The hyperphosphorylation of human tau and its aggregation into neurofibrillary tangles are central pathogenic events in familial tauopathies and Alzheimer's disease. However, the cellular consequences of neurofibrillary tangle formation in vivo have not been directly studied because cellular models of human neurofibrillary degeneration have been unavailable until recently. Incorporation of human tau into filaments in vivo and the association of filamentous tau with cytodegeneration were first demonstrated experimentally with the overexpression of human tau in identified neurons (anterior bulb cells) in the lamprey central nervous system. In this system, filamentous tau deposits are associated with the loss of dendritic microtubules and synapses, plasma membrane degeneration, and eventually the formation of extracellular tau deposits and cell death. Here we show that human tau hyperphosphorylation in anterior bulb cells is spatiotemporally correlated with a highly stereotyped sequence of degenerative stages closely resembling those seen in human neurofibrillary degeneration. Hyperphosphorylated tau deposits first appear in the distal dendrites and somata, together with degenerative changes that begin in distal dendrites and progress proximally over time. This sequence is independent of the tau isoform used, the presence of epitope tags and the method used to overexpress tau, and thus has important implications for the cytopathogenesis of human neurofibrillary disease.


The intracellular accumulation of hyperphosphorylated, filamentous tau protein is a hallmark of a large group of neurodegenerative conditions that are collectively responsible for the overwhelming majority of cases of dementing illness among elderly humans. The presence and distribution of neurofibrillary tangles (NFTs) and neurofibrillary tangle formation are hallmarks of Alzheimer's disease and other neurodegenerative conditions, raising considerable interest in the cellular mechanisms underlying NFT formation as a possible point of therapeutic intervention in neurofibrillary degenerative disease (NFD).

Studies of the early stages of NFD in human autopsy material suggest that abnormal tau deposits develop in a stereotyped spatiotemporal sequence, with the earliest changes being seen in the distal dendrites of vulnerable neurons, and the appearance of evenly distributed, granular tau deposits that have been phosphorylated at the AT8/Tau-1 site. Throughout time, large deposits of filamentous, highly phosphorylated tau (NFTs) fill the somata of such neurons, which eventually die, leaving extracellular tombstone NFTs consisting of highly modified tau filaments. Unfortunately, very little is known concerning the time course and the precise sequence of cellular events required to produce these lesions in vivo. The total time required for NFT formation and neuronal death can only be determined indirectly from autopsy studies, and such estimates vary from several months to up to 20 years. In particular, it is unclear which of the observed cellular changes play early, causal roles in the cascade of events leading to NFD, and which are merely consequences of more central events. For instance, both axonal and dendritic degeneration are widespread in Alzheimer's disease and other neurofibrillary degenerative conditions, yet it is unknown whether one or the other of these loci is the primary point of attack in NFD or if both dendritic and axonal changes are secondary to pathology affecting the entire cell. Similarly, it is unclear if NFD is primarily a cell-autonomous process, affecting only the cell containing the developing tangle, or if trans-synaptic mechanisms play a critical role. Finally, it is still unclear if the development of argyrophilia and/or tau feeding lines is a hallmark of a large group of neurodegenerative conditions that are collectively responsible for the overwhelming majority of cases of dementing illness among elderly humans.
phosphorylation at any one site plays a significant role in NFT formation and neurofibrillary degeneration in vivo, and if so, whether these changes are a cause or consequence of the polymerization of tau into filaments. The inability of neuropathologists to address these questions directly and effectively is primarily because of the impracticality of following individual neurons in which NFTs are developing on a prospective basis in humans and/or murine or other mammalian models in situ.\textsuperscript{16–18}

Throughout the past few years, we have developed a unique cellular model of tau filament/NFT formation for studying the cytopathological changes that accompany chronic overexpression of human tau that circumvents many of the difficulties outlined above. This cellular model of tau-induced NFD consists of giant neurons [anterior bulbar cells (ABCs)] in the hindbrain of the ammocoete sea lamprey, \textit{Petromyzon marinus}, that have been induced to overexpress human tau by the injection of plasmids containing constructs in which tau expression is driven by the cytomegalovirus (CMV) promoter. With this system, we showed for the first time that overexpression of the shortest human tau isoform (htau23) \textit{in vivo} can cause the incorporation of human tau into filaments, the phosphorylation of the PHF1 and Tau1 epitopes (serines 396 to 404 and 199 to 202, respectively), and gross degenerative changes in the soma and dendrites including the externalization of human tau deposits.\textsuperscript{19} We have since characterized the intracellular behavior of human tau filaments in ABC somata and dendrites and their association with dendritic microtubule (MT) and synaptic loss.\textsuperscript{20}

In the present study, we have performed a systematic analysis of the degenerative changes caused by tau overexpression throughout time in ABCs, and have correlated these changes both with the length of time after vector injection and with the appearance of multiple AD-related epitopes on tau, including the PHF1, AT8, AT100, and TG3, and ALZ50 sites, in ABCs expressing human tau. We have also compared the cytopathological changes induced by the longest human tau isoform (htau40) to those caused by htau23, and have compared htau23 and htau40 constructs containing Green Fluorescent Protein (GFP) fusions to those that express htau23 and htau40 alone. Finally, we have used a novel method of overexpressing tau in ABCs using self-replicating mRNAs derived from Semliki Forest Virus (SFV) to achieve chronic tau overexpression in ABCs in addition to the plasmid injection method used previously. We show that chronic overexpression of all of these constructs in ABCs induces the same stereotyped sequence of cytodegenerative changes throughout time, with the earliest and most severe changes occurring in the distal-most dendrites. Moreover, this sequence of degenerative changes is spatiotemporally correlated with the appearance of several AD-related phosphoepitopes. However, only the phosphorylation of the PHF1 epitope accompanies or precedes the earliest morphological changes induced by human tau overexpression in ABCs. These results bear a strong resemblance to sequences of cellular degeneration proposed for human NFD,\textsuperscript{8,10} and their significance and implications for the cellular mechanisms responsible for human NFD is discussed.

\section*{Materials and Methods}

\subsection*{Human Tau Constructs Overexpressed in ABCs}

All constructs used in this study are shown in Figure 1A. The human tau sequence was either fused with the coding sequence for GFP at the tau N terminal (for htau23—clone 93)\textsuperscript{20} or at the tau C terminal (for htau40—clone 139) as shown in Figure 1A. The plasmid pRC/CMVn123c\textsuperscript{19} was used to overexpress htau23 without an epitope tag. Htau40...
was expressed without an epitope tag by injecting mRNA purified by standard methods from the SFV-htau40 vector into ABCs as described in Figure 1B. Human tau expression was driven by the CMV promoter as a single transcript in all of the constructs used.

**Overexpression of Human Tau in ABCs**

Plasmid microinjection was performed as described, and surgery and preparation for fixation and immunohistochemistry was performed as described in Hall and Kosik. Briefly, the hindbrains of anesthetized ammocoete lampreys 8 to 11 cm in length were exposed and the somata of ABCs identified and injected under visual guidance. The brain was maintained under a constant flow of Ca++-free lamprey Ringers solution at all times, and the membrane potential of injected ABCs was monitored during injection. Microinjection of SFV-derived constructs was performed using a technique similar to that used for plasmids, but with modifications to ensure RNase-free conditions (ie, autoclaving of all stock solutions, electrode holder caps and electrode glass; addition of diethyl pyrocarbonate to stock solutions). In addition, the shanks of all micropipettes used for microinjecting SFV mRNAs were briefly flamed and allowed to cool before use. Otherwise, surgical procedures for all operations were identical to those described above and in Hall and Kosik. All procedures were performed under general anesthesia, which was accomplished by immersing the lampreys in a saturated aqueous solution of benzocaine for 10 to 20 minutes. Approximately 120 lampreys were used in this study, yielding 97 expressing cells. Sixty-seven of these cases were identified by established morphological criteria for identifying distal dendrites of axotomized ABCs in transverse thin sections through the lamprey hindbrain. In sections processed for immunoelectron microscopy, lamprey brains were fixed for 2 hours in 4% paraformaldehyde/0.05% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, and then transferred to phosphate-buffered saline containing 2 mol/L sucrose/0.2 mol/L glycine for 30 minutes followed by immersion in liquid nitrogen. Frozen ultrathin sections were then cut at −120°C with a cryo-diamond knife and transferred to Formvar-coated grids. Immunolabeling was performed on the grids by standard methods with 0.5% fish skin gelatin (Sigma Chemical Co., St. Louis, MO) as a blocking agent. A 1:10 dilution of anti-GFP antiserum (Clontech, Palo Alto, CA) was then used to identify tau filaments. Protein A-gold/anti-rabbit secondary (10 nm, Sigma) was then used to label filaments for the electron microscopy. Grids were then stained in 0.3% uranyl acetate for 10 minutes at 0°C and examined on a JEOL 1200EX electron microscope.

**Immunocytochemistry and Antibodies**

Lamprey brains were fixed and sectioned as described. Immunochemistry was performed on 10-μm transverse sections of paraffin-embedded lamprey heads that had been fixed by immersion in FAA (10% formalin, 10% glacial acetic acid, and 80% ethanol). The mAbs PHF1 (1:100), ALZ50 (1:25), TG3 (1:25), AT8 (1:100), and AT100 (1:100) were used to identify phosphoepitopes on httau23 and httau40. PHF1, ALZ50, and TG3 were kindly provided by Dr. Peter Davies (Department of Pathology, Albert Einstein College of Medicine, Bronx, NY), and AT8 and AT100 were purchased from Innogenetics Corp. (Leuven, Belgium). None of these mAbs cross-reacted with endogenous epitopes in FAA-fixed lamprey brain. Sections were deparaffinized in Histoclear and then photographed if they contained fluorescent profiles of ABC somata and dendrites. They were then rehydrated, quenched in excess H2O2, washed, and incubated with primary overnight at 4°C. Biotinylated secondary antibodies were then applied and antibody staining revealed with an Biotin/Extravidin/HRP kit (Sigma Chemical Co.), with diaminobenzidine as the chromagen.

**Results**

In this study, we have characterized the development of neurofibrillary pathology and assess its correlation with changes in the phosphorylation state of human tau in ABCs using a panel of mAbs directed at phosphoepitopes in regions of tau that flank the MT-binding repeats. We found that all of these mAbs recognize subsets of the human tau expressed in ABCs, but that none of them label as heavily or as extensively as PHF1. Moreover, we found no significant differences in the patterns of human tau immunostaining and/or cytodegeneration that were attributable to the effects of using different splice variants of tau (ie, httau23 and httau40) and different expression techniques on the response of ABCs to human tau overexpression. All of the constructs used resulted in the same stereotyped pattern of degenerative changes, which were in turn spatiotemporally correlated with the immunolabeling patterns of the anti-tau mAbs listed above and in Figure 1C. These patterns are described in detail below.

**The Earliest Changes Because of Human Tau Overexpression Appear in the Distal Dendrites of ABCs and Occur before Tau Hyperphosphorylation**

Lightly expressing ABCs (Figure 2C) and cells examined within a few days of plasmid injection often showed no
10 cytopathological changes at all. These cells showed PHF1/GFP staining in a granular, evenly distributed pattern throughout their somata and dendrites, and did not label with any of the other mAbs directed at tau phosphoepitopes (ie, AT8, TG3, AT100) or with ALZ50. Most of these cells exhibited slight swelling of some distal den-
drites and many of them tended to have stronger PHF1 staining in their distal-most dendrites than elsewhere. Cellular profiles were otherwise normal, and nuclei were tau-negative. No lightly expressing ABCs exhibited clearly fibrillar deposits, but such cells frequently showed heavier immunolabel localized to the plasma membrane, especially in distal dendrites (Figure 2C). We observed a total of 30 cells fitting this description, which were examined between 4 and 56 days after vector injection.

Human Tau Hyperphosphorylation First Occurs in ABC Distal Dendrites and Somata, and Is Correlated with Dendritic Swelling and Beading

Sixty-seven ABCs examined between 8 and 79 days after mRNA or plasmid injection were found to have expressed human tau heavily enough so that clear alterations to their normal somatodendritic morphology were evident. These alterations usually took the form of a pronounced swelling and/or beading of most or all of their distal dendrites. When examined in the electron microscope, swollen dendritic tips were found to contain aggregations of membrane-bound organelles mixed with tau filaments and some MTs (Figure 3; also see Hall et al20). Of the 63 cells immunostained with mAbs directed against phosphoepitopes other than PHF1, 45 exhibited immunolabeling of one or more of the following mAbs: ALZ50 (25 of 57 cells sampled) TG3 (13 of 36 cells sampled), AT100 (4 of 10 cells sampled), or AT8 (24 of 57 cells sampled). These hyperphosphorylated deposits were highly localized, occurring in either the soma and/or the distal dendrites (Figure 4), except in cells exhibiting severe degeneration, where they were distributed throughout the cell (Figure 5). A typical example of a somatic deposit of hyperphosphorylated human tau at a relatively early stage of degeneration is shown in Figure 4; examples of distal dendritic deposits are shown in Figures 3 and 4. Although somatic hyperphosphorylated tau deposits tended to have a fibrillar appearance (Figure 4), much of the distal dendritic staining seemed to be localized to membranous structures. This was particularly true of ALZ50 staining (see Figures 4 and 5B), although examples of ALZ50-positive fibrillar deposits were also found (Figure 4, asterisk). All of the mAbs used labeled both fibrillar and membranous structures. Immunostaining for ALZ50, TG3, AT8, or AT100 appeared in the distal dendrites of 10 cells that did not exhibit somatic hyperphosphorylated tau deposits, whereas only two cells were found that exhibited the reverse pattern, suggesting that distal dendritic changes involving tau hyperphosphorylation usually preceded the onset of somatic tau hyperphosphorylation. Proximal dendrites were hardly ever labeled with any phosphoepitope-specific mAb other than PHF1 unless cytodegenerative changes were present throughout the cell as well (Figure 5). There was no clear temporal sequence of immunolabeling of human tau deposits with

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*Figure 3.* Swollen dendritic tips produced by localized aggregations of membrane-bound organelles are the first morphological changes seen as the result of human tau overexpression in ABCs. **A:** A swollen dendritic tip from a lightly expressing (stage 1) ABC immunostained with PHF-1. **B:** Electron micrograph of a similar swollen dendritic tip showing pronounced aggregations of membranous organelles. **Inset** region is shown at high magnification at right. **C:** These aggregations contain MTs (carets), tau filaments (arrows), and mitochondria (m), small vesicles (v), and smooth endoplasmic reticulum (sER) oriented in an apparently random arrangement, with some clumping of both vesicles and mitochondria. Scale bars: 10 μm (**A**), 5 μm (**B**), and 500 nm (**C**).
the four mAbs that were used to identify hyperphosphorylated tau deposits. Although ALZ50 was the only PHF-tau marker other than PHF1 recognizing tau in some ABCs, TG3 and/or AT8 staining appeared to precede ALZ50 in others. Moreover, there was no significant difference between the proportions of cells labeling with each mAb (other than PHF1), suggesting that the events triggering the onset of hyperphosphorylation may make each of these epitopes equally likely to appear.

The onset of human tau hyperphosphorylation in distal dendrites was invariably either preceded or accompanied by at least one of two characteristic dystrophic changes seen with heavy chronic human tau overexpression in ABCs. These were the localized distension of the distal-most dendrites, and the localized collapse of these dendrites at multiple sites, resulting in a series of swellings interspersed with constrictions causing the affected dendrites to resemble beads on a string (Figures 4 and 5A). Of the 34 ABCs that showed only distal dendritic swelling and beading and no other morphological abnormalities, 19 did not exhibit any hyperphosphorylation-related epitope (ie, AT8, TG3, ALZ50, AT100) at all.

Those ABCs at this stage of degeneration that did exhibit tau hyperphosphorylation invariably had it limited to distal dendritic and somatic regions (Figure 4). Proximal dendrites and somata were grossly unaffected by the morphological changes in the distal dendrites of these cells, exhibiting smooth, unbroken profiles (see Figure 4 for examples).

**Figure 4.** Swelling and beading of dendritic tips is characteristically accompanied by tau hyperphosphorylation in ABCs showing a moderate degree of tau-induced degeneration (stage 2). Each micrograph shows immunolabel with a different mAb: PHF1 (A), ALZ50 (B), AT8 (C), TG3 (D). A and C: Adjacent sections from the same ABC (28 days after injection of pRc123c). B and D are from different cells 28 and 34 days, respectively, after the injection of this plasmid. Note that although some sections show somatic tau deposits (asterisks), and all cells show immunolabeling in distal dendrites (arrows), there is no labeling of dendritic shafts (small carets) in the proximal dendritic field by any mAb except PHF1. Note the parallel nature in the development of morphological degenerative changes (ie, distal dendritic beading, A, and the appearance of AT8, ALZ50, and TG3 immunolabel in these areas) Also note that although many of the distal dendritic deposits of hyperphosphorylated tau appear to be membranous, some are fibrillar in appearance (C, arrow). Scale bar: 25 μm.

**Progressive Degeneration of the Proximal Dendrites and Soma Is Spatiotemporally Correlated with Human Tau Hyperphosphorylation**

Thirty ABCs showed more extensive and severe degeneration than that described above, exhibiting degenerative changes involving proximal as well as distal dendrites. In these cases, tau hyperphosphorylation was also extended to these regions (Figure 5). In most of these cells, extracellular human tau deposits that immunolabeled with PHF1 were also visible (see Hall et al19 and
These deposits were clearly located outside of the ABC plasma membrane, but some immunostaining appeared to be in the extracellular space (Figure 5A) whereas other deposits appeared to be inside of ependymal and/or glial cells (Figure 5, E and F). Some extracellular tau deposits also labeled with TG3, Figure 5.
ALZ50, or AT8, but usually less extensively than with PHF1. Such cells exhibited beading, distortion, and loss of dendrites throughout the dendritic field. As with the milder cases of degeneration described above, human tau deposits in severely degenerated ABCs appeared to be either membranous or fibrillar, with the former predominating in dendritic tips and the latter most prominent in the soma and proximal dendrites. There was no sign that immunolabeling of any epitope (other than PHF1) was more widespread or intense than the others.

We found that these severely degenerated ABCs (in which the entire soma and dendritic field was involved) could be divided into relatively mild cases, in which the nucleus was still present and the somatodendritic profile was still clearly defined, and the most severe cases, where the expressing ABCs no longer exhibited a recognizable nucleus or a continuous plasma membrane. Axonal profiles were still present in most cases and often appeared grossly normal, although extracellular PHF1 staining was occasionally found around axonal profiles (not shown). Nuclei, when present, were invariably tau immunopositive, unlike their appearance in milder cases of tau-induced degeneration (Figures 2E, 5A, and 5C, asterisks).

Proposed Staging of Tau-Induced Neurodegeneration in ABCs

To determine whether the differences between mildly and severely degenerated ABCs described above were because of a progressive sequence of degenerative changes over time, we re-examined sections from all of the ABCs used in this study and assigned each to a stage of neurofibrillary degeneration based entirely on morphological criteria. We used only cells that had either a PHF1- or GFP-immunolabeled slide through their somata, proximal dendrites, and distal dendrites for staging purposes. Sixty-seven of the 97 cells examined in this study met these criteria and were staged as described below by a person who was blind to the age and identity of each cell examined. We thus specifically excluded the length of time that human tau was expressed in ABCs and the presence of hyperphosphorylated tau as staging criteria, unlike their appearance in milder cases of tau-induced degeneration (Figures 2E, 5A, and 5C, asterisks).

Stage of Neurofibrillary Degeneration

PHF-1 HYPER-P

Stage 1
PHF-1 label is light, granular, and rare
Stage 2
PHF-1 label becomes heavy and may be heterogeneous and fibrillar
Stage 3
Hyperphosphorylated tau staining throughout dendritic field
Stage 4
Nuclear absent

Figure 6. Stages of neurofibrillary degeneration caused by chronic human tau overexpression in ABCs. The most prominent and/or typical changes seen in degenerating ABCs that are overexpressing human tau. Stages were assigned to 67 ABCs according to morphological changes visible in immunostained sections on the basis of PHF1 and anti-GFP immunolabeling only, without reference to either the presence of tau hyperphosphorylation, the clone or technique being used to induce tau overexpression, or the length of time after injection. Although the presence and/or pattern of tau hyperphosphorylation was not an a priori criterion for assigning cells to a particular stage, the close correlation between the patterns of neurodegeneration (shown by PHF1 label at left) and hyperphosphorylated tau label (defined as immunolabeling with AT8, TG3, AT100, or ALZ50) is shown for each stage (right).

There was a clear progression through stages 1 to 4 over time after injection (Figure 7A), with the average ABC spending ~10 days each in stages 1 to 2, and somewhat longer in stage 3. A clear positive correlation (P < 0.05 or better, chi-square test) between the time of human tau expression and the stage of degeneration reached in ABCs was retained when the cells were sorted according to: 1) tau isoform used, 2) the presence or absence of an epitope tag, and 3) whether expression was achieved with a plasmid or with the SFV mRNA vector (Figure 7B). Thus the presence of a GFP epitope tag, the method used for overexpression, and the isoform used (three versus four MT-binding repeats) did not significantly affect either the pattern or overall time course of degeneration. Moreover, both htau23 and htau40 produced virtually identical patterns of phosphotau labeling as well, with PHF1 present at highest concentrations in dendrites in stage 1 cells, and throughout the cells in all later stages.
The stages of neurofibrillary degeneration in ABCs exhibit striking points of resemblance to the staging of neurofibrillary changes in human hippocampal pyramidal cells proposed by Braak et al. and Braak and Braak. These investigators based their studies on autopsy material from persons who did not show widespread neurofibrillary pathology, allowing them to assign stages of cellular degeneration without reference to extracellular or synaptic influences. They found that the mildest degree of degeneration featured the appearance of tau immunostaining in an even, granular distribution throughout the soma and dendrites, followed by the expansion and distortion of the distal-most apical and basolateral dendrites. Stages 2 and 3 of Braak et al., in which distal dendritic tufts show swelling, distortion, and beading at the same time as fibrillar tau deposits (ie, early NFTs) are forming in the somata of hippocampal pyramidal cells, closely resemble stage 2 in ABCs (Figure 4). More severe stages of degeneration in the hippocampus, as in ABCs, feature the breakdown of somatic morphology, nuclear loss, and the appearance of extracellular tau deposits. On the other hand, there are features of the NFD modeled in ABCs that are not identical to human neurofibrillary disease. For instance, the order of appearance of specific phosphoepitopes appears to be somewhat different in ABCs and in incipient human NFD, with PHF1 rather than AT8 appearing throughout the cell at pretangle stages (stages 1 and 2) in ABCs. AT8 appeared in tau-expressing ABCs at a time when morphological degeneration was widespread in the distal dendrites, and did not precede overt morphological changes as they did in the human NFD.

Comparison of Neurofibrillary Degeneration in ABCs and Human NFD

In this study, we show that a single, stereotyped sequence of events emerges with the staging of tau-induced NFD in ABCs. This sequence is unaffected by the presence of epitope tags, independent of the isoform used, and is tightly correlated with the immunolabeling pattern of several epitopes (ALZ50, TG3, AT8, AT100) that are associated with tau hyperphosphorylation and neurodegeneration in human neurofibrillary disease. This pattern of neurodegeneration seems to be specific to human tau, as overexpression of lamprey neurofilament protein from a construct derived from the same parent plasmid causes accumulation of neurofilaments in ABCs, but no cytodegeneration. There are key features of this sequence that may have important implications for the cellular mechanisms responsible for the development of human NFD including the similarity that tau-induced neurofibrillary degeneration in ABCs bears to NFD cytopathology in human neurons, and the fact that it can be produced simply by overexpressing human tau. These features are particularly noteworthy as they suggest that simple accumulation of human tau can instruct a neuron to degenerate in a highly stereotyped way that is conserved among vertebrates and is specific to human tau. It is thus likely that a detailed investigation of the interactions between human tau and the cytophysiology of ABCs will provide critical insights into the mechanism of tau-induced neurofibrillary degeneration in humans that are unavailable from more complex and less accessible systems.
Hyperphosphorylation and Neurofibrillary Degeneration in ABCs

Human tau has been induced to form filaments under a variety of conditions in vitro and in vivo. These studies have suggested several possible mechanisms by which tau deposits of filamentous tau might form in NFD. However, although there is abundant in vivo and in vitro evidence that the phosphorylation of human tau protein regulates its ability to bind to and stabilize MTs and good reason to suppose that tau filament formation plays a critical role in tau-induced cytodegeneration, there is no clear evidence that the hyperphosphorylation of tau is required for either tau filament formation in vivo or the cytodegeneration caused by NFD. We showed in a previous study of tau-induced NFD in ABCs that human tau forms large numbers of filaments throughout ABC somata and dendrites by 10 days after plasmid injection, at a time when all of the cells expressing that construct (clone 93–htau23 with an N terminal fusion of EGFP) are in either stages 1 or 2 (see Figure 7A). Because there is little if any tau hyperphosphorylation outside of the distal-most dendrites before stage 3, it seems unlikely that tau hyperphosphorylation is prerequisite for tau filament formation, at least in ABCs. Immunolabeling for PHF1 alone, by contrast, occurs very early after the onset of tau expression in ABCs, and precedes the first morphological signs of degeneration. Thus, it is possible that the phosphorylation of the PHF1 site (but not other AD-related phosphoepitopes) is a necessary preliminary for tau filament formation and consequent degenerative changes in ABCs. Constitutive phosphorylation of the other AD-related sites might occur as a consequence of filament formation, especially if the incorporation of tau into filaments were to block access to tau phosphoepitopes by phosphatases that normally dephosphorylate tau at these sites. However, the tight association of tau hyperphosphorylation with the progress of degenerative changes in ABCs, murine, and ovine model systems, and human tauopathies suggests that it plays an important but as yet obscure role in NFD cytopathogenesis.

The Dendrites as an Initial Focus for Neurofibrillary Pathology

The other major implication of these data for the pathological mechanisms underlying NFD at the cellular level is the localization of the first pathological changes to the dendritic tips. This is particularly interesting in light of the membranous nature of most of the distal dendritic tau deposits and the involvement of membranous organelles in the swelling of dendritic tips that initiate the degeneration process in ABCs, and suggests a number of possible routes by which human tau overexpression might initiate a degenerative cascade. For instance, recent studies have implicated the N-terminal domain of human tau as a potential point of interaction between tau and the plasma membrane, and tau may also compete with kinesin in its interactions between membranous organelles and MTs under conditions where tau is overexpressed, leading to the disruption of kinesin-mediated transport of membranous organelles. Either of these interactions might plausibly lead to accumulations of membranous organelles both in the cell body and at dendritic tips, especially because dendritic MTs have mixed polarity.

The contrast that we found between the time of onset of gross morphological changes to dendritic tips and axons may also have important implications for the issue of where the initial changes leading to NFT formation and neurofibrillary degeneration occur in human NFD. Our results lend some support to the proposal that degenerative changes in the dendrites precede axonal degeneration in NFD. Although the dendritic tips began to show PHF1 immunolabel and signs of swelling during stage 1, overt changes to ABC axonal morphology suggestive of degeneration were not seen until stage 3. Normal looking axons were seen in a number of stage 2 to 3 cases that were processed for electron microscopy, where dendritic degeneration was later shown to be severe, with extensive MT and synapse loss. This is in agreement with earlier work in this system where the phosphorylation of...
Implication of These Findings for Interpreting the Role of Tau Mutations in Producing Human NFD

Finally, the observation that htau23 and htau40 produces virtually identical effects when overexpressed in ABCs has possible implications for understanding the roles played by the characteristic tau isoform differences between neurofibrillary lesions of clinically defined tauopathies and the mechanism of action of some tau mutations that induce NFD in humans. One of the most puzzling issues raised by the recent identification of mutations affecting the tau coding sequence and tau splicing has been the difficulty in accounting for the widely different neuropathology resulting from these mutations, some of which appear to merely change the relative frequency of tau isoforms with three versus four MT binding repeats. For instance, although we found that htau23 and htau40 produce patterns of degeneration that are indistinguishable from one another when overexpressed in ABCs, two recent reports of the effects of overexpressing htau23 and htau40 in mice showed quite different patterns of pathology. These differences emphasize the following points: 1) the likely importance of cell-type specificity in determining how the presence or absence of a particular tau isoform produces cell-type-specific susceptibility and patterns of neurofibrillary pathology in familial tauopathies, and 2) that the underlying cellular mechanisms responsible for tau-induced neurofibrillary degeneration are not isoform-dependent and depend on interactions between human tau and highly conserved mechanisms of neuronal physiology. Thus although the development of murine models that faithfully reproduce the characteristic pathological features associated with the various familial tauopathies will be essential for understanding their pathogenesis, there is also clearly a complementary need for further intensive study of the fundamental cell biological mechanisms responsible for tau-induced neurofibrillary degeneration at the level of a single, identified neuron. The large cell size, unique accessibility, and stereotyped morphology and physiology of ABCs should make them ideal for this purpose.

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