Parkinsonism and impaired axonal transport in a mouse model of frontotemporal dementia

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Frontotemporal dementia (FTD) is characterized by cognitive and behavioral changes and, in a significant subset of patients, Parkinsonism. Histopathologically, FTD frequently presents with tau-containing lesions, which in familial cases result from mutations in the *MAPT* gene encoding tau. Here we present a novel transgenic mouse strain (K3) that expresses human tau carrying the FTD mutation K369I. K3 mice develop a progressive histopathology that is reminiscent of that in human FTD with the K369I mutation. In addition, K3 mice show early-onset memory impairment and amyotrophy in the absence of overt neurodegeneration. Different from our previously generated tau transgenic strains, the K3 mice express the transgene in the substantia nigra (SN) and show an early-onset motor phenotype that reproduces Parkinsonism with tremor, bradykinesia, abnormal gait, and postural instability. Interestingly, motor performance of young, but not old, K3 mice improves upon L-dopa treatment, which bears similarities to Parkinsonism in FTD. The early-onset symptoms in the K3 mice are mechanistically related to selectively impaired anterograde axonal transport of distinct cargos, which precedes the loss of dopaminergic SN neurons that occurs in aged mice. The impaired axonal transport in SN neurons affects, among others, vesicles containing the dopamine-synthesizing enzyme tyrosine hydroxylase. Distinct modes of transport are also impaired in sciatic nerves, which may explain amyotrophy. Together, the K3 mice are a unique model of FTD-associated Parkinsonism, with pathomechanistic implications for the human pathologic process.

tau | Alzheimer | transgenic | NFT | memory

How neuronal dysfunction and cell loss is brought about in neurodegenerative diseases such as Alzheimer disease (AD) and frontotemporal dementia (FTD) is only partially understood. In familial cases of FTD with tau aggregation (i.e., FTDP-17), mutations were identified in the *MAPT* encoding the microtubule (MT)-associated protein tau (1), and in FTD cases without tau aggregation, they were identified in *PGRN* encoding progranulin (2, 3). Of the 42 known MAPT mutations, several have been expressed in transgenic mice. The mice reproduce selective aspects of the disease which is, in part, determined by the choice of promoter and tau isoform, inclusion of FTDP-17 mutations, the integration site, and copy number of the transgene (4).

In FTD and AD, tau becomes increasingly hyperphosphorylated, i.e., more phosphorylated at physiological sites and, in addition, *de novo* at pathological sites (5). Hyperphosphorylation detaches tau from MTs, and makes it prone to form filamentous inclusions, including neurofibrillary tangles (NFTs) in AD and FTD, and Pick bodies in Pick disease (PiD) (6–9). However, it is only partly understood how aggregated tau interferes with cellular functions.

Here we report a novel transgenic mouse strain that expresses K369I mutant human tau in neurons (K3 mice). This mutation has been identified in a patient with a PiD neuropathology (10). Different from previously generated tau transgenic strains, K3 mice express the transgene in the SN, in addition to other brain areas. The mice develop memory impairment and an early-onset motor phenotype reminiscent of Parkinsonism. Sciatic nerve ligations and an analysis of SN neurons assisted in identifying impaired axonal transport of distinct cargos as pathomechanism.

Results

Hyperphosphorylation and Deposition of Tau in K3 Mice. K3 transgenic mice express K369I mutant human tau driven by the neuron-specific mThy1.2 promoter [Fig. 1*A* and supporting information (SI) *Text*] . K369I tau is expressed in several brain areas including cortex, hippocampus, and basal ganglia (Fig. 1*B*). In K3 forebrains, total tau protein levels are 2.9-fold higher than in WT controls (Fig. 1 *C* and *D*). Tau is phosphorylated at multiple sites including AT8, AT180, AT270, and pS422 (Fig. 1 *D–F*). However, reminiscent of human PiD, tau is not phosphorylated at the 12E8 (S262/S356) phosphoepitope, even in old (>12 months) K3 mice (Fig. 1 *D* and *G*). In frontal cortex of K3 mice, ovoid intraneuronal tau aggregates are predominant, resembling Pick bodies. Like in PiD, they are identified by Bielschowsky, but not Gallyas, silver impregnation (Fig. 2*A*). Their numbers increase with age (Fig. 2*B*). Antibodies pS422 and AT100 reactive with pathologically phosphorylated tau (11, 12) stained them as well, and the numbers of tau-positive inclusions correlate with these of Bielschowsky-positive deposits (Fig. 2 *A* and *C*). Thiazin red staining for fibrillar tau (13) co-localized with AT8 immunostaining of inclusions (Fig. 2*D*). A Western blot analysis of sarkosyl extractions (Fig. 2*E*) revealed high levels of insoluble tau in K3 mice, P301L transgenic pR5 mice (14), and WT tau-transgenic ALZ17 mice (15). It was much more phosphorylated at the PHF1 epitope in K3 than in P301L transgenic pR5 mice (14), whereas again, the 12E8 epitope was not phosphorylated (Fig. 2*E*). As in the human K369I mutation carrier (10), tau in K3 mice was ubiquitinated (Fig. S1). Together, this shows that, histopathologically, the K3 mice model FTD with the K369I mutation.

Memory Deficits in K3 Mice. To test memory functions of K3 mice we used the novel object recognition task (16, 17). Here, the time spent exploring two objects on the first test day is equal, whereas on the second test day mice with normal memory will spend more time exploring a novel object. At 2 months of age, K3 and

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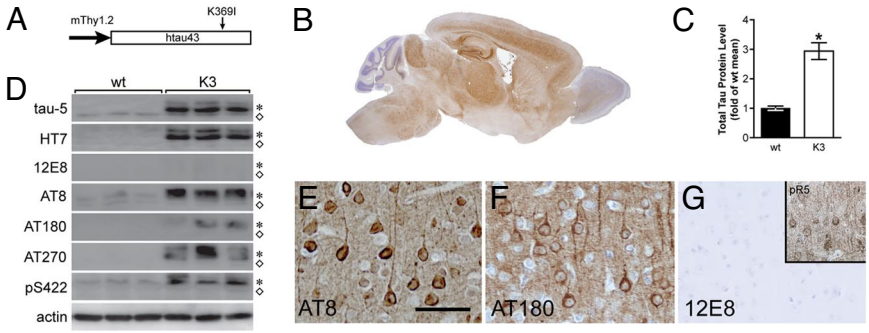

Fig. 1. K3 mice model tau hyperphosphorylation of FTD with the K369I mutation. (*A*) K3 mice express human tau (htau43) carrying the pathogenic K369I mutation under control of the neuron-specific murine (m)Thy1.2 promoter. (*B*) Immunohistochemistry for human tau reveals widespread expression of transgenic tau in brain (brown) in a 3-month-old K3 mouse. No staining is observed in WT controls (not shown). (*C*) K3 mice show 2.94 ± 0.28-fold higher total cerebral tau level than WT controls, as determined by quantification of Western blots, using the total tau-specific tau-5 antibody (*, $P < 0.0001$). (*D*) Western blotting reveals that in the cortex of K3 mice transgenic tau (asterisk) is over-expressed compared with endogenous tau (open diamonds) as shown with tau-5. WT littermate controls are included for comparison. Transgenic tau is identified by human tau-specific antibody HT7. Whereas transgenic tau is phosphorylated at epitopes AT8, AT180, AT270, and pS422, the 12E8 epitope is not. (*E–G*) Transgenic tau in cortical neurons is phosphorylated at multiple sites, including AT8 and AT180 (brown). As in human PiD, the 12E8 site of tau is not phosphorylated in K3 mice. (*Inset*) 12E8-positive staining of a cortical neuron from a P301L tau transgenic pR5 mouse. (Scale bar, 100 μm.)

WT littermates showed a similar preference for the novel object, whereas at 4 months, the memory of K3 mice was impaired as revealed by a lack of preference (Fig. 3*A*). Thus, K3 mice are characterized by impaired working memory as early as 4 months of age.

Motor Symptoms of K3 Mice Are Ameliorated by L-Dopa. Already at 4 weeks of age, K3 mice had repeated and prolonged resting phases in the open field, and displayed an intensive progressive tremor (Fig. 3*B*). Furthermore, they clasped their hind and front limbs when lifted by the tail (Fig. 3*C*), and showed abnormal gait with significantly reduced footstep length (Fig. 3 *D* and *E*). At 2 months, K3 mice showed reduced locomotor activity and rearing in the open field (Fig. 3*F*). This complex early-onset motor phenotype of K3 mice is highly reminiscent of Parkinsonism in humans, which is defined by tremor, bradykinesia, abnormal gait, and postural instability. Hence, we assessed possible alterations of the dopaminergic system pharmacologically. K3 mice reacted with a strong cataleptic response to lower doses of the dopamine antagonist haloperidol than WT littermates (Fig. 3*G*). Postural instability of K3 mice as determined by pronounced impairment of motor coordination on the challenging beam could be ameliorated with L-dopa (Fig. 3*H*).

Age-Dependent Loss of Dopaminergic Neurons in K3 Mice. In K3 mice, Parkinsonism reflects expression of transgenic tau in dopaminergic SN neurons, whereas our P301L transgenic mice, which lack motor symptoms (14), show hardly any transgenic tau expression in the SN (Fig. S2). To determine whether K3 mice have an underlying loss of dopaminergic SN neurons, we stereologically analyzed sections of K3 mice and WT littermates at ages from 3 to 24 months for tyrosine hydroxylase (TH)-immunoreactive neurons. Whereas numbers were not different at 3 months of age, they were significantly reduced in 12- and 24-month-old K3 brains (Fig. 4). Hence, although there is a progressive loss of dopaminergic SN neurons, the presence of motor deficits despite unaltered numbers of TH-positive cells in young K3 mice indicates an early functional impairment of dopaminergic SN neurons that precedes degeneration as a cause of Parkinsonism.

Amyotrophy in K3 Mice in the Absence of Overt Neurodegeneration. Tau overexpression has been shown to cause non-Parkinsonian motor phenotypes and amyotrophy in transgenic mouse models, generally associated with Wallerian spinal cord degeneration in the presence of high expression levels of the transgene in α-motor neurons of the spinal cord (15, 18–21). K3 mice progressively gain less weight that results from amyotrophy, as the weight of peripheral organs, except for muscles, and body length are indistinguishable from those of WT mice throughout the entire lifespan (K3 mice are 26.5% ♂ and 30% ♀ lighter than WT littermates; $P < 0.001$). Although muscles of K3 mice did not express K369I tau, they showed first signs of atrophy at 4 weeks of age that progressed with age (Fig. S3*A*). The α-motor neurons of the spinal cord express K369I tau (Fig. S3*B*), but showed no signs of neuronal loss, even at 1 year of age (Fig. S3*C*). Furthermore, tau-expressing peripheral nerves such as sciatic nerve and spinal cord were free of degenerative signs (Fig. S3*D*). Neuromuscular junctions stained for synaptophysin and with α-bungarotoxin for the acetylcholine receptor revealed no differences between K3 and WT muscle (Fig. S3*E*), whereas staining for the mitochondrial structural protein porin was reduced, possibly indicating a functional impairment of the neuromuscular junctions in K3 mice (Fig. S3*E*). Taken together, amyotrophy and early-onset Parkinsonism in K3 mice occur in the absence of overt neurodegeneration.

Morphological Correlates of Impaired Axonal Transport in K3 Mice. Bielschowsky silver-positive axonal swellings and spheroids have been identified as a morphological correlate of disrupted axonal transport in an AD mouse model and in the AD brain (22, 23). We found similar progressive changes in K3 but not WT brains (Fig. S4). Already at 2 months of age, a few axonal swellings were detectable in the nigrostriatal projection, and at 5 months spheroids occurred. These became larger and more numerous at 10 months of age, indicating a progressive axonal pathology.

Tau Impairs Axonal Transport of TH in Dopaminergic Neurons. The dopaminergic nigrostriatal system is formed by SN pars compacta (SNc) neurons that project to the striatum to inhibit involuntary movement (24). To produce dopamine, the rate-limiting enzyme TH is synthesized in the SNc and transported in anterograde fashion to synapses in the striatum, converting tyrosine to L-dopamine. In 3-month-old K3 mice with motor deficits but without SN degeneration, we found increased TH in the SN compared with controls, and drastically reduced TH in

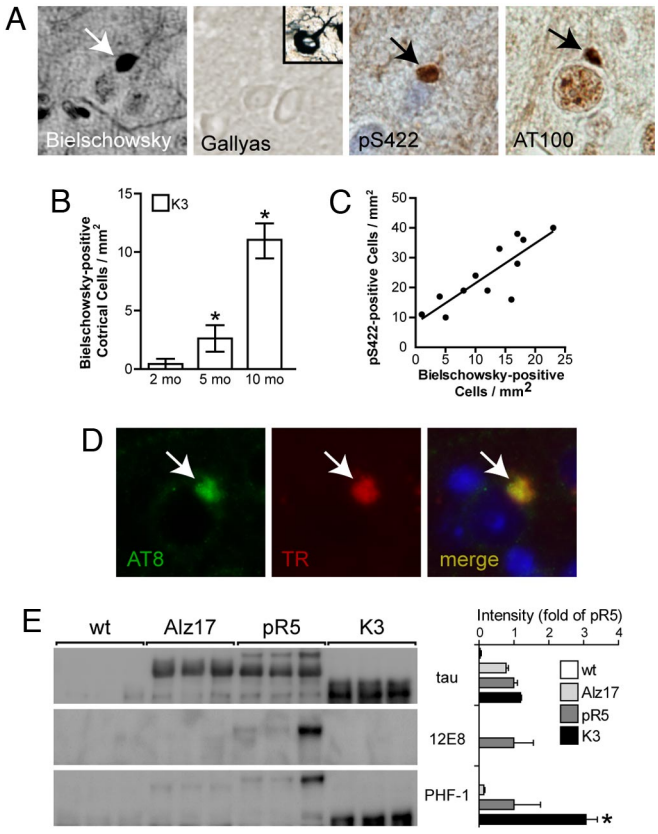

Fig. 2. K3 mice show a progressive histopathology reminiscent of FTD with the K369I mutation. (*A*) The intraneuronal ovoid inclusions are Bielschowsky silver-positive (black; arrow), and Gallyas silver-negative (*Inset*, Gallyas-positive human NFT). The round Pick body-like lesions are also reactive with antibodies pS422 and AT100 for pathologically phosphorylated tau. The inclusions are 12E8-negative (not shown). No staining is found in controls (not shown). (*B*) Numbers of Bielschowsky-positive cells in K3 mice increase with age (*, $P < 0.01$ vs. previous age-group). (*C*) Numbers of Bielschowsky- and pS422-positive cells correlate in K3 brains ($P < 0.001$). (*D*) Staining with the AT8 antibody (green) and Thiazin red (TR) for fibrillar deposits co-localized (yellow) in intraneuronal lesions in K3 brains. (*E*) Comparison of sarkosyl-insoluble tau in brains of WT, WT tau-expressing ALZ17, P301L mutant tau-expressing pR5, and K3 mice. Western blotting reveals insoluble tau in ALZ17, pR5, and K3 mice. Insoluble tau is phosphorylated at the 12E8 epitope only in pR5 mice, whereas phosphorylation at the PHF-1 epitope is most abundant in K3 mice (*, $P < 0.05$).

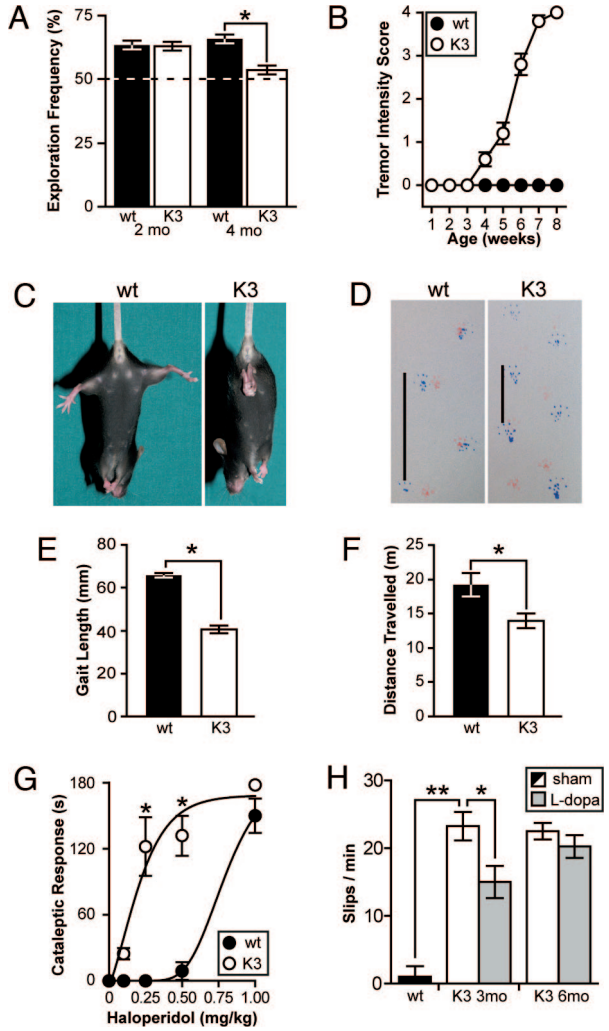

Fig. 3. K3 mice present with early-onset memory deficits and Parkinsonism-like motor deficits that can be partially ameliorated with L-dopa. (*A*) At 2 months of age, K3 mice (white bars) explore novel objects with the same preference as WT littermates (black bars). At 4 months of age, their memory is impaired as indicated by an equal exploration of both the known and the novel object (*, $P < 0.001$). (*B*) At 4 weeks of age, K3 mice (white dots) present with a slight tremor that becomes progressively more intense, until at 8 weeks of age all K3 mice suffer from severe tremor. No tremor is observed in WT littermates (black dots). Scoring of tremor is described in *Methods*. (*C*) Already at 6 weeks of age, K3 mice, but not WT littermates, clasp their hind limbs when lifted by the tail. (*D* and *E*) Abnormal gait shown by coloring of paws. Bars indicate foot step length that is 38.3% shortened in K3 mice versus WT littermates (*, $P < 0.0001$). (*F*) Already at 2 months of age, the locomotor activity of K3 mice (white bar) is reduced as they travel less than WT littermates (black bar) in the open field arena. This difference persists throughout age (*, $P < 0.05$). (*G*) Experimental induction of catalepsy with the dopamine antagonist haloperidol (administered i.p.) in the bar test reveals a four-times-increased sensitivity of the dopaminergic system in K3 mice versus WT littermates (*, $P < 0.001$). (*H*) The challenging beam task shows a pronounced postural instability of K3 mice compared with WT littermates, as indicated by repeated slipping when crossing a narrow beam (**, $P < 0.0001$). Single doses of L-dopa ameliorate the balance deficit of 3-month-old K3 mice (gray bar; *, $P < 0.05$). The L-dopa responsiveness is lost when K3 mice reach 6 months of age.

striatal synapses compared with controls (Fig. 5 *A* and *B*). Numbers of TH-positive synaptic boutons of SNc neurons were not reduced, suggesting that less TH is transported along the nigrostriatal projection. Reduced availability of TH can cause reduced dopamine levels. We found that, already at 6 weeks of age, K3 mice had a significant ($P < 0.05$) 15.3 ± 3.9% (mean ± SD) decrease of basal dopamine levels compared with WT controls.

Tau has previously been reported to interfere with axonal transport in cell culture, resulting in an altered distribution of organelles (19, 25). Hence, we assessed the distribution of TH in dopaminergic SN neurons of K3 mice stained for TH (Fig. 5*C*). Compared with WT controls, SN neurons of K3 mice that express transgenic tau showed a reduced TH intensity in growth cones, along with enrichment in cell bodies, supporting the notion of reduced TH transport (Fig. 5 *D–F*). Taken together, young K3 mice have less TH in striatal synapses and thus lower basal dopamine levels.

Selective Axonal Transport Impairment in K3 Mice. To further address transport defects in the dopaminergic system of K3 mice, we compared levels of different cargos transported between SN and striatum by Western blotting (Fig. 5 *G* and *H*). In the striatum of K3 mice, the markers of amyloid β precursor protein (APP)-containing vesicles, APP, PS1, and Gap43 (26), the APP family member amyloid β precursor-like protein 2 (APLP2), the

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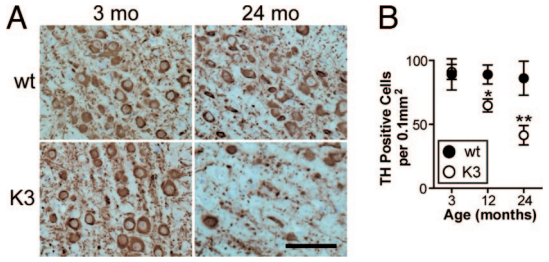

Fig. 4. Progressive loss of dopaminergic SN neurons in K3 mice. (*A*) Immunohistochemistry of dopaminergic TH (brown)-containing neurons reveals that, not at 3, but at 24 months of age, significantly fewer cells are TH-positive in K3 than in WT SN. (Scale bar, 50 μm.) (*B*) At 3 months of age, numbers of TH-positive cells on serial sagittal sections are equal in WT and K3 SN; however, at 12 and 24 months of age, numbers of TH-positive cells are significantly reduced in K3 mice (*, $P < 0.01$; **, $P < 0.0001$).

motor proteins kinesin heavy chain and kinesin light chain, and the scaffold protein Jip1 were markedly reduced. Mitochondrial respiratory chain protein complexes NADH-ubiquinol oxidoreductase (Co-I) and ATP synthase (Co-V) were also reduced. No changes were found for synaptophysin and synaptotagmin, markers of different transport cargos (27, 28), APLP1, and the motor protein kinesin-III. As levels of these markers were not reduced in the K3 SN, this suggests selectively impaired axonal transport in the nigrostriatal system.

To directly address axonal transport *in vivo*, we ligated the sciatic nerve in K3 mice and WT littermates, taking advantage of transgene expression in spinal cord α-motor neurons (Figs. S3*B* and S5 *A* and *B*). Ligation causes accumulation of anterogradely transported cargos proximal and retrogradely transported cargos distal to the ligation site (26, 29). In ligated WT nerves, APP, Gap43, kinesin heavy chain (Kif5B), kinesin light chain (KLC), and Jip1 accumulated proximal to the ligation site, different from K3 mice in which accumulation of APP, Gap43, and Jip1 was significantly reduced. Co-V accumulated proximally and distally of the ligation in WT nerves, representing bidirectional transport of mitochondria. In ligated K3 nerves, however, Co-V accumulated only in the distal part, suggesting impaired anterograde and unaffected retrograde transport of mitochondria in K3 mice. A tau dependency of this impairment is further supported by immunohistochemistry as only K369I tau-expressing axons show reduced numbers of mitochondria (Fig. S5*C*). Retrograde transport was unaffected, as assessed also by striatal injections of FluoroGold, a retrogradely transported dye (Fig. S6). Accumulation of marker proteins for other transport cargos and motor proteins at the ligation sites in WT and K3 nerves was comparable. These included the slow transport marker tubulin (29), synaptic vesicle precursor proteins synaptophysin and synaptotagmin, motor protein kinesin-III (27), and calcitonin gene-related peptide (30). Together, this shows that tau specifically impairs the anterograde axonal transport of APP- and TH-containing vesicles and mitochondria, and leaves the transport of other cargos unaffected. Hence, early-onset amyotrophy and Parkinsonism in the K3 mice are both likely caused by impaired axonal transport.

Discussion

Possibly reflecting a unique expression pattern of the K369I tau transgene that included the SN, we obtained a novel mouse strain, K3, that not only models histopathological characteristics of FTD with the K369I tau mutation, but also memory impairment, amyotrophy, and Parkinsonism. We identified cargos such as TH-containing vesicles and mitochondria whose

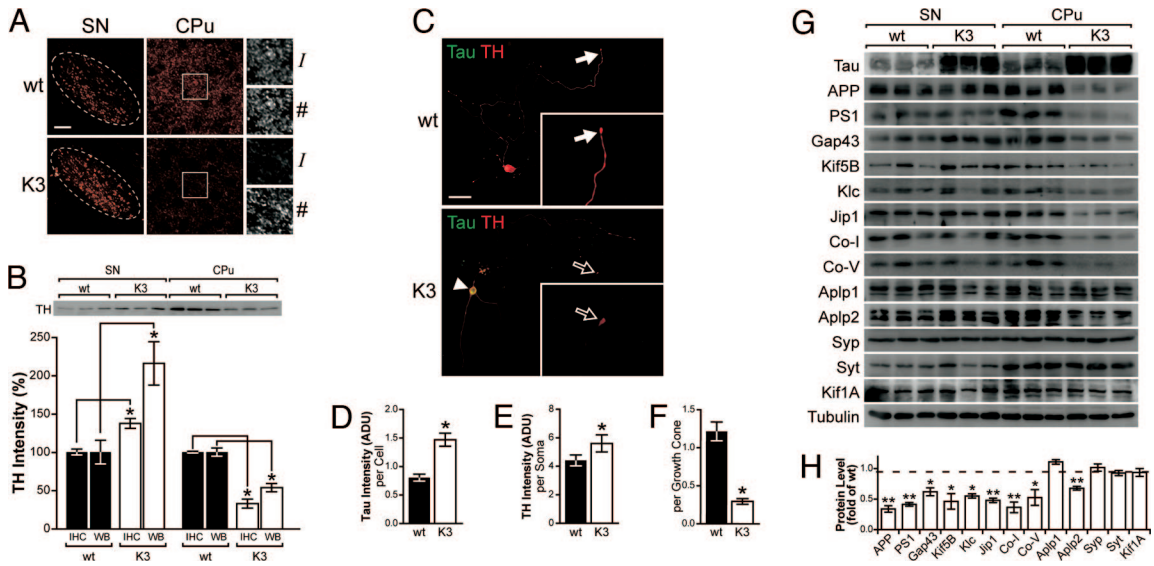

Fig. 5. TH transport is impaired in the nigrostriatal projection of K3 mice and in primary SN neuronal cultures established from them. (*A*) Staining for TH (red) on sagittal sections of 3-month-old K3 and WT mice reveals that TH intensity (*I*) is increased in the SN pars compacta (broken lines), and reduced in synaptic boutons of the striatum (CPu). Numbers of TH-positive synaptic boutons are unaltered in K3 mice (*Right*). (*B*) Quantification of immunohistochemical and Western blot (WB) data reveals that TH accumulates in K3 SN neurons (*, $P < 0.0005$). Consequently, TH is decreased in the K3 CPu (*, $P < 0.0001$), indicating that TH is not delivered properly to the axonal terminals of SN neurons in the K3 striatum. (*C*) Tau-expressing primary cultured K3 SN neurons (arrowhead) show a markedly decreased TH staining of axonal growth cones (*Inset*; open arrow) compared with WT neurons (arrow; *Inset*). This occurs in the absence of overt morphological alterations. (Scale bar, 50 μm.) (*D*) K3 SN neurons have 1.82-fold higher total tau levels than WT neurons (*, $P < 0.01$). (*E*) Quantification of fluorescence intensities shows increased TH in the soma of primary SN neurons of K3 mice compared with WT neurons (*, $P < 0.05$). (*F*) In contrast, TH fluorescence intensity is markedly decreased in growth cones of K3 compared with WT neurons (*, $P < 0.0001$). (*G*) SN and striatal (CPu) extracts from WT and K3 mice analyzed in parallel show a marked reduction of selective proteins in the CPu of K3 mice. Changes are found exclusively for motor proteins and markers of distinct vesicles and mitochondria (see *Results*). For comparison, protein levels are not reduced in the SN. (*H*) Quantification of protein levels in the CPu of K3 mice shown as expression (in fold) compared with WT (dashed line; *, $P < 0.05$; **, $P < 0.01$).

axonal transport was selectively impaired. Hence, the K3 mice are particularly suited in which to study the role of tau in impaired axonal transport, which has been implicated as a central pathomechanism in AD and related disorders (31).

The K369I mutation was initially identified in a human patient with a neuropathologic process indistinguishable from sporadic PiD (10). PiD is a subtype of FTD characterized by rapid disease progression with frontotemporal and limbic neuronal loss (9). In cases with generalized PiD, additional brain areas may be affected, such as the SN (32). The definition of PiD, however, has undergone several changes over time (33). While Pick body-like inclusions have been frequently seen in carriers of distinct MAPT mutations (10, 34, 35), the very existence of an autosomal-dominant PiD resulting from MAPT mutations has been questioned (36). PiD is generally characterized by a frontal syndrome and the presence of Pick bodies made mainly of 3R tau (37, 38). Pick bodies are also 12E8- and AT100-negative (39–41). For comparison, K3 mice develop ovoid tau inclusions that are 12E8-negative but AT100-positive. In this respect, they model the human K369I histopathology, as there the inclusions are also 12E8-negative but AT100-positive (10). The K3 mice do, however, model other aspects of sporadic PiD, such as ubiquitination and the presence of Bielschowsky- but not Gallyas-positive tau aggregates.

The Pick body-like inclusions, Parkinsonism, and amnesia that characterize K3 mice are part of the human PiD syndrome, although in the K369I case, no Parkinsonism has been reported, possibly reflecting the absence of a tau pathologic process in the SN (data not shown). Similarly, amyotrophy is absent in the human case. However, as expression of the K369I transgene in the sciatic nerve also caused an impaired transport of distinct cargos associated with a pathologic process, this may reveal a central pathomechanism.

AD and FTD both present with a progressive decline of memory function leading to dementia, although in FTD it is often preceded by behavioral changes and motor symptoms such as Parkinsonism (9). The K3 mice present with early-onset memory impairment at an age of 4 months, before Bielschowsky-positive tau deposits are detectable. This is in line with the observation that memory deficits precede histopathological changes in other tau-expressing strains (21, 42). Pathological tau thus interferes with distinct cellular mechanisms causing neuronal dysfunction before tau is sequestered into deposits. Consequently, recovery of memory function has been observed in mice when expression of transgenic tau was suppressed, despite a persistent accumulation of NFTs, suggesting that tau mediates neuronal dysfunction independent of NFT formation (43). We speculate that the memory impairment in the K3 mice may also be caused by impaired axonal transport, in agreement with previous findings in AD brain and APP transgenic mice (31).

K3 mice develop a complex motor phenotype and amyotrophy as early as 4 weeks of age, which, again, is before tau aggregates. Early-onset tremor, bradykinesia, muscle rigidity, and postural instability establish K3 mice as a unique mouse model for Parkinsonism in FTD. Interestingly, as in the treatment of Parkinsonism in patients with FTD, the symptoms of K3 mice are partially reversible with L-dopa at an early, but not late, stage of disease.

Increasing evidence suggests that neuronal dysfunction resulting from failure of axonal transport is an important pathomechanism in neurodegeneration, including AD (31, 44). The functional impairment of K3 mice is accompanied by progressive morphological changes including axonal swellings and spheroids that are histopathological correlates of disrupted axonal transport (22, 23), preceding loss of SN neurons. *In vivo* experiments revealed that tau can directly inhibit axonal transport (18). Although mechanistically not fully understood, it has been proposed that increases in tau, as found in AD and FTD, decrease binding of motor proteins to MT tracks (45, 46). In K3 mice, we found impaired TH transport in the nigrostriatal system. This suggests that the motor deficits are, at least in part, a result of early-onset neuronal dysfunction caused by impaired axonal distribution of TH in the nigrostriatal system, and thus, reduced basal dopamine levels. Similar to K3 mice, viral expression of both WT or P301L tau in the SN of rats caused reduced TH immunoreactivity in the striatum, decreased dopamine levels, and formation of axonal spheroids that preceded neuronal loss by several months (23). Therefore, it is not unlikely that, in the rat model, neuronal dysfunction is also a result of tau-impaired axonal transport, as in K3 mice. Reduced striatal synaptic supply of transported cargos other than TH is likely to contribute to the K3 phenotype, as we have shown that transport of additional cargos such as APP-containing vesicles or mitochondria is also affected. Finally, sciatic nerve ligations revealed a similarly impaired axonal transport in a second, unrelated system. Neuronal dysfunction resulting from impaired anterograde axonal transport is therefore likely to cause amyotrophy in the K3 mice, in the absence of overt neurodegeneration.

In conclusion, K3 mice are a unique model for aspects of PiD, but mainly for FTD-associated Parkinsonism and memory impairment. Future therapeutic strategies that target transport impairment in tauopathies may be beneficial in the treatment of L-dopa-resistant Parkinsonism in FTD.

Materials and Methods

Mice. A human K369I mutant tau cDNA, lacking exon 3 and containing four MT-binding domains, was cloned together with a Kozak consensus sequence into a murine Thy1.2 expression vector as described (14). Transgenic mice were produced by pronuclear injection of B6D2F1×B6D2F1 oocytes (47). Founders were identified by PCR using primers 5′-GGGTGTCTCCAATGCCTGCTTCT-TCAG-3′ and 5′-AAGTCACCCAGCAGGGAGGTGCTCAG-3′. Strains were established from founder mice, and the K3 strain was backcrossed 8 times onto a C57BL/6 background. pR5 mice express P301L mutant tau (14), whereas ALZ17 mice express WT human tau (15). Animal experiments were approved by the Animal Ethics Committee of the University of Sydney.

Cell Culture and Immunocytochemistry. For nigrostriatal primary cultures, SN and striatum were prepared separately from neonatal mice. The tissue was dissected in Hanks' balanced salt solution and incubated with 2.5 mg/ml trypsin in the presence of 1 mg/ml DNaseI (Sigma) for 20 min at 37°C. Subsequently, it was triturated in plating medium containing DMEM/10% FBS (Gibco) using fire-polished glass Pasteur pipettes. SN cells (2×10^5) were plated overnight at 37°C on polyD-lysine (0.1 mg/ml; Sigma)-coated 12-mm glass coverslips mounted in the center of a 12-well culture plate well. Subsequently, 1×10^4 striatal cells were plated in a ring around the coverslips to support SN cell growth. After 2 h, the plating medium was replaced by 1 ml of Neurobasal medium containing B27 supplement and Glutamax (Gibco). For immunofluorescence staining, cells were fixed with 4% paraformaldehyde in 80-mM Pipes, 1 mM $MgCl_2$, and 1 mM EGTA (pH 6.8) after 7 days. Cells were permeabilized with 0.1% Triton in PBS solution and stained with antibodies for TH and tau.

Statistics. Statistical analysis was performed with Prizm 4 software for Windows (GraphPad) using Student's *t* test. All values are given as mean ± SE.

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