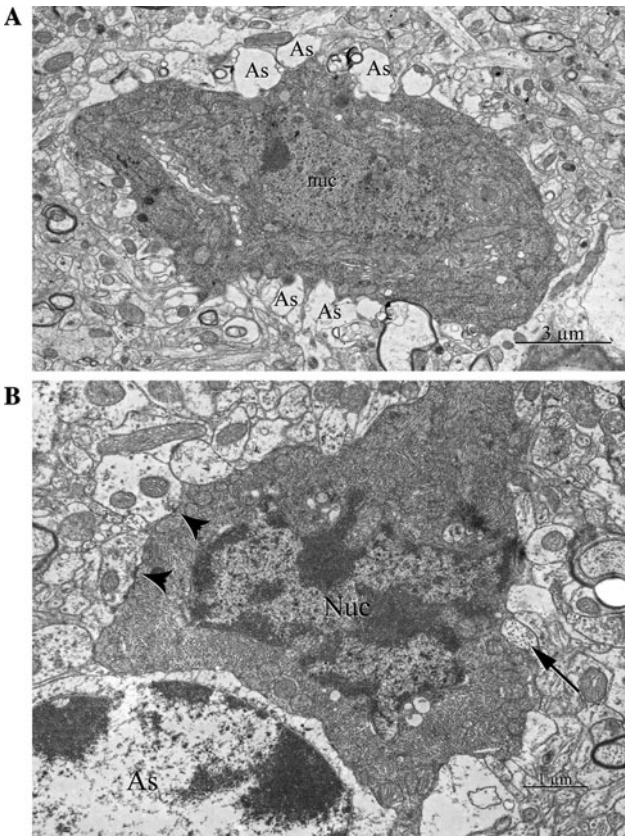

Fig. 7 Two dark neurons are shown. **a** The shrunken appearance, irregular nucleus (*nuc*), jagged outline, and swollen astrocytic processes (*As*) surrounding this neuron suggest that it is degenerating. There are no tau filaments present within the cytoplasm, and this is true for all dark 4-month-old layer V neurons at this age. Dark neurons without tau are also present in older rTg4510 animals, but occur throughout the cortex. 4-month-old rTg4510 mouse cortex. **b** Only one clearly degenerating neuron was found to contain neurofibrillary tangles in this study. This neuron's cytoplasm is completely filled with tau filaments, and the cell's jagged outline, irregular nucleus (*nuc*), and shrunken appearance indicate it is dying. However, two axosomatic synapses are still present (*arrowheads*). The nucleus of an astrocyte (*As*) appears at *lower left*, and some tau filaments are visible in a neuronal process to the right of the dark neuron (*arrow*). No ghost tangles are observed in rTg4510 mice, despite the deaths of NFT-positive cells. 13-month-old rTg4510 mouse cortex

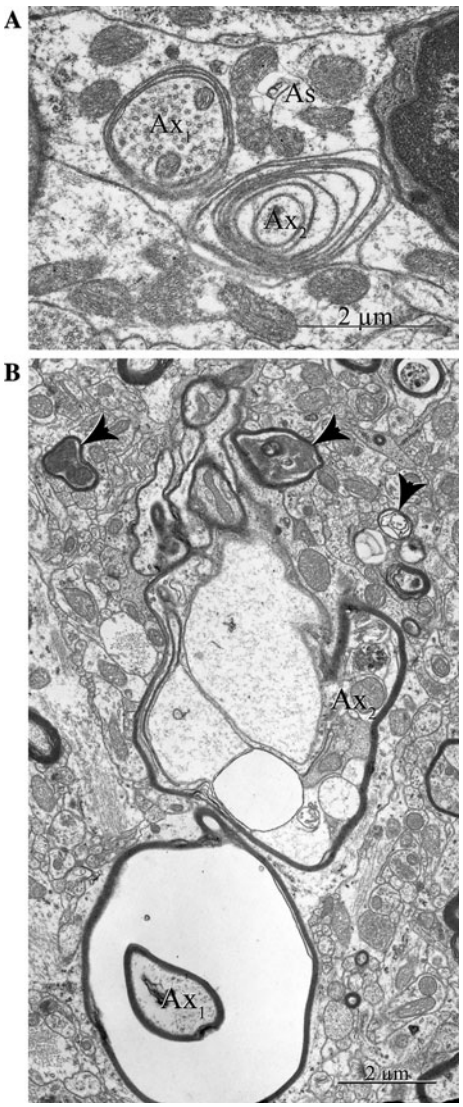

Fig. 8 Common myelin sheath abnormalities are shown. **a** The lamellae of the myelin sheath at lower right (Ax_2) are separated from each other, so that the sheath is loose. Its companion axon (Ax_1) has a normal appearance. The dark cytoplasm and nucleus of an endothelial cell is visible at right, and both axons are surrounded by an astrocytic process (*As*). 9-month-old rTg4510 cortex. **b** The inner myelin lamellae of the axon at bottom (Ax_1) are detached from the outer lamellae, and a large space separates them. At top is another axon (Ax_2) with a fragmented appearance and numerous membrane-bound compartments within it. Additional degenerating nerve fibers are indicated by arrowheads. 13-month-old rTg4510 cortex

consistent with similar observations by de Calignon et al. (2009) on rTg4510 mice. The tendency of NFTs to persist as ghost tangles after cell death is well documented in human disease, as well as in some other murine tauopathy models (Schindowski et al. 2006). It might be supposed that microglia phagocytose degenerating neurons that contain tau filaments, but while the microglial cells observed in this study all showed numerous phagocytic inclusions in their cytoplasm, suggesting they are in an activated state and engaged in clearing debris from the cortex, no tau debris was observed within them. A possible explanation can be found in the fact that most ghost tangles are immunoreactive primarily for the 3R isoform of tau, and almost never the 4R isoform (Iseki et al. 2006); consequently, rTg4510 mice, which express the 4R isoform exclusively, would not be expected to form ghost tangles, since the 4R isoform apparently does not persist after cell death (de Calignon et al. 2009). The ultimate fate of intracellular tau in the rTg4510 mutant is an unresolved question and deserves further study.

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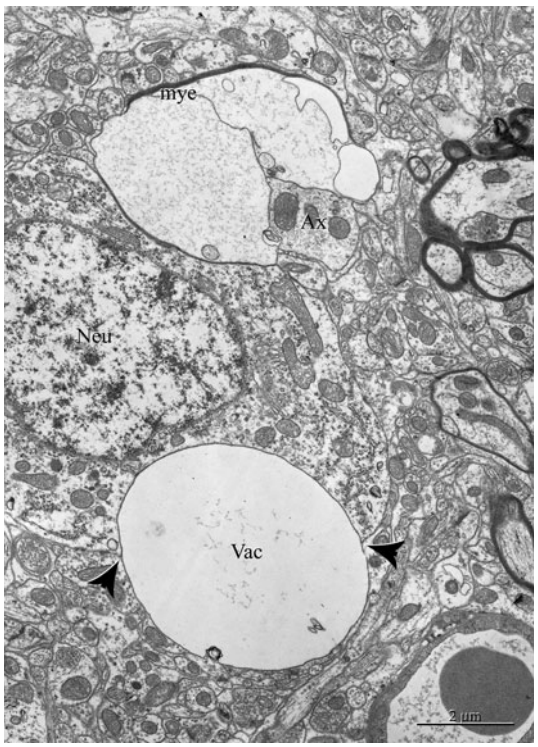

Fig. 9 This vacuole (*vac*) is deeply indenting the surface of the neuron (*Neu*), the nucleus of which is at *center left*. The neuronal cytoplasm extends almost halfway around the vacuole (*arrowheads*). Above the vacuole, a swollen axon (*Ax*), with a fragmented appearance and a myelin sheath of varying thickness (*mye*), indents the neuron to a lesser degree. 13-month-old rTg4510 mouse cortex

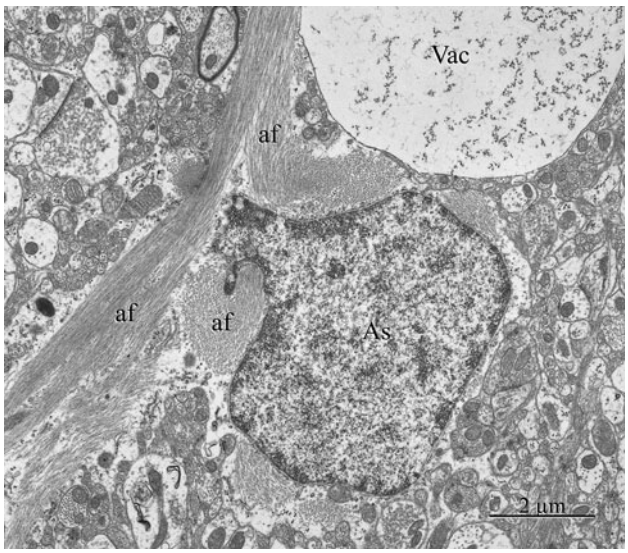

Fig. 10 Hypertrophic astrocytes such as this are common in rTg4510 cortex. A large vacuole is visible at the top of the image (*Vac*), and the nucleus of the astrocyte (*As*) is immediately below it. Several large bundles of astrocytic filaments (*af*) extend throughout the astrocyte and into its processes. 13-month-old rTg4510 mouse cortex

Though NFTs were not observed, the dark layer V cells visible at 4 months may represent the beginnings of tau-related neuronal cell death in the cortex. These dark cells are spread throughout layer V and are not seen in other cortical layers at this age. This is of interest, since histological techniques reveal hyperphosphorylated and insoluble tau as early as 2.5 months of age in rTg4510 mice (de Calignon et al. 2009; Spires et al. 2006). At later ages, neurons with dark cytoplasm become more common and occur in all cortical layers, and it assumed that such neurons are dying. Yet interestingly, as pointed out, only one dark neuron with tau filaments in its cytoplasm has been encountered, and this was in a 13-month-old mouse.

The prevalence of degenerating nerve fibers increases with age in TG animals. Similar observations in other mouse models of tauopathy have led to the hypothesis that mutant tau brings about neuronal cell death by collapsing the microtubule pathways of the cell and, by extension, intracellular transport (Hasegawa et al. 1998; Hong et al. 1998). However, as previously discussed, even cells with massive amounts of aggregated tau in their cytoplasm can continue to function (de Calignon et al. 2009; Rocher et al. 2009; Santacruz et al. 2005). Nevertheless, signs of impaired intracellular transport are apparent in rTg4510 mice, since with age axons that are enlarged and contain accumulations of mitochondria become increasingly common. At the same time, other axons have an increased density of microtubules, though such axons show no obvious signs of degeneration. These axons could represent an early stage of tau pathology, as the normal function of tau is lost, but at present, their significance is unknown.

In TG animals at 13 months, disrupted myelin sheaths are extremely common. The most frequently encountered pathology is the splitting of the lamellae of myelin sheaths, resulting in myelin disruption and, sometimes, swelling of the nerve fibers. Myelin disruption is also common in many other TG mouse models of tauopathy (Adams et al. 2009; Desai et al. 2009; Higuchi et al. 2002; Leroy et al. 2007; Lewis et al. 2000; Lin et al. 2003b; Lin et al. 2005). Somewhat less common but much more striking are the large, highly irregular, swollen myelinated nerve fibers that generate profiles in which it is difficult to identify the axon. These swellings are structurally very similar to the "axonal spheroids" described by other authors, and they are a frequently noted feature of both human and mouse model tauopathy, although the detailed features of the pathology differ from animal model to animal model. Tau aggregates in swollen fibers have not been encountered in the present study, and this is in contrast to the large NFTs reported by Lin et al. (2003b) and Probst et al. (2000) inside myelinated axons of mice in other mutant lines. A possible explanation for the presence of degenerating nerve fibers in the neuropil of the rTg4510 mutant is simply that their parent neurons

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are degenerating, but the relative lack of dying neurons in the cortex at any one time points towards an axon-specific pathology.

Possible end-stages of these degenerating myelinated nerve fibers are the large vacuoles that are present in the cortex at 9 and 13 months. Some studies use the term "axonal spheroids" to refer to these structures, as well as the debris-filled swellings described above, but the word "vacuole" is used here to emphasize the uncertainty of their origins. Debris-filled swellings are usually irregular in shape, while the profiles of empty, large vacuoles are always circular. The concept that some vacuoles arise from degenerating myelinated nerve fibers is supported by the presence of vacuoles surrounded by thin myelin sheaths. Another possibility is that some of the large vacuoles arise from degenerating neuronal cell bodies, but despite an intensive search, no synapsing axon terminals, or the remains of such terminals, have been found attached to the membranes bounding large vacuoles, as might be expected if some vacuoles are the remains of neuronal cell bodies.

In contrast to findings from several other TG mouse lines (Higuchi et al. 2002; Lin et al. 2003a), no tau has been observed in astrocytes in the rTg4510 mutant. On the other hand, glial filaments become abundant in the cytoplasm of cortical astrocytes, and this hypertrophy is probably a reaction to the widespread pathology in the surrounding neuropil. A similar reactive gliosis occurs in both human tauopathy (Schindowski et al. 2006) and TG mouse models of tauopathy (Lewis et al. 2001; Lewis et al. 2000) in response to the massive insult of tau pathology, so this finding is not surprising, though the degree of hypertrophy is striking.

In summary, the rTg4510 mutant mouse shares many similarities to other murine tauopathy models, such as large, intraneuronal aggregates of insoluble tau protein in some neurons, a loss of neurons leading to behavioral deficits, and swollen myelinated nerve fibers. However, in the dorsal premotor cortex of rTg4510 mice, tau is largely segregated into the somatic compartment of the neuron; no extracellular tau is visible, nor is present in glial cells. This is notable, because although tau-filled neurons are dying in rTg4510 mice, no "ghost tangles" are present, as they are in human disease, leaving unclear the mechanism of the breakdown pathway and ultimate fate of abnormal tau in these mice. As shown, there is a dramatic loss of cortical thickness with age, especially in the thickness of layer I, and as demonstrated in previous studies of this mutant in our laboratory, this coincides with a decline in dendritic length and complexity, especially in the apical tuft of pyramidal cells. Despite changes to their dendritic arbors, TG neurons appear otherwise healthy whether or not they possess NFTs, reinforcing a key finding in rTg4510 that filamentous tau is not toxic in and of itself.

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