Molecular mechanisms of chronic traumatic encephalopathy
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The prevalence of neurodegenerative disorders is rapidly increasing. While Alzheimer’s disease and dementia generally correlate with longer lifespans, neurodegenerative disorders like chronic traumatic encephalopathy often affect individuals at young age. Historically, the underlying disease mechanisms of these chronic disorders—the slowly changing biochemical composition during aging and the repeated, rapidly changing biomechanical environment during head impact—have been viewed as distinct events. Recent studies suggest that Alzheimer’s disease and chronic traumatic encephalopathy share common degenerative pathways on the molecular and cellular levels. Here we examine this current trend and explore the molecular, cellular, tissue, and organ level mechanisms of neurodegeneration through the lens of biomedical engineering. Understanding the underlying disease mechanisms across the spatio-temporal scales of neurodegeneration provides new opportunities to modulate, slow down, and possibly revert molecular dysfunction, axonal death, tissue atrophy, and loss of brain function.

Chronic traumatic encephalopathy is more than just a disorder associated with boxing
Historically, the impact of repeated head injuries on unsteady gait and mental confusion were first reported in professional boxers in 1928, where the symptoms became collectively known as punch-drunk syndrome [10]. A decade later, upon recognizing that these symptoms were in fact chronic and worsened in time, the term chronic traumatic encephalopathy was coined [11]. Single incidences of chronic traumatic encephalopathy were reported in a soccer player, a circus clown, and a head banger; yet, it was not until the autopsy of a deceased professional football player more than half a century later that the condition became associated with widely popular contact sports [12]. We now know that its symptoms typically do not occur until years, if not decades, after the initial injury [13]. This uncertainty has provoked a growing anxiety amongst affected individuals: Throughout the past decade, dozens of former NFL players have been diagnosed with the condition and hundreds have pledged to donate their brains for scientific studies [14]. With this new insight, the definition of chronic traumatic encephalopathy has rapidly evolved [15] and its clinical symptoms have been collectively summarized as research diagnostic for criteria traumatic encephalopathy syndrome [16]. We now broadly associate chronic traumatic encephalopathy with repeated head impacts in a variety of sports including American football, boxing, wrestling, rugby, hockey, and soccer [17], as well as blast impacts on the battlefield [18]. Although the public awareness of chronic traumatic

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A brief history of chronic traumatic encephalopathy
Chronic traumatic encephalopathy is a progressive degenerative disease
Every year, more than 40 million people worldwide experience a mild traumatic brain injury or concussion [1]. Recent studies suggest that even mild concussions, if repetitive, can trigger progressive neurological degeneration, a condition that is now widely recognized as chronic traumatic encephalopathy [2]. The current media hype around chronic traumatic encephalopathy has created a new level of awareness and increasingly more head injuries are now associated with the condition [3]. Yet, to date, the only method to reliably diagnose chronic traumatic encephalopathy is post mortem histopathology, where it manifests itself through an accumulation of hyperphosphorylated tau protein [4], progressive axonal failure, and gradual structural degradation [5]. Strikingly, axonal failure and structural degradation appear to be shared, at least in part, by traumatic brain injury [6] and a number of other neurodegenerative diseases [7] including Parkinsonism [8] and Alzheimer’s disease [9]. However, the molecular mechanisms of neurodegeneration remain poorly understood.
encephalopathy has drastically increased throughout the past decade, and we have made important advances in diagnosing the condition, we still know surprisingly little about its prevalence, incidence, and risk factors [4].

**Chronic traumatic encephalopathy spans across multiple spatial and temporal scales**

From systematic case studies, we now know that chronic traumatic encephalopathy is characterized through a well-defined, ordered, and predictable progression of abnormally phosphorylated tau protein throughout the nervous system [5]. The pathophysiology of chronic traumatic encephalopathy is closely correlated with other abnormally aggregated proteins including transactive response DNA-binding protein 43, amyloid beta, and alpha-synuclein, which are associated with frontotemporal dementia, amyotrophic lateral sclerosis, Alzheimer’s disease, and Parkinson’s disease [8]. This supports the emerging view that chronic traumatic encephalopathy—a condition associated with the deposition of misfolded protein with age—shares several characteristic neuropathological and clinical hallmarks with many other forms of neurodegeneration [19]. Figure 1 highlights the underlying spatial and temporal spectrum of these neurodegenerative diseases: Traumatic brain injury takes place on extremely short temporal scales and manifests itself as biomechanical damage on the larger spatial scales, top left [6]. Alzheimer’s disease, by contrast, takes place on extremely long temporal scales and manifests itself as biochemical damage associated with the hyperphosphorylation of tau on the smaller spatial scales, bottom right [20]. Chronic traumatic encephalopathy initially originates in isolated focal perivascular hyperphosphorylated tau lesions at the depths of cortical sulci [4], from where it gradually propagates across the spatial and temporal scales to eventually affect the entire brain [21]. It manifests itself histopathologically through biomechanical and biochemical damage in the form of neurofibrillary tangles, breakdown of the tau-microtubule complex, diffuse axonal injury, and marked gray and white matter atrophy [22]. These observations raise the important questions if and how repeated head injuries affect tau kinase and phosphatase, and how tau lesions gradually propagate across the brain.

**Spatial and temporal determinants of neurodegeneration**

**On the molecular level, neurofibrillary tangles are a hallmark of neurodegeneration**

Neurofibrillary tangles are aggregates of hyperphosphorylated tau protein. In the healthy axon, tau is an intrinsically disordered protein that is subject to a complex array of posttranslational modifications [23]. By binding to microtubules, tau contributes directly or indirectly to key structural and regulatory function: Within individual microtubules, tau modulates microtubule polymerization, controls microtubule structure, and regulates axonal transport [24]; within the axon, tau promotes the assembly of individual microtubules into well-organized, evenly spaced bundles [25]. Two competing hypotheses have recently emerged to explain the remarkable dense and regular packing of microtubules within the axon: the polymer brush hypothesis and the cross-bridging hypothesis [26]. In the polymer brush hypothesis, dense packing is a result of repulsive forces between tau proteins of neighboring microtubules and compressive forces induced by periodically spaced actin rings underneath the axonal plasma membrane. In the cross-bridging hypothesis, the regular distance between neighboring microtubules is a result of tensile forces induced by the formation of an electrostatic zipper between two tau proteins of neighboring microtubules. Despite its critical importance for axonal stability and intracellular transport, the precise mechanism by which tau regulates microtubules packing remains insufficiently understood [27]. We do know though, that phosphorylation, the site-specific addition of a phosphate group, can modulate tau’s affinity to bind to microtubules [23]. Hyperphosphorylation reduces tau’s ability to bind to microtubules, which destabilizes microtubules, causes tau to clump together in neurofibrillary tangles, and disrupts intracellular function [7]. Neurofibrillary tangles have long been recognized as the...
primary marker of Alzheimer’s disease [20]. While neurofibrillary tangles are rare in severe traumatic brain injury, they are increasingly recognized in mild traumatic brain injury. Their elevated concentration in the cerebrospinal fluid has recently been proposed as an in vivo biomarker of chronic traumatic encephalopathy [22]. However, phosphorylated tau pathology is not unique to chronic traumatic encephalopathy alone: Pathologies that share common neurodegenerative pathways associated with misfolded tau protein have collectively become known as tauopathies [7]. Since its discovery more than four decades ago [28], tau has evolved from a plain microtubule stabilizer to a multifunctional protein with critically important regulatory functions in the nervous system [23]. Yet, the precise mechanisms of tau-mediated neurotoxicity are still far for being completely understood, and it remains controversial whether misfolding of tau into neurofibrillary tangles is a cause or consequence of neurodegeneration [29].

On the cellular level, breakdown of the tau-microtubule complex induces diffuse axonal injury

The axonal cytoskeleton is made up of microtubules, neurofilaments, and microfilaments. Microtubules are hollow cylinders composed of α- and β-tubulin heterodimers that form thirteen laterally joined protofilaments. With a diameter of 24 nm and a stiffness of 2.0 GPa, microtubules are the strongest cytoskeletal filaments in eukaryotic cells [30]. They are critically important in many cellular processes; they provide structural stability and serve as highways for intracellular transport [31]. Unlike all other microtubules, axonal microtubules are coated with tau proteins that protect against depolymerization, stabilize their structure, and cross-link them to neighboring microtubules. The tau-microtubule complex plays a major role in regulating cytoskeletal mechanics, structure, and function [32]. Under physiological conditions, at moderate stretch and stretch rates, tau-microtubule dynamics make axons behave like rubber bands; they deform reversibly, and are almost entirely elastic [33]. Under pathological conditions, at high stretch and stretch rates, tau-microtubule interactions make axons brittle and their cytoskeleton becomes damaged [34]. Cytoskeletal damage disrupts axonal transport, the transport products build up at the site of breakage, the axon begins to swell, and will eventually break [31]. The bulb that forms close to the cell body upon retraction of the transected axon is a classical hallmark of diffuse axonal injury [8]. For a long time, the common belief was that mechanical forces would trigger the instantaneous breakdown of the tau-microtubule complex and induce immediate axonal rupture, a condition known as primary axotomy. More recent findings suggest that axonal failure is a gradual interplay of biomechanical and biochemical phenomena including the initial biomechanical injury within milliseconds of the insult followed by secondary biochemical events within hours or days, a condition known as secondary axotomy [35]. Yet, the precise sequence of events by which the axonal cytoskeleton degrades is currently unknown.

On the organ level, neurodegeneration results in pronounced gray and white matter atrophy

Unlike severe traumatic brain injury, chronic traumatic encephalopathy progresses gradually in time, suggesting that biochemical factors, and not just biomechanical forces, modulate its pathology [6]. Unlike Alzheimer’s disease, chronic traumatic encephalopathy is associated with tau abnormalities that begin focally, suggesting that biomechanical forces, and not just biochemistry, trigger its local initiation [4]. While it is widely accepted that axons gradually fail when exposed to extreme strain or strain rates [31], there is an ongoing controversy whether axonal stretching or axonal shearing is the critical failure mode [27]. When our brain is rapidly accelerated, different tissue types respond differently to mechanical loading. Recent studies have shown that brain tissue displays a gradual stiffness gradient within its white matter tissue [36] and a discrete stiffness jump across the gray-and-white-matter interface [37]. Diffuse axonal injury lesions occur primarily at the gray-and-white-matter interface [38] where mechanical stress fields experience a discrete jump, supporting the notion that biomechanical factors modulate the initial foci of chronic traumatic encephalopathy. Computational brain models created from medical images can help us understand how mechanical forces propagate across the brain and identify critical regions of elevated axonal stretch, strain, or stress [39].

Figure 2 shows that hyperphosphorylated tau pathology is initially restricted to discrete regions located around small vessels at the depths of the cerebral sulci [5]. This suggests that damage to the cerebral vasculature at the time of injury plays a critical role in chronic traumatic encephalopathy. From these multiple discrete epicenters, neurofibrillary pathology gradually propagates to the superficial layers of the adjacent cortex, and then diffuses widely across the frontal and temporal lobes, the diencephalon, and the brainstem [16]. At the tissue level, it induces a gradual loss of neurons. At the whole organ level, cell death translates into enlarged ventricles, pronounced gray and white matter atrophy, and an overall reduction in brain mass [14]. A recent consensus panel defined these features to be comparable to—but regionally distinct from—the spatio-temporal propagation of other tauopathies including Alzheimer’s disease [4]. Understanding the role of biomechanics in chronic traumatic encephalopathy could hold the key to identify the mechanistic origin of neurodegeneration [40] and explain its unique histopathological traces in comparison to other non-mechanical tauopathies [8].
Challenges & opportunities

How can we diagnose chronic traumatic encephalopathy?

A first step towards better understanding neurodegeneration is reaching a consensus on how to diagnose it during an individual's lifetime [41]. Currently, the only method to reliably diagnose chronic traumatic encephalopathy is post-mortem neuropathology with a complete autopsy and immunohistochemical analysis [4]. While the clinical diagnosis of severe traumatic brain injury is relatively straightforward, the lack of reliable in vivo biomarkers for chronic traumatic encephalopathy implies that we can neither make definitive statements about the degree of disease progression in a single individual, nor estimate its prevalence in the general population [42]. Improving diagnostics is a critical step towards identifying early markers of chronic traumatic encephalopathy, increasing treatment outcomes, and preventing disease progression [43]. On the molecular level, tau composition in the cerebrospinal fluid holds promise as a cost-efficient in vivo biomarker of neurodegeneration [44]; not only as a quantitative marker in advanced disease progression, but also as a prognostic marker in the earliest stages when clinical expression is weak [22]. On the tissue level, neurofibrillary tangles around small vessels at the depths of cortical sulci are the single most defining neuropathological biomarker of chronic traumatic encephalopathy [4]. On the whole brain level, neuroimaging is offering a wide variety of in vivo biomarkers including the loss of regional activity, regional anisotropy, and regional tissue volume derived from functional magnetic resonance imaging, diffusion tensor imaging, and volume magnetic resonance imaging, respectively [22]. Another promising technology that is gaining popularity in neuroimaging, magnetic resonance elastography, suggests the loss in tissue stiffness as an in vivo biomarker in multiple sclerosis [45], Alzheimer's disease [46], and frontotemporal dementia [47]. Probably the most promising imaging technique today is positron emission tomography [48], which provides two independent ligand-based in vivo biomarkers, one selective for amyloid and one selective for tau [49]. Despite significant progress towards identifying early predictive biomarkers of neurodegeneration, the diagnosis of neurodegenerative disorders remains challenging because of the partial overlap of their clinical presentations and symptoms [50]. This is particularly true for chronic traumatic encephalopathy, where studies are naturally subject to selection bias [8].

How can we predict the effects of chronic traumatic encephalopathy?

Computational modeling is emerging as a powerful tool to simulate the mechanistic origin of neurodegeneration and characterize failure thresholds and safety limits of molecular, cellular, and organ level damage. On the molecular level, coarse-grained molecular dynamics simulations [51] or rigidity analysis [52] allow us to virtually probe potential...
failure mechanisms of the binding interfaces [53], in the case of chronic traumatic encephalopathy the tau—tau and tau-microtubule binding sites. On cellular level, representative volume elements of cellular substructures [34] and discrete axonal models of tau—tau and tau-microtubule mechano-kinetics [54] can predict critical stretch and stretch rates at the onset of axonal breakdown. On the whole brain level, personalized finite element models can correlate impact kinematics to axonal strains and strain rates [55]. For example, simulations based on head kinematics of football and hockey players have revealed a close correlation between mean and maximum strains and strain rates and alterations in fractional anisotropy [56]. With recent advances in neuroimaging, we can now not only personalize individual loading scenarios [57], but also the anatomic model of the head [58], and its regional stiffness variations [59]. Simulations of mechanically-induced brain damage naturally reveal stress discontinuities at the tissue-vasculature-interface and at the interface between gray and white matter [60], regions that have traditionally been correlated with first lesions of diffuse axonal injury [38]. Personalized simulations can now help identify brain regions at risk for individual impact scenarios [61] and correlate individual strain profiles to the broad spectrum of symptoms associated with chronic traumatic encephalopathy [16]. The ability to accurately simulate and reliably predict the effects of mechanical forces on neurodegeneration opens numerous opportunities in biomedical engineering [62], from improving helmets in contact sports [63] and optimizing protective devices in bicycling [64] to reducing brain damage during neurosurgery [39]. While most existing approaches model the brain only at a single spatial or temporal scale, rapid advancements in multiscale modeling provide a computational platform to integrate knowledge from different disciplines [65] and virtually probe how neurodegeneration propagates bio-chemo-mechanically [66] across the spatial and temporal scales [67]. A multimodal model of neurodegeneration can predict the onset of neurodegeneration from integrating modes of biomarker changes throughout life and provide mechanistic links between normal aging, early aging, and multiple mild traumatic brain injuries as illustrated in Figure 3.

**How can we treat chronic traumatic encephalopathy?**
The most important question—in addition to preventing neurodegeneration—is whether there is realistic hope of treating it. The answer may lie in understanding neurodegeneration at the molecular level [29], where tau proteins aggregate through nucleation, templating, growth, multiplication, and spread. Each of these mechanisms represents a potential target for therapeutic intervention [68]. Therapies that target tau phosphorylation, tau aggregation, and microtubule...
stabilization are already in clinical trials [7], while tau reduction strategies are still in the preclinical stage [23]. Recent evidence in mice suggests that cis antibody treatment could prevent tauopathy development and spread, and restore many structural and functional sequelae [69]. In addition, the release of neurofibrillary tangles into the extracellular space, their diffusion across the extracellular space, and their uptake by other cells [70] could be valuable targets to reduce spatial propagation [68]. Understanding the pathways of neurodegeneration will likely require a multifaceted approach [8] in which neurobiologists, neuroradiologists, and neuroscientists join forces to gain a more holistic picture of neurodegenerative diseases [24]. By applying engineering principles and design concepts to biology and medicine, the field of biomedical engineering could play a pivotal role when addressing this complex challenge [71]. Our current understanding of the spatial and temporal spectrum of neurodegeneration converges towards a view in which traumatic encephalopathy covers a landscape of phenomena between traumatic brain injury and Alzheimer’s disease [19], and shares common early disease mechanisms [69]. We are only beginning to understand the complex interplay of neurodegeneration. Biomedical engineering has the potential to become a key player in the quest to diagnose, predict, and treat neurodegenerative disease.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


This study summarizes results of a national consensus panel in the quest to unify neuropathological criteria for chronic traumatic encephalopathy using 25 cases of various tauopathies; it identified an abnormal accumulation of hyperphosphorylated tau as a pathognomonic lesion of chronic traumatic encephalopathy.


This review summarizes the current knowledge of tau-related neurodegeneration and discusses the potential for mechanism-based treatment based on preventing tau aggregation and propagation.


This highlights controversies in the study of severe and mild traumatic brain injury, and advocates the hypothesis that chronic traumatic encephalopathy is an environmental modification of the broad spectrum of pathologies associated with normal aging.


This publication reviews the clinical manifestations of chronic traumatic encephalopathy from 202 published cases and correlates its clinical symptoms to diagnostic criteria for traumatic encephalopathy syndrome.


Molecular mechanisms of chronic traumatic encephalopathy van den Bedem and Kuhl


This study characterizes the tau-microtubule complex using synchrotron small angle X-ray scattering and computational modeling of different tau isoforms to explain dense microtubule packing in crowded axon environments.


This study addresses the current controversy between the polymer brush hypothesis and the cross-bridging hypothesis and explains the regular, dense microtubule packing within the axon via long-range cross-bridging effect of tau using a combined analytical and numerical approach.


This publication highlights the central role of tau in neurodegeneration, discusses the interplay between tau phosphorylation and tau aggre- gation, and systematically compares current tau-directed therapies in neurodegeneration.


This study proposes a new computational model of tau-microtubule interaction to systematically study the effects of strain and strain rates on axonal failure; it explains mechanistically why axons fail at high strain rates but remain intact under low strain rates, even when stretched to twice their initial length.


This publication proposes brain stiffness as a non-invasive in vivo biomarker for neurodegeneration and uses magnetic resonance elastography to demonstrate that the stiffness of the whole brain, the frontal lobe, and the temporal lobe is significantly lower in individuals with frontotemporal dementia than in cognitively normal controls.


This study compares different strategies to diagnose tauopathies in vivo via neuroimaging, which would enable a more precise spatial and temporal characterization of the molecular mechanisms of neurodegeneration in different types of tauopathies.


   This publication highlights recent advances and discusses the potential of biomedical engineering in understanding mild traumatic brain injury across the molecular, cellular, and whole organ scales.


   This publication interprets neurodegeneration within the wide class of diseases characterized through misfolded proteins that aggregate into seeds, form pathogenic assemblies, and spread in a prion-like manner; within this prion paradigm, the authors identify new therapeutic strategies for a large class of currently intractable neurodegenerative diseases.


   This study is the first to identify cistauosis, the formation of cisphosphorylated tau protein, as an early marker of tauopathy, and introduce a cis antibody, which blocks the process of cistauosis and prevents tauopathy development and spread.
