Review

Tau Biology, Tauopathy, Traumatic Brain Injury, and Diagnostic Challenges

Rudy J. Castellani[a],* and George Perry[b]
[a]Departments of Pathology and Neuroscience, West Virginia University School of Medicine, Morgantown, WV, USA
[b]College of Sciences, University of Texas, San Antonio, San Antonio, TX, USA

Abstract. There is considerable interest in the pathobiology of tau protein, given its potential role in neurodegenerative diseases and aging. Tau is an important microtubule associated protein, required for the assembly of tubulin into microtubules and maintaining structural integrity of axons. Tau has other diverse cellular functions involving signal transduction, cellular proliferation, developmental neurobiology, neuroplasticity, and synaptic activity. Alternative splicing results in tau isoforms with differing microtubule binding affinity, differing representation in pathological inclusions in certain disease states, and differing roles in developmental biology and homeostasis. Tau haplotypes confer differing susceptibility to neurodegeneration. Tau phosphorylation is a normal metabolic process, critical in controlling tau's binding to microtubules, and is ongoing within the brain at all times. Tau may be hyperphosphorylated, and may aggregate as detectable fibrillar deposits in tissues, in both aging and neurodegenerative disease. The hypothesis that p-tau is neurotoxic has prompted constructs related to isomers, low-n assembly intermediates or oligomers, and the "tau prion". Human postmortem studies have elucidated broad patterns of tauopathy, with tendencies for those patterns to differ as a function of disease phenotype. However, there is extensive overlap, not only between genuine neurodegenerative diseases, but also between aging and disease. Recent studies highlight uniqueness to pathological patterns, including a pattern attributed to repetitive head trauma, although clinical correlations have been elusive. The diagnostic process for tauopathies and neurodegenerative diseases in general is challenging in many respects, and may be particularly problematic for postmortem evaluation of former athletes and military service members.

Keywords: Dementia pugilistica, phosphorylated tau, repetitive head trauma, tau, tauopathy

SUMMARY OF PURPOSE

The primary purpose of this review is to highlight the complexity of tau in health and disease, and to point out the many uncertainties concerning its role in pathogenesis as well as diagnostic interpretation. It is intended to foster a more circumspect approach to molecular and clinical neuroscience with respect to tau biology, and the avoidance of premature conclusions with respect to: 1) the role of tau phosphorylation as a primary neurotoxic process; and 2) the relationship between tau pathology at autopsy and clinical problems that may have been present during life.

IDENTIFICATION OF TAU AND THE TAU GENE

Tau was initially identified by Weingarten et al. as a heat stable protein factor that would convert

*Correspondence to: Rudy J. Castellani, MD, WVU Department of Pathology, PO Box 9203, Morgantown, WV 26506, USA. Tel.: +1 304 293 3212; Fax: +1 304 293 1627; E-mail: Rudolph.castellani@hsc.wvu.edu.

6S dimers of tubulin into 36S rings necessary for microtubule polymerization [1]. They named this factor tau (τ) for its ability to induce tubule formation. Phosphorylation of tau was found to promote a conformational change favoring depolymerization of the tubulin assembly [2–4]. Brion et al. first reported immunohistochemical evidence of tau in paired helical filaments (PHF) in 1985 [5]. Later the same year, Grundke-Iqbal et al. [6] reported that bovine tau preparations reacted with antibodies to Alzheimer PHF, and that affinity purified antibodies labeled neurofibrillary tangles (NFT) and dystrophic neurites, but not amyloid-β (Aβ) plaques. Neve et al. [7] subsequently used cDNA clones for tau and mapped the tau gene to 17q21.

The tau gene on chromosome 17q21.31 spans 16 exons of approximately 150 kilobases of genomic DNA. In human brain, alternative splicing of exons 2 and 3 results in three isoforms with either 0, 1, or 2 inserts of 29 amino acids (0N, 1N, 2N) [7–11] (Table 1). Each of the three isoforms may contain 3 repeats (3R) or 4 repeats (4R) of the microtubule binding domain encoded on exon 10, resulting is six isoforms. 1N, 0N, and 2N isoforms comprise 54%, 37%, and 9% of tau in human brain, while 3R and 4R tau species are expressed in roughly equal amounts among 0N, 1N, and 2N tau [12–14]. Expression of tau isoforms is developmentally regulated and tissue specific [9, 15, 16]. In the human fetus, only the shortest isoform (3R, 0N) is expressed, while the same isoform is downregulated in the adult brain. Tau phosphorylation is also developmentally regulated, being high until the end of synaptogenesis, compared to the adult human brain in which only 2–4 mole phosphate are attached per molecule of tau protein [17].

The developmental shift in isoforms roughly coincides with the formation of synapses [18]. The 3R, 0N isoform that predominates during development shows the least microtubule binding affinity, and switches to a relative increase in 4R tau species over time, suggesting pressure for greater microtubule binding affinity in the developed brain, and perhaps a role for the 3R tau species in neuroplasticity or in response to injury. Increased 3R tau during cellular stress, as well as the persistence of fetal tau in the adult brain [19], support this concept.

The regulation of tau binding appears to occur by alternative splicing and post-translational modifications. Tau also has a short reaction time with microtubules [20], which might explain why a protein in such abundance within the axon does not interfere with axonal transport. There is evidence that tau has two binding sites for microtubules. Microtubule binding repeats bind protofilaments at the taxol-binding site of beta-tubulin. The proline-rich region binds a protofilament anchoring the projection domain on the surface of the microtubule [21].

It is interesting that exon 10 is constitutively expressed in rodents [22], but is regulated in humans [8, 9]. This may in part underlie human susceptibility to tauopathy compared to rodents. The relative microtubule instability conferred by human 3R tau in response to cellular stress favors a depolymerized phosphorylated species, compared to rodents in which microtubule binding is maintained in a steady state because of constitutive expression of exon 10.

Faulty regulation of exon 10 splicing in humans, and the resulting imbalance of 3R and 4R tau expression, is suggested as a pathogenic basis for human tauopathy [12]. Excessive inclusion of exon 2 and exon 3 has also been reported in gliopathy and spinal cord degeneration [23], although it remains to be determined whether cell specific expression of exon 2 and exon 3 is the basis for this finding.

Two tau haplotypes, referred to as H1 and H2, occur because of a 900 kb inversion polymorphism [24–26]. The H1 haplotype and the H1/H1 genotype is suggested to be a risk factor for progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), argyrophilic grain disease, and idiopathic Parkinson's disease [24, 27–32]. The H2 haplotype is associated with increased expression of exon 3 in grey matter, suggesting that the inclusion of exon 3 might be protective against neurodegeneration [33]. The H1/H2 genotype confers a greater risk of developing dementia before the age of 45 years in individuals with Down's syndrome [32, 34].

Normal tau function

The primary function of tau within the brain appears to be the binding of tubulin to promote polymerization and stabilization of microtubules [1]

Table 1
Genetic heterogeneity of tau

Differential regulation of exons 2, 3, and 10 in development and disease (6 isoforms)
Regulated 3R and 4R tau with different microtubule binding affinities
Two haplotypes (H1 and H2) that confer disease susceptibility
Pathogenic mutation causes frontotemporal dementia phenotype

(Table 2). Tau stabilizes and stiffens microtubules such that it supports the lengthy axon. Interactions with tubulin are dynamic processes with equal binding properties to both polymerized and non-polymerized tubulin, which regulates neurite polarity, axonal sprouting, and neuroplasticity, i.e., morphogenesis, and regulates axonal transport through interactions with motor proteins [35–41]. Microtubule binding confers a conformational change [3, 4], influences other diverse cellular processes [42–45], and interacts with other natively unfolded protein such as TDP-43, FUS, and alpha-synuclein [46–48]. A number of studies suggest alternative functions, including cell cycle regulation via tyrosine kinase, plasma membrane interaction, and synaptic function [42, 43, 46, 49].

Physiologic tau phosphorylation is therefore integral to life across species as a productive response to a variety of stressors including insulin dysfunction, glucose deprivation, starvation, hypothermia, hibernation, anesthesia, and glucocorticoids, among other conditions [50–57] (Table 3). Physiologic tau phosphorylation may also regulate subcellular localization of tau, which in turn may influence signaling cascades or synaptic function [58, 59]. A number of post-translational modifications apart from phosphorylation also occur which may have functional implications [58]. Among these are O-glycosylation, advanced glycation and the Maillard reaction, ubiquitination, nitration, SUMOylation, prolyl-isomerization, acetylation, and truncation [60–67].

Studies increasingly suggest a role for physiologic tau phosphorylation in synaptic function [59]. Tau is normally present at both pre- and post-synaptic sites [68], and accumulates as hyperphosphorylated tau at these sites in AD [69]. Whether tau diffuses across the synapse under normal conditions is an open question. Synaptic stimulation nevertheless induces site-specific, subsynaptic tau phosphorylation [70–72]. Tau mRNA has also been identified in axons and at subsynaptic sites, suggesting a role for local translation of tau in maintaining axonal integrity and synaptic function [73, 74]. Tau may also modulate signaling of synaptic neurotransmitter receptors, with post-synaptic tau phosphorylation acting as a "synaptic brake" via a complex and incompletely resolved mechanism. Glycogen synthase kinase 3 beta (GSK3β)-mediated tau phosphorylation, for example, may regulate neurotransmitter receptor endocytosis and negatively influence long term depression [59, 72].

The biology of hibernation is interesting in that tau protein transitions to a PHF-like phosphorylated state, involving epitopes typically related to tau phosphorylation in AD. Yet the phosphorylation state is completely reversible upon arousal from torpor and return to euthermic conditions [75]. This tends to suggest that tau phosphorylation in AD is a reactive phenomenon rather than a primary toxic process, and raises the issue of whether controlled hyperphosphorylation of tau confers cellular protection.

Tau has been shown to bind filamentous actin of dendritic spines as further evidence of its role in cytoskeletal integrity [76, 77]. Other studies have localized tau to the nucleus and the centrosome, in addition to the mitotic spindle microtubules of dividing cells [78–80], suggesting that tau phosphorylation might be involved in nucleus-cytoplasm translocation and cell cycle transition. Tau can also bind DNA, whereas tau phosphorylation may prevent DNA binding [81, 82]. Nucleolar organization and protection of genomic DNA is still another potential function [83, 84]. Tau is found in association with RNA as part of a ribonucleoproteome, complexing with RNA and a variety of proteins [48, 85, 86]. Finally, tau is also expressed in astrocytes and oligodendrocytes, the latter with all six isoforms, although with a lesser degree of microtubule binding [87–89]. Oligodendrocyte tau appears to be involved in microtubule stability during morphogenesis and myelination [90].

Table 2
Some physiologic functions of tau

Stabilization of microtubules
Actin binding and cytoskeletal integrity
Regulating neurite polarity
Axonal sprouting
Neuroplasticity
Axonal transport
Cell cycle regulation
Plasma membrane interaction
Synaptic transmission ("synaptic brake")

Table 3
Some stimuli for tau phosphorylation

Insulin dysfunction
Glucose deprivation
Starvation
Hibernation
Hypothermia
Anesthesia
Glucocorticoids
Opiates
Alcohol

Tau protein phosphorylation and hyperphosphorylation

Normal tau is a highly soluble natively unfolded protein [91–93], which contrasts with hyperphosphorylated p-tau in NFT which is highly insoluble [94]. The latter should be distinguished from physiologic tau phosphorylation, which is an ongoing dynamic process in the brain, and a necessary, tightly regulated process [75]. Phosphorylation regulates interactions involved in subcellular distribution and axonal transport [95, 96], organelle delivery to the somatodendritic compartment [97], neurotransmitter receptors [98], apolipoprotein E [99], Src kinases [49, 100, 101], and Pin1 [102]. Because of its high number of serine and threonine residues, tau protein is an excellent substrate for protein kinases, especially proline-directed kinases such as GSK3β [75]. Tau phosphorylation by cyclin dependent kinases and mitogen activated protein kinases [103–105], emphasize the role of tau metabolism in cellular division and proliferation. Non-proline directed kinases are also involved [106]. GSK3β and cdk5 may play a relatively more prominent role in tau phosphorylation in the human brain [75]. Interconnection of the kinase network, promiscuity of protein kinases, and the tendency of phosphorylation sites to cluster present technical challenges to the study of tau phosphorylation *in vivo*. The phosphorylation yield at any given site is low and can be difficult to assess. Site directed mutagenesis results in complex alterations in ionic properties, which limits the significance of experimental findings [75].

Numerous phosphatases dephosphorylate tau *in vitro* [43, 107], especially PP2A which is thought to also play a role *in vivo* [108]. Activity of tau protein phosphatases is further regulated by endogenous inhibitors, which themselves are subject to regulatory phosphorylation [75], emphasizing the complexity of tau phosphorylation.

The broad property of "hyperphosphorylation" is a hallmark of tau aggregates in AD, numerous other tauopathies, and aging [17, 109, 110]. Many phosphorylation sites occupied in PHF tau may be occupied in the normal brain [75]. In advanced disease, most of the approximately 39 potential AD phosphorylation sites [111, 112] are phosphorylated, with total phosphate content in p-tau pathological aggregates three times that of physiologic tau [17, 113]. One study in transgenic mice reports that pathological hyperphosphorylation is characterized by an increase in the proportion of phosphorylation at given residues, rather than an increase in the total number of phosphorylated residues [58], suggesting that tau "hyperphosphorylation" reflects an exaggerated physiologic phosphorylation, rather than disorganized phosphorylation at random sites receptive to phosphate groups. Still other studies suggest a role for molecular isomerism catalyzed by proline isomerase, with cis isomers of the Thr231 proline motif of p-tau variously labeling lesional brain tissue in AD and former professional athletes, as well as acutely traumatized murine neurons and axons in acute or recent trauma in humans [114, 115]. Trans isomers of p-tau are said to be "physiological" [114], although their specific role in the diversity of cellular tau functions is unclear.

It is noteworthy that antibodies used in p-tau analyses *in vitro* and *in vivo* react to highly selective epitopes, each with functional and pathological implications. The widely used monoclonal antibody AT8, for example, is used to identify tau phosphorylation at Ser 202, Thr 205, and Ser 208, which in turn identifies a wide spectrum of tau aggregates including the "pre-tangle" in autopsy brain [116]. Pretangle aggregates are not otherwise apparent using histologic dyes such as hematoxylin and eosin, or silver impregnation techniques such as Bielschowsky silver. For this reason, p-tau as identified by AT8 immunohistochemistry may lack any associated pathological alteration (such as a morphologically identifiable NFT). Pathology with a hypothesized link to repetitive traumatic brain injury (TBI) for example is often entirely immunohistochemical, with no tissue reaction that would otherwise suggest that an injury has taken place. This tends to raise questions about p-tau immunoreactivity as an indicator of cell death with repetitive TBI exposure. This may also explain the lack of eloquence regarding p-tau and clinical signs [117–120]. Phosphorylation at Thr 212 and Ser 214, identified in tissues by monoclonal antibody AT100, may be a better indicator of more advanced pathology [121], less sensitive than AT8 but more specific for pathological aggregates.

Decomposition and associated artifacts are synonymous with postmortem human brain analyses, and may be underappreciated. It is known, for example, that postmortem changes in the phosphorylation state is a dynamic process, with dephosphorylation of p-tau occurring rapidly postmortem, in a site-specific manner [122–128]. P-tau autopsy tissues may preferentially label buried epitopes, i.e., resistant to degradation. The patterns of immunoreactivity

in the human brain may therefore be skewed toward postmortem artifact and away from solubility or *in vivo* biological relevance.

Hyperphosphorylation of tau may result from an imbalance in the activity of tau protein kinases and tau phosphatases, which in turn may be necessary for the formation of pathological fibrils. The conversion of physiologic tau to filamentous tau is believed to be a multi-step processes, with microtubule detachment as the initial step [129–131]. A number of biological mechanisms have been suggested [132–136]. Higher concentrations of tau may also influence conformational changes necessary for fibril formation [129]. Interestingly, 3R tau is said to facilitate twisted paired helical filaments such as those seen in classical AD NFT, while 4R tau has a tendency to assemble into straight filaments such as described in PSP [137]. Whether fibrillar or PHF tau signifies cytotoxicity, versus a productive response to the aging process or cellular stress, remains an open question [138]. Direct experiments verifying a feed-forward pathological cascade are sparse, with some studies showing no correlation between NFT accumulation and length of microtubules [139]. Still other studies demonstrating adduct formation (e.g., advanced glycation, advanced lipid peroxidation), and sequestration of redox active transition metals, may indicate that p-tau aggregation, up to and including PHF tau, is a productive response to cellular stress [140, 141].

Studies in recent years have increasingly implicated soluble, low-n tau assembly intermediates as the toxic or biologically active species [142–149]. The same concept is invoked for Aβ in AD [150]. This again suggests that the most readily identifiable postmortem lesions detected by immunohistochemistry may be the least biologically relevant. In one inducible transgenic model, progression of insoluble tau pathology was noted after suppression of mutated tau gene expression, during the process of functional recovery [144].

"Tauopathy"

The broad term "tauopathy" was first suggested in 1997 for a familial degenerative tauopathy [151] and is often used to connote diverse neurodegenerative diseases characterized by p-tau accumulations with various morphologies and clinical correlates (Table 4). To the extent that tauopathy implies the accumulation of p-tau as a rate-limiting factor for disease pathogenesis, the terminology may be unfortunate. A convincing case could be made that p-tau

Table 4
Diseases with tau neuropathology

Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17)
Alzheimer's disease
Aging
Primary age-related tauopathy
Aging-related tau astrogliopathy
Progressive supranuclear palsy
Pick's disease
Argyrophilic grain disease
Corticobasal degeneration
Progressive subcortical gliosis
Amyotrophic lateral sclerosis/parkinsonism-dementia complex
Diffuse neurofibrillary tangles with calcification
Dementia pugilistica
Tangle-only dementia
Down syndrome
Gerstmann-Straussler-Scheinker disease
Hallervorden-Spatz disease
Creutzfeldt-Jakob disease
Globular glial tauopathy
Niemann-Pick disease type C
Prion protein cerebral amyloid angiopathy
Subacute sclerosing panencephalitis
Myotonic dystrophy
Non-guanamian motor neuron disease with neurofibrillary tangles
Postencephalitic parkinsonism
Meningioangiomatosis
Tuberous Sclerosis

is a disease response, perhaps even a productive disease response [117, 138]. The term "tauopathy" may be subclassified into "primary" tauopathy, in which p-tau accumulation is the major pathological finding, or "secondary" tauopathy, in which some other protein deposit occurs (e.g., Aβ, prion protein) [75]. P-tau in sporadic primary tauopathies may not correlate with neuronal loss in some diseases [120]. Rigorously defined, true primary tauopathies may be limited to frontotemporal lobar degenerations associated with pathogenic mutations of the tau gene (MAPT) on chromosome 17 (FTDP-17) [152]. Like familial AD with APP mutations, the role of tau mutation in the molecular pathology is unclear. Some studies suggest that MAPT mutation causes chromosomal instability and aneuploidy [153, 154], rather than the elaboration of a toxic tau species *per se*.

Sporadic tauopathies are currently classified as frontotemporal lobar degeneration-tau (FTLD-tau), which encompasses Pick disease, PSP, and CBD [120]. Interestingly, MAPT remains the most substantial association by genome wide association analysis [155], and patients with MAPT tau mutation have clinical and pathological features that overlap with PSP and CBD [156, 157]. CBD and PSP clinical

Table 5
Experimental and other limitations of the tau prion concept

Inefficiency of templating in culture
Methods for generating neurotoxic species not standardized
Fibril characteristics necessary for seeding are poorly defined
No consensus standards for tau seeding in culture
Relevance of mutant tau
Selective isoform expression experimentally versus nonselective expression *in vivo*
Tau expression promiscuity in transgenic animals
Tau leakiness
Axonal (as opposed to perikaryal) expression of tau
No natural tauopathy in rodents
Phenotypic propagation of neurodegeneration as a function of strain is not demonstrated
Does not explain selective vulnerability
Contradicted by early appearance of tau in structures with diffuse projections

phenotypes tend to contain lesions composed mainly of 4R tau, which supports dysfunctional microtubule binding as a factor in neurodegeneration. Pick disease phenotype (the least common of the sporadic FTLD-tau phenotypes), on the other hand, contains lesions comprised of 3R tau. Given the tendencies toward tau isoform specificity in FTLD-tau, it is tempting to suggest specific isoforms as therapeutic targets [158]. Such a construct would require p-tau as inherently toxic, however, which is not established.

The tau prion

Clavaguera et al. first demonstrated the elaboration of tau filaments following injection of wild-type mice with tau derived from P301S transgenes, raising the issue of protein-only transmission of phenotypic characteristics [159]. Relevance to human disease nevertheless requires the presumption that p-tau is neurotoxic in the human brain *in vivo*, which remains an open question. Conceptualizing the tau prion is challenging, and involves putative processes such as seeding, templating, spread, strain variation, transcellular propagation, trans-synaptic propagation, functional connectivity, selective vulnerability, and prion-like, each with a level of imprecision [160]. Pliability of definition is evident with terms such as "infectious prions", "non-infectious prions", "quasi-prions", and "transcellular prionoids" [161]. The tau prion nevertheless provides a framework for neurodegeneration based on non-mendelian, horizontal transmission of deleterious information, which is at issue in genuine prion disease. Protein-only transmission of phenotypic information in yeast is well-characterized [162]. By the prion analogy, p-tau would template or seed brain tissue, confer adverse biological properties on naïve tau molecules, and perpetuate an autocatalytic neurodegenerative cascade.

Kaufman et al. provide evidence for seeding phenomena and strain variation in tauopathy [163, 164], although their relationship with neurodegeneration in progressive tauopathies is unclear.

There are some limitations of the tau prion concept (Table 5). In an early seminal study by Frost et al., extracellular tau at supraphysiological levels templated intracellular tau in less than 2% of the cells, while the conformationally templated p-tau in cell culture showed little, if any, resemblance to NFT [165]. Guo and Lee used recombinant 4-R tau [166], which has weak amyloid-like properties in human tauopathies compared to mixed 3R and 4R tau in AD. Sonication is often used to generate neurotoxic species for *in vitro* analyses, which is not standardized across laboratories. Characteristics of tau fibrils necessary for seeding experiments are poorly defined. Consensus standards for tau seeding in cell culture studies do not exist [160]. Many studies employ mutant tau, which is of doubtful relevance to sporadic disease since MAPT mutations do not occur in the overwhelming majority of human tauopathies. Most studies on tau propagation also utilize truncated tau [160], mutated or not, rather than full length tau. While reasonable in theory given C-terminally truncated tau in the synapse, it generally ignores the multiple isoforms in humans with variable splicing of the C-terminus and N-terminus. Many studies use recombinant tau rather than tau filaments derived from human disease, raising an issue of biological relevance. Propagation studies that rely on selective expression of specific isoforms do not take into account the fact that expression of 3R and 4R isoforms occurs in all human tauopathies, regardless of whether the predominant form in pathological lesions is 3R, 4R, or a mixture of both [160]. Transgenic constructs relying on conditional expression of tau [167] may have unaccounted for promiscuity

(e.g., expression outside the entorhinal cortex), tau "leakiness," [160] or axonal tau mRNA and expression outside the cell body [73, 74].

Transgenic mice expressing, or overexpressing, a single isoform of wild-type human tau, do not develop tauopathy or neurodegeneration [160], while various other experimental constructs show tau expression without neurodegeneration [168, 169]. Tau prions have yet to be re-derived and re-injected with phenotypic changes in subsequent passages, separating tau prions from conventional prion disease. It may finally be pointed out that p-tau accumulates initially within neurons of the locus ceruleus, appearing as early as childhood [170]. Since the locus ceruleus is said to be "unsurpassed" among brain regions in the diffuseness of its connections [171, 172], trans-synaptic or transcellular neurodegeneration appears to be limited in the aging process *in vivo*.

Age-related p-tau

Recent analyses of primary age-related tauopathy (PART) [118] and aging-related tau astrogliopathy (ARTAG) [173] have expanded the spectrum age-related p-tau accumulation patterns. PART is, in essence, an accumulation of p-tau within medial temporal lobe and subcortical structures, with little or no Aβ deposition. Clinical symptoms range from no symptoms to mild symptoms involving the memory domain. Most cases previously referred to as "tangle only dementia" or "tangle-predominant senile dementia" are likely within the PART spectrum. It is noteworthy that classic studies of dementia pugilistica (DP) describe neurofibrillary degeneration in a similar distribution [174], which raises the possibility of coincidental p-tau pathology.

The related condition ARTAG, refers to p-tau accumulation within astrocytes, with a tendency for subpial, subependymal, and perivascular areas, and in subcortical white matter. Like PART, ARTAG has no predictable clinical substrate and overlaps substantially with pathology hypothesized as a substrate for repetitive neurotrauma [173]. PART is said to be primarily neuronal although tau astrogliopathy may co-exist with PART. Interestingly, PART is a mixed 3R/4R tauopathy whereas ARTAG is a 4R tauopathy, suggesting some degree of cell type specificity. This may in part explain why AD is a mixed 3R/4R tauopathy, while PSP and CBD, with an abundance of 4R tau, have prominent glial p-tau accumulation. A recent study of 687 postmortem brains from a spectrum tauopathies suggested variable distribution patterns of ARTAG, and differing pathogenesis possibly related to cerebrospinal fluid circulation or mechanical forces. The clinical significance of these patterns was not studied. The issue of spread among astrocytes was raised but remained speculative [175].

TBI and tauopathy in athletes and military service members

Athletes

The relationship between repetitive TBI and neurodegenerative tauopathy has been poorly understood for decades. It remains theoretical and is problematic for a number of reasons [176] (Table 6). The TBI component of the equation itself presents a significant challenge for study. Mathematical thresholds for parenchymal and vascular injury are impossible to quantify (reviewed in [177]). TBI repetition is largely undefined, and the role of TBI repetition of whatever extent on injury thresholds or putative tauopathy is unknown. Given myriad conditions associated with p-tau accumulation, as well as the numerous biological processes associated tau phosphorylation, it is

Table 6
Limitations of the TBI-progressive neurodegenerative tauopathy concept

Neurological signs attributed to early 20th century boxing were not progressive in most cases
Index case of DP at autopsy was most likely familial AD in a former boxer
Index case of putative DP-like disease in a football player depicted age-related changes [184]
Putative disease process is currently defined solely by immunohistochemistry (no clinical correlate required; no neurodegeneration (neuron or axon loss) required)
TBI in athletes is inferred from participation; otherwise undefined and impossible to quantitate
Athletes in modern case series were neurologically asymptomatic or had known neurodegenerative diseases in most cases
National Football League cohort has less cancer, fewer suicides, lower mortality, and better cardiovascular health compared to controls (no evidence of a pervasive, fatal disease related to occupational exposure)
Studies suggesting AD risk with mild TBI are inconsistent (no risk or modest risk)
AD is not confirmed pathologically in studies showing AD risk with moderate or severe TBI (dementia from structural brain injury in some cases not excluded)
No longitudinal data exists demonstrating TBI, latency, clinical neurodegeneration, and neurodegenerative pathology

nevertheless expected that TBI at some level of severity may stimulate physiologic tau phosphorylation, and even that p-tau inclusions may appear over time following TBI in some instances, for reasons not fully elucidated.

TBI-neurodegenerative disease theory began with the investigation of boxers in the early part of the 20th century, some of whom demonstrated neurological signs and a putative condition known in boxing circles as "punch drunk." [178] Signs included dysarthria, gait disturbance, tremor, and cognitive impairment, as well as dementia in some cases (later termed 'dementia pugilistica' by Millspaugh [179]). It is important to note that neurotrauma exposure in boxers of this era was extreme [180, 181]. Many hundreds of fights over a lengthy career, with additional exposure in boxer booths and as sparring partners for elite fighters, were commonplace. Medical oversight was limited, with no mandatory exclusion times. Boxers often used light gloves. Little care was taken to match evenly weighted or evenly skilled boxers, and there was little inclination to stop fights short of incapacitating and often multiple TBI of a range of severities. The emergence of neurological manifestations of TBI in this setting is therefore not surprising.

Theoretical constructs suggesting a relationship between TBI and chronic disease have evolved considerably. Concussion-related hemorrhage was suggested by Martland as a pathological substrate for Punch Drunk, but was discarded by the 1940s. Martland included autopsy data of acute TBI in a non-boxer to support his theory, although there was no mention of NFT or other neurodegenerative inclusions. The case itself depicted gross features of diffuse axonal injury, suggesting conflation with severe, acute TBI. Later pathogenic theory by Millspaugh also emphasized acute traumatic injury with no mention of NFT [179], suggesting some difficulty with an explanation for chronic disease and disease progression. Interestingly, the first report of microscopic neuropathology by Brandenburg and Hallervorden in 1954 [182] indicated in retrospect a case of early-onset, and likely familial autosomal dominant AD, having nothing to do with boxing. The depiction of lesions that could aptly be regarded as "cotton wool" plaques, early-onset dementia, and extensive cerebral amyloid angiopathy suggest presenilin 1 mutation [183].

In 1973, Corsellis and colleagues [174] reported neuropathological features in 15 boxers from the early 20th century, most of whom had severe neurologic impairment. The findings included NFT,

prominent in the medial temporal lobe and out of proportion to plaque pathology (distinguishing DP from AD), loss of pars compacta neurons of the substantia nigra, scarring of the cerebellar tonsils, and fenestrated cavum septum pellucidi. Thin fornices and atrophy of the mammillary bodies were common in this series. Vascular disease with infarcts and other co-morbidities such as contusions, neurosyphilis, and cavernous malformation were also present. The NFT assumed primary importance, however, eventually placing DP in the lexicon of tauopathies, and solidifying the notion of TBI-induced progressive degenerative tauopathy, perhaps prematurely. Careful examination of the clinical data and neuropathology from historical cases casts doubt on the concept of a progressive AD-like or PD-like neurodegenerative disease, or otherwise progressive degenerative tauopathy following a period of latency, even among early 20th century boxer with extreme levels of neurotrauma exposure [176].

The Corsellis et al. series was reported prior to the advent of immunohistochemistry (i.e., the concept of tauopathy), although case material from that series was relied upon for later immunohistochemical studies [185–188], likely because of reduced neurotrauma exposure in boxers and few new DP cases. Studies in recent years more often include asymptomatic boxers [189] (notwithstanding one death from acute TBI), or boxers who became symptomatic from other neurodegenerative diseases [190–193]. Boxing-related neuropathology has also become progressively more subtle, limited to immunohistochemical reactivity in some cases [194]. In essence, there has been a shift in case material from men with unambiguous neurological signs due to head trauma from boxing, to deceased men who happened to have boxed.

Attempts to link TBI to neurodegenerative tauopathy in non-boxers from 2005 forward follow a similar pattern. Subjects either lacked neurological signs attributable to TBI or had other neurodegenerative diseases. Diagnosis instead relies only on brain p-tau interpretation [195, 196]. Identification of p-tau in some cases requires whole brain screening with free-floating immunohistochemistry of 50 μm hemispheric sections obtained from a sledge microtome [198]. Given the ubiquity of p-tau with age [170], the high frequency of p-tau deposits in former American football players is not surprising [197], nor is the fact that p-tau patterns attributed to TBI are described in people with no history of neurotrauma [198–202]. Data from other athlete cohorts are sparse, but tend to be similar. Autopsy studies of p-tau in

former soccer players, for example, describe p-tau in athletes with either no neurological signs [203], or neurological signs attributable to known neurodegenerative diseases [204–208].

In summary, available scientific evidence does not demonstrate a causal link between athletic participation and progressive neurodegenerative tauopathy in athletes, or for that matter a risk for genuine neurodegenerative disease [209], either from TBI inferred from athletic participation or athletic participation in general. Such a link would also be in conflict with epidemiological data demonstrating that NFL athletes in particular have better overall health (lower cancer rates, lower mortality, better cardiovascular health), and lower suicide rates [210], notwithstanding a modest increase in AD and amyotrophic lateral sclerosis (ALS) [211], which may be explained by the lower mortality. Indeed, the superior overall health of the NFL cohort, combined with the reported high frequency of focal p-tau immunoreactivity, indicate axiomatically that the focal p-tau as described has no clinical impact across the group as a whole.

Military service members

Military service members are vulnerable to TBI because of the nature of armed conflict and military training, and also because of the increased use of improvised explosive devices (IEDs) [212]. Most military service-related TBIs since 2006 have been associated with IED blasts [213]. Case reports and small case series have likewise described focal p-tau immunoreactivity patterns, hypothesized to be due to blast-related TBI sustained in the service [214, 215]. Reports have gone so far as to suggest that post-traumatic stress disorder may share common neurobiological underpinnings with neurodegenerative tauopathy [214–216].

Some studies have suggested that military service-related TBI is a risk for AD specifically. A study of World War II veterans suggested that AD risk was increased in subjects with a history of moderate or severe TBI in a dose dependent fashion, with moderate TBI conferring roughly two-fold risk, and severe TBI conferring a roughly four-fold risk [217]. The study did not find an AD risk with mild TBI, which is in line with one systematic review [218]. However, one recent, large-scale case-control study of US veterans concluded that mild TBI without loss of consciousness conferred a modest risk for dementia as well as AD specifically [219]. The risk was higher in mild TBI with loss of consciousness, and higher still with moderate or severe TBI, again suggesting

a dose-dependent relationship between TBI and AD. A recent large cohort study of civilians in Denmark concluded that mild TBI conferred a modest risk for both dementia and AD [220].

Causal assertions from epidemiological studies remain hypothetical, however. The risks are overall modest as noted. The dose–response relationship between AD and TBI severity is also problematic in that severe TBI causes dementia and reduced life expectancy, while AD increases exponentially after middle age. Small relative risks in this setting may be due to misclassification of TBI-related dementia as AD in subset of cases. For example, Lewin et al. [221] studied 75 severely head-injured patients and found that patients often had dementia from TBI, with few surviving more than a decade. In another study, of 288,009 hospitalized survivors of TBI, 124,626 developed long-term disability including dementia [222]. Accurately assessing AD risk in this setting may therefore require pathological confirmation (generally not available in large scale epidemiological studies), since moderate and severe TBI often include structural brain damage [219] (e.g., contusion, laceration, diffuse axonal injury), which may in turn cause "dementia." To date, no longitudinal study demonstrating the sequence of TBI, a period of latency, clinical neurological deterioration, and autopsy-confirmed AD has been presented. More research is needed before the null hypothesis—that the reported dose-response relationship with service-related TBI and AD is a statistical artifact—can be rejected.

Blast-related TBI has emerged as a major cause of morbidity and mortality in military service. Blasts have been the most common cause of injury in American soldiers since 2006; of the ∼1 million veterans screened for TBI between 2007 and 2015, 8.4% reported TBI, the majority of which were mild and associated with blast [213]. Injury to the brain associated with blasts is heterogeneous [212]. Primary blast injury due to positive and negative pressure waves, secondary injury due to shrapnel, tertiary injury due to acceleration of the head and body across the war theater, and quaternary injury due any downstream pathology, including burns, lung injury, mass effect from brain swelling, ischemic brain injury, etc., are components of the blast injury complex. Neuropathological sequelae of primary blast injury are unclear, although early data suggest astroglial scarring at sites of differing tissue density (gray-white interface, periventricular tissue, perivascular areas, subpial areas) [223]. P-tau proteinopathy was

inconsistent in this series, arguing against the hypothesis of blast-induced tauopathy.

Tau immunohistochemistry, TBI, and diagnostic challenges

Given the complexities of tau biology as well as the unproven concept that TBI causes neurodegenerative tauopathy, limitations in the diagnostic process, for which there is a paucity of literature, may not be fully appreciated (Table 8). Pathological assessment and tissue sampling typically involve a multitude of brain regions, the standards for which are variable and evolving. Antibodies in common use for immunohistochemistry react with only one of a large number of candidate epitopes, and may have differing reactivities as a function of epitope, time, and lesion type [224]. Human tissue is limited to postmortem brain, which is by definition partly decomposed and subjected to phosphatase activity as noted above. Epitopes that survive phosphatase activity are amplified by polymers [225], such that the tissue expression overestimates the true amount of p-tau. Cross reactivity is usually controlled for by omitting the primary antibody rather than by absorption with purified protein. The extent to which p-tau antibodies react with tau epitopes *per se* in any given case is, strictly speaking, unclear.

Neuropathological assessment by immunohistochemistry is therefore an entirely empirical exercise, permitting no conclusions about the nuances of tau pathobiology. The focus is instead on microscopic morphology [118, 173], which may be misleading as an indicator of disease or neurotoxicity (reviewed in [226]). P-tau immunohistochemistry also calls attention to selective vulnerability, for which there is no explanation. The questions of why, for example, the neurons of the cerebellar cortex are spared of p-tau even in end-stage neurodegenerative tauopathy, or

Table 7
Some p-tau microscopic lesions

Neurofibrillary tangle
Flame-shaped neurofibrillary tangle
Globus neurofibrillary tangle
Ghost tangle
Pre-tangle
Dystrophic neurite
Neuropil thread
Grain
Tufted astrocyte
Equivocal tufted astrocyte
Coiled body
Astrocytic plaque
Globular astroglial inclusion
Ramified astrocyte
Thorny astrocyte
Fuzzy astrocyte

why the neurons of the locus ceruleus or the basal nucleus of Meynert may accumulate p-tau early in life, separately or together, are unanswered. Factors responsible for AD variants such as limbic predominant AD and hippocampal sparing AD, are similarly elusive [160].

Added to the diagnostic challenges are limitless morphological variations and patterns of immunoreactivity [75, 173, 227] (Table 7). The NFT, dating to the early 20th century [228], was the primary lesion of interest until the identification of tau as the major protein component of NFT and the advent of immunohistochemistry. Neuropil threads, dystrophic neurites, and a variety of morphologic p-tau presentations within neurons in AD and FTLD-tau were described subsequently [120, 229]. Various morphological subtypes of glial inclusion are reported in recent literature, including tufted astrocytes, oligodendroglial coiled bodies, astrocytic plaques, globular astroglial inclusions, ramified astrocytes, "equivocal tufted astrocytes," thorn-shaped astrocytes, and granular or fuzzy astrocytes,

Table 8
Challenges in addressing TBI-p-tau theory at autopsy

Poor correlation of p-tau accumulations with clinical signs
Frequent lack of detailed TBI history
Evolving standards for sampling, immunohistochemistry, and diagnosis
Subjectivity in interpreting p-tau accumulations and tissue architecture
Broadening spectrum of benign, age-related p-tau patterns
Lack of guidelines for assessing vascular disease, metabolic derangements, polypharmacy
Unknown error rate between and within neuropathologists
Variable clinical characterization of individual cases during life
Absence of genetic data
Broad public misunderstanding of TBI consequences driven by scientifically naïve media
Absence of patient consequences for misdiagnosis at autopsy
Vulnerability to *ipse dixit* interpretation

each considered somewhat specific among the tauopathies [120, 227, 230–234]. The error rate in distinguishing among these descriptive morphologies, irrespective of clinical correlation, is entirely unknown.

Clinical correlations in neurodegenerative proteinopathy are also more limited than may be appreciated [235]. For example, AD proteinopathy cannot predict the level of cognitive dysfunction unless the pathology is end-stage [119]. Decedents with a substantial level of p-tau pathology in their medial temporal memory circuitry are often cognitively normal [118]. In the elderly, proteinopathy is virtually meaningless as a predictor of cognitive function by blinded analysis [236]. Proteinopathy cannot distinguish clinical Parkinson's disease from the clinical presentation of Lewy body dementia [237]. PSP p-tau pathology may be associated with CBD clinical manifestations, and vice versa. Both PSP and CBD p-tau pathology may occur in patients with the behavioral variant of frontotemporal dementia or primary progressive aphasia [238]. Patients with frontotemporal lobar degeneration may show signs of ALS, and patients with ALS may develop the spectrum of frontotemporal dementia phenotypes [238], none of which have been shown to correlate with proteinopathy burden with any degree of precision. The presence of neurodegeneration, i.e., neuronal/axonal degeneration, has an inconsistent relationship with p-tau pathology in CBD and PSP [120]. Clinical signs correlate more with neurodegeneration than proteinopathy [120], suggesting that proteinopathy may be epiphenomenal in some cases. This may explain substantial proteinopathy with intact cognition [118, 170, 173, 236], or the lack of a clinical correlate of p-tau pathology described in former athletes or military service members. There are also few guidelines for assessing metabolic derangements, numerous medications, and microvascular disease [239], which influence cognition independent of proteinopathy.

Some guidance is available in terms of consensus recommendations [118, 119, 173, 238, 240–242], although these tend to be provisional and subject to repeated modification. Because of the nature of consensus guidelines, i.e., the formal recognition that the science is unresolved, their application in neuropathology tends toward precision (consistency in pathological assessment), rather than accuracy in identifying clinical disease. This is reflected in consensus recommendations for AD, in which the preferred terminology is "Alzheimer's disease neu-

ropathologic change," irrespective of clinical findings during life [119]. Similarly, consensus recommendations for frontotemporal lobar degeneration are concerned with patterns of neuropathology rather than clinical subtypes [242].

Added to the bewildering array of pathological lesions and clinical correlations, is the human element. The breadth of circumstances associated with prospective case material, and interpretation for the sake of diagnosis for interested families, may be considerable. Any given case may present with little or no clinical information, and variably rigorous clinical disease classification during life. The specialization of treating physicians may vary from general family practitioners to neurologists with specific expertise in dementia and movement disorders. Genetic data is often not available. Imaging studies may cloud the diagnostic process by suggesting some conditions over others based on variable image acquisition sequences and soft anatomical data.

The diagnostic process may be even more challenging in the arena of presumed repetitive neurotrauma. TBI history may be absent or incomplete, or inferred from a history of athletic participation or military service. Surviving next-of-kin may believe that recent onset of psychiatric signs is due to sport participation many decades prior. A family may be struggling with an inexplicable neurodegeneration in a family member. They may believe that concussion causes suicidal ideation or neurodegeneration because of scientifically naïve media reporting [243–245], or they may be unwilling or unable to accept that a family member took his or her own life. Families may demand that certain items appear or not appear on death certificates, or they may be interested in seeking damages from a third party, which may in turn lead to profligate tissue sampling and p-tau immunostaining in an attempt to confirm a desired diagnosis. The diagnostic process further takes place in the autopsy setting, in which misdiagnosis has no impact on the patient. These factors taken together may encourage *ipse dixit* interpretation, and present major challenges to objective and accurate disease classification.

Conclusions

The foundation for tau toxicity theory dates to Alzheimer's description of the NFT in 1906. It began in earnest with the identification of tau, a protein co-factor involved in the polymerization of tubulin, as a major protein component of

the NFT. Subsequent pathogenic theory, including kinase-phosphatase imbalance, soluble assembly intermediates, and prion-like propagation, is rooted in the concept that pathological lesions represent neurotoxicity. The limiting factor for this neurotoxicity bias may be the light microscope. But for the visible microscopic inclusions, p-tau neurotoxicity theory and the extensive literature that now accompanies it, would not exist. The question nevertheless remains whether neurodegenerative inclusions embody a dynamic, primary etiopathogenesis, or instead downstream epiphenomena that distract from a more fundamental upstream biology.

Investigations from multiple perspectives including molecular, genetic, experimental, pathological, and clinical, indicate complex tau biology that bridges normal metabolic processes, neurodevelopment, healthy aging, and neurodegenerative disease. Normal tau, including tau phosphorylation, is necessary for development, cell cycle activity, synaptic function, and neuroplasticity. P-tau in postmortem brain may tend toward buried epitopes, insolubility, and limited biological meaning. Clinicopathological correlations with inert p-tau inclusions are fraught with imprecision. Neurodegeneration in the true sense, that is degeneration of neurons with an associated tissue reaction, is the better clinical correlate, while p-tau immunoreactivity in the absence of neurodegeneration and clinical signs may be extensive. P-tau neuropathology is ultimately a superficial indicator of tau pathophysiology, and may be misleading in terms of cause and effect.

Uncertainties in the repetitive TBI-tauopathy paradigm are considerable. TBI definitions are widely variable. Thresholds for mechanical tissue injury are impossible to quantify. Human data in athletes are limited to case reports and heterogeneous case series with little to no TBI history other than that inferred from participation. Brain tissue interpretation may require expensive, labor-intensive research methodology that has yet to be validated for diagnostic purposes. The concept of a decades' long period of latency between TBI exposure and neurodegenerative disease is often asserted, but has not been convincingly demonstrated, even in boxers. P-tau, especially p-tau identified in postmortem brain, is debatable as a driver of clinical disease, and is in part an artifact of postmortem decomposition. Importantly, epidemiological data indicate better overall health in the NFL athlete cohort, compared to the control population, casting doubt on the idea of a pervasive neurodegenerative disease from athletic participation. In former military service members, epidemiological studies of dementia or AD (and associated tauopathy) risk with mild TBI show modest risk or no risk, which essentially precludes causality. The dose-dependent risk of AD with TBI severity is based on large-scale epidemiology with no pathological confirmation of tauopathy. To these uncertainties and contrary data are added the tau prion concept, soluble low-n assembly intermediates, and geometric isomers, making for limitless theoretical possibilities and the potential for constructs more metaphysical than biological.

Finally, the diagnostic process with respect to tauopathy in postmortem brain is problematic. Clinical correlation is poor, standard methodology for postmortem brain examination is lacking, diagnostic error rates are unknown, and there may be external influences that degrade diagnostic accuracy. Such challenges, along with the consequence-free environment of postmortem diagnosis, may risk autopsy confirmation of individual preferences rather than genuine neurodegenerative disease.

DISCLOSURE STATEMENT

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