Editor's Summary

Blast Brain: An Invisible Injury Revealed

Traumatic brain injury (TBI) is the "signature" injury of the conflicts in Afghanistan and Iraq and is associated with psychiatric symptoms and long-term cognitive disability. Recent estimates indicate that TBI may affect 20% of the 2.3 million U.S. servicemen and women deployed since 2001. Chronic traumatic encephalopathy (CTE), a tau protein-linked neurodegenerative disorder reported in athletes with multiple concussions, shares clinical features with TBI in military personnel exposed to explosive blast. However, the connection between TBI and CTE has not been explored in depth. In a new study, Goldstein et al. investigate this connection in the first case series of postmortem brains from U.S. military veterans with blast exposure and/or concussive injury. They report evidence for CTE neuropathology in the military veteran brains that is similar to that observed in the brains of young amateur American football players and a professional wrestler. The investigators developed a mouse model of blast neurotrauma that mimics typical blast conditions associated with military blast injury and discovered that blast-exposed mice also demonstrate CTE neuropathology, including tau protein hyperphosphorylation, myelinated axonopathy, microvascular damage, chronic neuroinflammation, and neurodegeneration. Surprisingly, blast-exposed mice developed CTE neuropathology within 2 weeks after exposure to a single blast. In addition, the neuropathology was accompanied by functional deficits, including slowed axonal conduction, reduced activity-dependent long-term synaptic plasticity, and impaired spatial learning and memory that persisted for 1 month after exposure to a single blast. The investigators then showed that blast winds with velocities of more than 330 miles/hour—greater than the most intense wind gust ever recorded on earth—induced oscillating head acceleration of sufficient intensity to injure the brain. The researchers then demonstrated that blast-induced learning and memory deficits in the mice were reduced by immobilizing the head during blast exposure. These findings provide a direct connection between blast TBI and CTE and indicate a primary role for blast wind—induced head acceleration in blast-related neurotrauma and its aftermath. This study also validates a new blast neurotrauma mouse model that will be useful for developing new diagnostics, therapeutics, and rehabilitative strategies for treating blast-related TBI and CTE.

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**TRAUMATIC BRAIN INJURY**

**Chronic Traumatic Encephalopathy in Blast-Exposed Military Veterans and a Blast Neurotrauma Mouse Model**

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Blast exposure is associated with traumatic brain injury (TBI), neuropsychiatric symptoms, and long-term cognitive disability. We examined a case series of postmortem brains from U.S. military veterans exposed to blast and/or concussive injury. We found evidence of chronic traumatic encephalopathy (CTE), a tau protein–linked neurodegenerative disease, that was similar to the CTE neuropathology observed in young amateur American football players and a professional wrestler with histories of concussive injuries. We developed a blast neurotrauma mouse model that recapitulated CTE-linked neuropathology in wild-type C57BL/6 mice 2 weeks after exposure to a single blast. Blast-exposed mice demonstrated phosphorylated tauopathy, myelinated axonopathy, microvasculopathy, chronic neuroinflammation, and neurodegeneration in the absence of macroscopic tissue damage or hemorrhage.

Blast exposure induced persistent hippocampal-dependent learning and memory deficits that persisted for at least 1 month and correlated with impaired axonal conduction and defective activity-dependent long-term potentiation of synaptic transmission. Intracerebral pressure recordings demonstrated that shock waves traversed the mouse brain with minimal change and without thoracic contributions. Kinematic analysis revealed blast-induced head oscillation at accelerations sufficient to cause brain injury. Head immobilization during blast exposure prevented blast-induced learning and memory deficits. The contribution of blast wind to injurious head acceleration may be a primary injury mechanism leading to blast-related TBI and CTE. These results identify common pathogenic determinants leading to CTE in blast-exposed military veterans and head-injured athletes and additionally provide mechanistic evidence linking blast exposure to persistent impairments in neurophysiological function, learning, and memory.

**INTRODUCTION**

Blast exposure from conventional and improvised explosive devices (IEDs) affects combatants and civilians in conflict regions around the world (1–4). Individuals exposed to explosive blast are at increased risk for traumatic brain injury (TBI) (2, 5–15) that is often reported as mild (16 cf., 17). Blast-related TBI represents a neuropsychiatric spectrum disorder that clinically overlaps with chronic traumatic encephalopathy (CTE), a progressive tau protein–linked neurodegenerative disease associated with repetitive concussive injury in athletes (18–21). Neuropathological hallmarks of CTE include widespread cortical foci of perivascular tau pathology, disseminated microgliosis and astrogliosis, myelinated axonopathy, and progressive neurodegeneration. Clinical symptoms of CTE include progressive affective lability, irritability, distractability, executive dysfunction, memory disturbances, suicidal ideation, and in advanced cases, cognitive deficits and dementia.

Blast exposure is a known precipitant of brain injury in animals (22–37) and humans (38–42) and has been linked to CTE neuropathology in a single case report by Omalu et al. (43). Despite growing awareness of blast-related TBI, the mechanisms of injury and biological basis underpinning blast neurotrauma and sequelae remain largely unknown and a matter of significant controversy. Given the overlap of clinical signs and symptoms in military personnel with blast-related TBI and athletes with concussion-related CTE, we hypothesized that common biomechanical and pathophysiological determinants may trigger development of CTE neuropathology and sequelae in both trauma settings. Here, we combine clinicopathological correlation analysis and controlled animal modeling studies to test this hypothesis.
RESULTS

CTE neuropathology in blast-exposed military veterans and athletes with repetitive concussive injury

We performed comprehensive neuropathological analyses (table S1) of postmortem brains obtained from a case series of military veterans with known blast exposure and/or concussive injury (n = 4 males; ages 22 to 45 years; mean, 32.3 years). We compared these neuropathological analyses to those of brains from young amateur American football players and a professional wrestler with histories of repetitive concussive injury (n = 4 males; ages 17 to 27 years; mean, 20.8 years) and brains from normal controls of comparable ages without a history of blast exposure, concussive injury, or neurological disease (n = 4 males; ages 18 to 24 years; mean, 20.5 years). Case 1, a 45-year-old male U.S. military veteran with a single close-range IED blast exposure, experienced a state of disorientation without loss of consciousness that persisted for ~30 min after blast exposure. He subsequently developed headaches, irritability, difficulty sleeping and concentrating, and depression that continued until his death 2 years later from a ruptured basilar aneurysm. His medical history is notable for a remote history of concussion associated with a motor vehicle accident at age 8 years. Case 2, a 34-year-old male U.S. military veteran without a history of previous concussive injury, sustained two separate IED blast exposures 1 and 6 years before death. Both episodes resulted in loss of consciousness of indeterminate duration. He subsequently developed depression, short-term memory loss, word-finding difficulties, decreased concentration and attention, sleep disturbances, and executive function impairments. His neuropsychiatric symptoms persisted until death from aspiration pneumonia after ingestion of prescription analgesics. Case 3, a 22-year-old male U.S. military veteran with a single close-range IED blast exposure 2 years before death. He did not lose consciousness, but reported headache, dizziness, and fatigue that persisted for 24 hours after the blast. He subsequently developed daily headaches, memory loss, depression, and decreased attention and concentration. In the year before his death, he became increasingly violent and verbally abusive with frequent outbursts of anger and aggression. He was diagnosed with posttraumatic stress disorder (PTSD) 3 months before death from an intracerebral hemorrhage. His past history included 2 years of high school football and multiple concussions from fist fights. Case 4, a 28-year-old male U.S. military veteran with two combat deployments, was diagnosed with PTSD after his first deployment 3 years before death. His history was notable for multiple concussions as a civilian and in combat, but he was never exposed to blast. His first concussion occurred at age 12 after a bicycle accident with temporary loss of consciousness and pre/posttraumatic amnesia. At age 17, he experienced a concussion without loss of consciousness from helmet-to-helmet impact injury during football practice. At age 25, he sustained a third concussion during military deployment with temporary alteration in mental status without loss of consciousness. Four months later at age 26, he sustained a fourth concussion with temporary loss of consciousness and posttraumatic amnesia resulting from a motor vehicle–bicycle collision. Afterward, he experienced persistent anxiety, difficulty concentrating, word-finding difficulties, learning and memory impairment, reduced psychomotor speed, and exacerbation of PTSD symptoms. He died from a self-inflicted gunshot wound 2 years after his last concussion. The athlete group included Case 5, a 17-year-old male high school American football player who died from second impact syndrome 2 weeks after sustaining a concussion; Case 6, an 18-year-old high school American football and rugby player with a history of three to four previous concussions, one requiring hospitalization, who died 10 days after his last concussion; Case 7, a 21-year-old male college American football player, who played as a lineman and linebacker but had never been diagnosed with a concussion during his 13 seasons of play beginning at age 9, and who died from suicide; and Case 8, a 27-year-old male professional wrestler who experienced more than 9 concussions during his 10-year professional wrestling career who died from an overdose of OxyContin. The normal control group included Case 9, an 18-year-old male who died suddenly from a ruptured basilar aneurysm; Case 10, a 19-year-old male who died from a cardiac arrhythmia; Case 11, a 21-year-old male who died from suicide; and Case 12, a 24-year-old male who died from suicide.

Neuropathological analysis of postmortem brains from military veterans with blast exposure and/or concussive injury revealed CTE-linked neuropathology characterized by perivascular foci of tau-immunoreactive neurofibrillary tangles (NFTs) and glial tangles in the inferior frontal, dorsolateral frontal, parietal, and temporal cortices with predilection for sulcal depths (Fig. 1, A to X). NFTs and dystrophic axons immunoreactive for monoclonal antibody CP-13 (Fig. 1, A to I, L, Q, R, and U, and fig. S1) directed against phosphorylated tau protein at Ser202 (pS202) and Thr205 (pT205), monoclonal antibody AT8 (Fig. 1S) directed against phosphorylated tau protein at Ser202 (pS202) and Thr205 (pT205), and monoclonal antibody Tau-46 (Fig. 1T) directed against phosphorylation-independent tau protein were detected in superficial layers of frontal and parietal cortex and anterior hippocampus. Evidence of axon degeneration, axon retraction bulbs, and axonal dystrophy were observed in the subcortical white matter subjacent to cortical tau pathology (Fig. 1, M and U to X, and fig. S1). Distorted axons and axon retraction bulbs were prominent in perivascular areas. Large clusters of LN3-immunoreactive activated microglia clusters (Fig. 1, K and P) were observed in subcortical white matter underlying focal tau pathology, but not in unaffected brain regions distant from tau lesions. Neuropathological comparison to brains from young-adult amateur American football players (Fig. 1, C, D, G, and H) with histories of repetitive concussive and subconcussive injury exhibited similar CTE neuropathology marked by perivascular NFTs and glial tangles with sulcal depth prominence in the dorsolateral and inferior frontal cortices. The young-adult athlete brains also revealed evidence of robust astrocytosis and multifocal axonopathy in subcortical white matter. Clusters of activated perivascular microglia were noted in the subcortical U-fibers. Neuropathological findings in the military veterans with blast exposure and/or concussive injury and young-adult athletes with repetitive concussive injury were consistent with our previous CTE case studies (20, 21) and could be readily differentiated from neuropathology associated with Alzheimer’s disease, frontotemporal dementia, and other age-related neurodegenerative disorders. Control sections omitting primary antibody demonstrated no immunoreactivity. By contrast, none of the brains from the four young-adult normal control subjects demonstrated phosphorylated tau pathology, axonal injury, subcortical astrocytosis, or microglial nodules indicative of CTE or other neurodegenerative disease (fig. S2).

Blast exposure induces traumatic head acceleration in a blast neurotrauma mouse model

We developed a murine blast neurotrauma model to investigate mechanistic linkage between blast exposure, CTE neuropathology, and neurobehavioral sequelae. Our compressed gas blast tube was designed to accommodate
Fig. 1. CTE neuropathology in postmortem brains from military veterans with blast exposure and/or concussive injury and young athletes with repetitive concussive injury. (A and E) Case 1, phosphorylated tau (CP-13) neuropathology with perivascular neurofibrillary degeneration in the frontal cortex of a 45-year-old male military veteran with a history of single close-range blast exposure 2 years before death and a remote history of concussion. Whole-mount section. Scale bar (E), 100 μm. (B and F) Case 2, phosphorylated tau (CP-13) neuropathology with perivascular neurofibrillary degeneration in the frontal cortex of a 34-year-old male military veteran with history of two blast exposures 1 and 6 years before death and without a history of concussion. Whole-mount section. Scale bar (F), 100 μm. (C and G) Case 6, phosphorylated tau (CP-13) neuropathology with perivascular neurofibrillary degeneration in the frontal cortex of an 18-year-old male amateur American football player with a history of repetitive concussive injury. Whole-mount section. Scale bar (G), 100 μm. (D and H) Case 7, phosphorylated tau (CP-13) neuropathology with perivascular neurofibrillary degeneration in the frontal cortex of a 21-year-old male amateur American football player with a history of repetitive subconcussive injury. Whole-mount section. Scale bar (H), 100 μm. (I) Case 1, phosphorylated tau (CP-13) immunostaining in the parietal cortex revealed a string of perivascular foci demonstrating intense immunoreactivity (areas enclosed by hash lines). Whole-mount section. (J) Case 1, phosphorylated neurofilament (SMI-34) immunostaining in adjacent parietal cortex section demonstrating colocalization of multifocal axonal swellings and axonal retraction bulbs surrounding small blood vessels (black circles) relative to perivascular tau foci (areas enclosed by hash lines). Whole-mount section. (K) Case 1, human leukocyte antigen–DR (HLA-DR) (LN3) immunostaining in adjacent parietal cortex section demonstrating colocalization of microglial clusters (black circles) relative to perivascular tau foci (areas enclosed by hash lines). Whole-mount section. (L) Case 1, high-magnification micrograph of phosphorylated tau (CP-13) immunostaining in the parietal cortex demonstrating string of perivascular phosphorylated tau foci. Whole-mount section. (M) Case 1, phosphorylated tau (PHF-1, brown) and phosphorylated neurofilament (SMI-34, red) double immunostaining in parietal cortex demonstrating axonal swellings and a retraction bulb (arrow) in continuity with phosphorylated tau neuritic abnormalities. Whole-mount section. Scale bar, 100 μm. (N) Case 1, phosphorylated neurofilament (SMI-34) immunostaining showing diffuse axonal degeneration and multifocal irregular axonal swellings in subcortical white matter subjacent to cortical tau pathology. Whole-mount section. (O) Case 1, phosphorylated neurofilament (SMI-34) immunostaining demonstrating perivascular axonal pathology and axonal retraction bulbs near a small cortical blood vessel. Whole-mount section. (P) Case 1, activated microglia (LN3) immunostaining showing a large microglial nodule in the subcortical white matter subjacent to cortical tau pathology. LN3 immunostaining was not observed in brain areas devoid of tau pathology. Whole-mount section. Scale bar, 100 μm. (Q) Case 2, phosphorylated tau (CP-13) immunostaining showing diffuse neuronal tau pathology (pre-tangles) in the hippocampal CA1 field. Whole-mount section. (R) Case 2, phosphorylated tau (CP-13) pathology in temporal cortex. Whole-mount section. (S) Case 1, phosphorylated tau (AT8) immunostaining showing diffuse neuronal tau pathology (pre-tangles) in the hippocampal CA1 field. Whole-mount section. (T) Case 1, phosphorylation-independent total tau (Tau-46) immunostaining in the frontal cortex. Whole-mount section. (U) Case 3, phosphorylated tau (CP-13) immunostained axonal varicosities in the external capsule of a 22-year-old male military veteran with a history of a single close-range IED blast exposure and remote history of concussions. Whole-mount section. (V to X) Case 3, SMI-34 immunostained axonal varicosities and retraction bulbs in the thalamic fasciculus and external capsule. Whole-mount sections.
mice (fig. S3) and allowed free movement of the head and cervical spine to model typical conditions associated with military blast exposure (tables S2 and S3 and figs. S3 to S7). Wild-type C57BL/6 male mice (2.5 months) were anesthetized and exposed to a single blast with a static (incident) pressure profile comparable in amplitude, waveform shape, and impulse to detonation of 5.8 kg of trinitrotoluene (TNT) at a standoff distance of 5.5 m and in close agreement with ConWep (Conventional Weapons Effects Program) (Fig. 2A and table S3) (44). The model blast is comparable to a common IED fabricated from a 120-mm artillery round and is within the reported range of typical explosives, blast conditions, and standoff distances associated with military blast injury (45).

To investigate intracranial pressure (ICP) dynamics during blast exposure, we inserted a needle hydrophone into the hippocampus of living mice and monitored pressure dynamics during blast exposure. We detected blast wavefront arrival times in the brain that were indistinguishable from corresponding free-field pressure (FFP) measurements in air (Fig. 2B) and in close agreement with ConWep measurement of an equivalent TNT blast (Fig. 2A and table S3). To investigate possible thoracic contributions to blast-induced ICP transients, we evaluated pressure tracings in the hippocampus of intact living mice (Fig. 2B) and compared results to the same measurements obtained in isolated mouse heads severed at the cervical spine (Fig. 2C). Blast-induced pressure amplitudes in the two experimental preparations were comparable to each other and to the corresponding FFP measurements in air, after accounting for the addition of the dynamic pressure on the head. Small differences in the pressure waveforms were within the expected range given frequency-dependent response characteristics of the transducers and differences in the two experimental preparations. We did not detect delayed blast-induced ICP transients in either preparation over recording times up to 100 ms. These observations indicate that blast wavefront transmission in the mouse brain is mediated without significant contributions from thoracovascular or hydrodynamic mechanisms.

In our experimental system, the blast shock wave traveling at ~450 m/s encountered the left lateral surface of the mouse head first, then traversed the ~11-mm skull width (46) in ~24 µs. The pressure differential associated with this traversal has an insignificant effect on skull displacement due to the short time interval. For the remainder of the waveform duration, the static pressures at the lateral surfaces of the skull are virtually identical and the corresponding transient effects are negligible. The air-skull impedance mismatch creates a back-reflected air shock as well as a rapidly moving (≥1500 m/s) transmitted shock wave, the latter taking a maximum of ~7 µs to traverse the cranium and cranial contents. Although the reflected and transmitted shock waves are large (~2.5 times greater than the 77-kPa incident overpressure), the ~7-µs traversal time of the skull-brain transmitted wave is short enough to allow rapid equilibration across the skull. Thus, the head acts acoustically as a “lumped element” (47, 48). The only significant pressure term remaining is the ~19-kPa peak dynamic pressure generated by blast wind. We concluded that an ICP transducer in the brain parenchyma should measure pressure differentials that do not differ by more than 19 kPa from FFP values, at least beyond the initial 30 µs after blast arrival. This analysis was confirmed by experimental measurements (Fig. 2B). Only the initial rise of the blast wave has a short enough time scale to be affected by propagation effects in the head, a prediction confirmed by the longer rise time of the ICP compared to the static FFP waveforms (Fig. 2, B and C). The remaining waveform components evenly distribute through the brain with amplitude and shape that approximate the static FFP (Fig. 2A).

The blast wave had a measured Mach number of 1.26 ± 0.04 (fig. S6), from which the calculated blast wind velocity was 150 m/s (336 miles/hour). Kinematic analysis of high-speed videographic records of head movement during blast exposure confirmed rapid oscillating acceleration-deceleration of the head in the horizontal and sagittal planes of motion (Fig. 2, D to G, and video S1). We calculated peak average radial head acceleration of 954 ± 215 krad/s² (Fig. 2G), corresponding to 100.2 N exerted on the head during blast exposure. Peak angular and centripetal acceleration were most significant during the positive phase of the blast shock wave. No appreciable head acceleration was detected after ~8 ms.

Single-blast exposure induces CTE-linked neuropathology, ultrastructural pathology, and phosphorylated tau proteinopathy in a blast neurotrauma mouse model

We hypothesized that blast forces exerted on the skull would result in head acceleration-deceleration oscillation of sufficient intensity to induce persistent brain injury (“bobblehead effect”). To evaluate this hypothesis, we studied brains from mice euthanized 2 weeks after exposure to a single blast or sham blast. Gross examination of postmortem brains from both groups of mice was unremarkable and did not reveal macroscopic evidence of contusion, necrosis, hematomata, hemorrhage, or focal tissue damage (Fig. 3, A to F, and fig. S8). In contrast, brains from blast-exposed mice showed marked neuropathology by immunohistological analysis (Fig. 3, H, J, L, Q, N, S, and T). Blast-exposed brains exhibited robust reactive astrogliosis throughout the cerebral cortex, hippocampus, brainstem, internal capsule, cerebellum, and corticospinal tract (Fig. 3, H and T) that was not observed in brains from sham-blast control mice (Fig. 3, G and O). Brains from blast-exposed mice also exhibited enhanced somatodendritic phosphorylated tau CP-13 immunoreactivity in neurons in the superficial layers of the cerebral cortex (Fig. 3I) that was not observed in the brains of sham-blast control mice (Fig. 3I). The cerebral cortex and CA1 field of the hippocampus in the brains of blast-exposed mice were also notable for clusters of chromatolytic and pyknotic neurons with nuclear and cytoplasmic smudging and beaded, irregularly swollen dystrophic axons (Fig. 3, L and Q) that were not observed in the brains of sham-blast control mice (Fig. 3, G and O). Brains from blast-exposed mice also exhibited enhanced somatodendritic phosphorylated tau CP-13 immunoreactivity in neurons in the superficial layers of the cerebral cortex (Fig. 3I) that was not observed in the brains of sham-blast control mice (Fig. 3I). The cerebral cortex and CA1 field of the hippocampus in the brains of blast-exposed mice were also noticeable for clusters of chromatolytic and pyknotic neurons with nuclear and cytoplasmic smudging and beaded, irregularly swollen dystrophic axons (Fig. 3, L and Q) that were not observed in the brains of sham-blast control mice (Fig. 3, G and O). Brains from blast-exposed mice also exhibited enhanced somatodendritic phosphorylated tau CP-13 immunoreactivity in neurons in the superficial layers of the cerebral cortex (Fig. 3I) that was not observed in the brains of sham-blast control mice (Fig. 3I). The cerebral cortex and CA1 field of the hippocampus in the brains of blast-exposed mice were also noticeable for clusters of chromatolytic and pyknotic neurons with nuclear and cytoplasmic smudging and beaded, irregularly swollen dystrophic axons (Fig. 3, L and Q) that were not observed in the brains of sham-blast control mice (Fig. 3, G and O). Brains from blast-exposed mice also exhibited enhanced somatodendritic phosphorylated tau CP-13 immunoreactivity in neurons in the superficial layers of the cerebral cortex (Fig. 3I) that was not observed in the brains of sham-blast control mice (Fig. 3I).
matolytic and pyknotic neurons throughout the stratum pyramidale and a marked paucity of dendritic profiles in the stratum radiatum (Fig. 4H and fig. S9, B and C) that was not evident in the brains of sham-blast control mice (Fig. 4A and fig. S9A). Blast-related ultrastructural microvascular pathology was notable for the presence of hydropic perivascular astrocytic end-feet (Fig. 4, I and J, and figs. S11, S13 to S16, S19C, and...
Fig. 3. Single-blast exposure induces CTE-like neuropathology in wild-type C57BL/6 mice. (A to F) Absence of macroscopic tissue damage (contusion, necrosis, hematoma, or hemorrhage) 1 day (A to C) or 2 weeks (D to F) after exposure to a single blast. Experimental blast conditions were compatible with 100% survival and full recovery of gross locomotor function. (G) Normal astrocytic glial fibrillary acidic protein (GFAP) immunoreactivity in a mouse brain 2 weeks after exposure to sham blast. Whole-mount sections. (H) Increased astrocytic GFAP immunoreactivity in the ipsilateral cortex (area enclosed by white hash line), bilateral thalamus (white asterisks), and bilateral hypothalamus (black asterisks) 2 weeks after single-blast exposure. Parenchymal atrophy with ventricular dilation was also observed (white arrowhead). Whole-mount sections. (I) Background phosphorylated tau (CP-13) immunostaining in superficial layers of the cerebral cortex 2 weeks after exposure to sham blast. (J) Phosphorylated tau (CP-13) immunostaining in superficial layers of the cerebral cortex 2 weeks after exposure to a single blast. Increased accumulation of phosphorylated tau in the brains of blast-exposed mice was confirmed by quantitative immunoblot analysis (Fig. 5). (K and P) Background phosphorylated neurofilament (SMI-31) immunostaining in the hippocampus 2 weeks after exposure to sham blast demonstrating normal-appearing CA1 pyramidal neurons with no detectable axonal pathology. (L and Q) Increased phosphorylated neurofilament (SMI-31) immunostaining in the hippocampus 2 weeks after exposure to single blast demonstrating pyknotic CA1 pyramidal neurons with nuclear smudging and injured axons with beaded, irregular swellings [arrowhead, (Q); enlargement shown in inset]. (M and R) Faint total tau (Tau-46) immunoreactivity in the soma and processes of pyramidal neurons in the hippocampal CA1 field 2 weeks after exposure to sham blast. (N and S) Increased total tau (Tau-46) immunoreactivity in the soma and processes of pyramidal neurons [arrowheads, (S)] in the hippocampal CA1 field 2 weeks after exposure to single blast. Biochemical abnormalities in total tau expression in the brains of blast-exposed mice were confirmed by quantitative immunoblot analysis (Fig. 5). (O) Faint activated microglial [Ricinus communis agglutinin (RCA)] immunoreactivity in the cerebellum 2 weeks after exposure to sham blast. (T) Increased activated microglial RCA immunoreactivity in the cerebellum indicative of brisk microgliosis [arrowheads, (T)] 2 weeks after exposure to single blast.
Pathologically swollen, edematous, and often highly vacuolated astrocytic end-feet were observed in association with dysmorphic capillaries marked by pathologically thickened, tortuous basal lamina and abnormal endothelial cells with irregularly shaped nuclei (Fig. 4L and S20). Perivascular processes in the hippocampi of blast-exposed mice often contained inclusion bodies, lipofuscin granules, myelin figures, and autophagic vacuoles (Fig. 4, I, L, and N, and figs. S11, S12, S14, and S17 to S19). Pericytes (Fig. 4, I and L, and figs. S11, S12, S14, and S16),...
microglial cells (fig. S18), dystrophic myelinated nerve fibers (Fig. 4K and figs. S15 to S17 and S19A), and "dark neurons" (Fig. 4M and figs. S20 to S22) with electron-dense cytoplasm and irregularly shaped nuclei were frequently observed in proximity to these abnormal capillaries in blast-exposed mice. By contrast, the brains of sham-blast control mice exhibited normal hippocampal cytoarchitecture without evidence of ultrastructural neuropathology (Fig. 4, A to G).

To confirm the presence of phosphorylated tau proteinopathy in the brains of blast-exposed mice, we performed immunoblot analysis of tissue homogenates prepared from brains harvested from mice 2 weeks after single-blast or sham-blast exposure (Fig. 5). Immunoblot analysis revealed a significant blast-related elevation of phosphorylated tau protein epitopes pT181 and pS202 detected by monoclonal antibody CP-13 (Fig. 5, A, B, and G) and pT205 detected by monoclonal antibody AT270 (Fig. 5, C, D, and I) that are associated with early neurodegenerative tau misprocessing. Blast-related tau phosphorylation was also detected when quantitated as a ratio of phosphorylated tau protein to total tau protein (Fig. 5, E, F, H, and J). In mice exposed to sham blast, all three of the major native murine tau isoforms (4R2N, 4R0N, and 4R1N) were evident (Fig. 5E). By contrast, immunoblot of brain homogenates prepared from mice exposed to a single blast revealed a tau protein isoform distribution pattern that was dominated by a

**Fig. 5.** Single-blast exposure induces increased brain tau protein phosphorylation in wild-type C57BL/6 mice. (A and B) Immunoblots of brain extracts from the left and right hemispheres of mice probed with monoclonal antibody CP-13 directed against phosphorylated tau protein (pS202/pT205) 2 weeks after exposure to sham blast (lanes 1 to 4) or single blast (lanes 5 to 8). Note the single broad band that migrated with an apparent molecular mass of 53 kD (arrows) in brains from mice in both groups. (C and D) Immunoblots of brain extracts from the left and right hemispheres of mice probed with monoclonal antibody AT270 directed against phosphorylated tau protein (pT181) using the same homogenates as in (A) and (B). (E and F) Immunoblots of brain extracts from the left and right hemispheres of mice probed with monoclonal antibody Tau 5 directed against total tau protein using the same homogenates as in (A) to (D). Unlike the results shown in the preceding panels, Tau 5 immunoblots revealed an apparent blast-related alteration in tau protein isoform distribution. (G) Densitometric quantitation of CP-13 phosphorylated tau protein (pS202/pT205) immunolabel in brain homogenates from mice exposed to single blast or sham blast 2 weeks before euthanizing. Mean values ± SEM in arbitrary densitometric units (a.u.). P < 0.005, two-tailed Student’s t test. (H) Densitometric quantitation of CP-13 phosphorylated tau protein (pS202/pT205) immunolabel in brain homogenates as a proportion of total tau protein (Tau 5) in brain homogenates from mice exposed to single blast or sham blast 2 weeks before euthanizing. Mean values ± SEM in arbitrary densitometric units. P < 0.05, two-tailed Student’s t test. (I) Densitometric quantitation of AT270 phosphorylated tau protein (pT181) immunolabel in brain homogenates from mice exposed to single blast or sham blast 2 weeks before euthanizing. Mean values ± SEM in arbitrary densitometric units. P < 0.05, two-tailed Student’s t test. (J) Densitometric quantitation of AT270 phosphorylated tau protein (pT181) immunolabel in brain homogenates as a proportion of total tau protein (Tau 5) in brain homogenates from mice exposed to single blast or sham blast 2 weeks before euthanizing. Mean values ± SEM in arbitrary densitometric units. P < 0.001, two-tailed Student’s t test.
single band corresponding to the intermediate-sized native tau isoform (4R1N; Fig. 5F). Phosphorylated tauopathy (Fig. 5, B and D) and tau isoform distribution abnormalities (Fig. 5F) were detected bilaterally, a finding consistent with blast-related CTE neuropathology and electrophysiological deficits. Blast-induced brain tau proteinopathy was confirmed by enzyme-linked immunosorbent assay (ELISA) analysis of tau protein phosphorylated at pSer199 (single blast, 40 ± 2 ng/ml; sham blast, 31 ± 2 ng/liter; P = 0.027, two-tailed Student’s t test).

**Single-blast exposure persistently impairs axonal conduction and long-term potentiation of activity-dependent synaptic transmission in the hippocampus**

We investigated the possibility that blast-related histopathological and ultrastructural abnormalities would be reflected in equally persistent functional impairments in hippocampal neurophysiology. Analysis of Schaffer collateral–evoked synaptic field potential input-output relations (Fig. S23B) did not reveal an effect of blast exposure on baseline synaptic transmission at either 2 weeks or 1 month after blast exposure (Fig. S24). However, axonal conduction velocity of CA1 pyramidal cell compound action potentials in the stratum alveus (fig. S23A) was significantly slowed 2 weeks after blast exposure, an effect that persisted for at least 1 month [Fig. 6, A and B; P < 0.05, repeated-measures multifactorial analysis of variance (ANOVA)].

Next, we examined the effect of blast exposure on stimulus- and cyclic adenosine monophosphate (cAMP)–evoked long-term potentiation (LTP) of synaptic strength at Schaffer collateral–CA1 synapses (fig. S23B), candidate mechanisms of memory storage. We found marked impairments of stimulus-evoked LTP in mouse slices prepared 2 weeks and 1 month after blast exposure (Fig. 6C; P < 0.05, repeated-

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**Fig. 6.** Single-blast exposure induces persistent impairments in axonal conduction velocity and LTP of synaptic transmission in wild-type C57BL/6 mice. (A) Conduction velocity measurements of first peak compound action potential delay as a function of distance between recording electrodes in CA1 pyramidal cell axons in the stratum alveus of hippocampal slices from mice exposed to single blast (red circles, n = 13) compared to sham blast (black circles, n = 11). Mean ± SEM for each group. (B) Representative stimulus-evoked compound action potentials at proximal and distal recording sites (solid and hash lines, respectively) in hippocampal slices from mice exposed to single blast (red) and sham blast (black). Arrows indicate peak negative velocities used to calculate conduction velocity. (C) Time course of LTP at Schaffer collateral–CA1 synapses evoked by TBS in hippocampal slices from mice exposed to single blast (red circles, n = 17) compared to sham blast (black circles, n = 11). Each point mean ± SEM fEPSP slope of n slices. (D) Time course of LTP at Schaffer collateral–CA1 synapses evoked by bath application of the adenylyl cyclase stimulant forskolin (50 μM) plus the type II phosphodiesterase inhibitor rolipram (10 μM; bar, FOR+ROL) in hippocampal slices from mice 2 weeks (squares, n = 9) and 4 weeks after exposure to single blast (red circles, n = 7) compared to each other and to sham blast (black circles, n = 11). Each point mean ± SEM fEPSP slope of n slices. (E) Time course of LTP at Schaffer collateral–CA1 synapses evoked by TBS in hippocampal slices from mice 2 weeks (blue squares, n = 10) and 4 weeks after exposure to single blast (red circles, n = 7) compared to each other and to sham blast (black circles, n = 11). Each point mean ± SEM fEPSP slope of n slices. (F) Time course of long-lasting potentiation at Schaffer collateral–CA1 synapses evoked by bath application of the adenylyl cyclase stimulant forskolin (50 μM) plus the type II phosphodiesterase inhibitor rolipram (10 μM; bar, FOR+ROL) in hippocampal slices from mice 2 weeks (squares, n = 12) and 4 weeks after exposure to single blast (red circles, n = 15) compared to each other and to sham blast (black circles, n = 19). Each point mean ± SEM fEPSP slope of n slices.
measures multifactorial ANOVA). When the 2-week and 1-month blast-exposed cohorts were examined independently, we found that the magnitude of posttetanic potentiation (PTP) immediately after application of theta-burst stimulation (TBS) was significantly less at the 2-week time point (Fig. 6E; P < 0.05, repeated-measures multifactorial ANOVA). Although PTP recovered by 1 month after blast, the magnitude of LTP 1 hour after tetanus was significantly reduced at both postblast time points (Fig. 6E; P < 0.05, repeated-measures multifactorial ANOVA). These results indicate that exposure to single blast impaired long-term activity-dependent synaptic plasticity for at least 1 month after blast exposure in our model. Next, we examined cAMP-dependent LTP of Schaffer collateral–CA1 field excitatory postsynaptic potentials (fEPSPs) induced by 15-min bath application of the adenylate cyclase activator forskolin (50 μM) plus the type II phosphodiesterase inhibitor rolipram (10 μM). In contrast to control slices, cAMP-LTP was profoundly attenuated 30 to 60 min after drug washout in hippocampal slices prepared from both left and right hemispheres of mice 2 weeks and 1 month after blast exposure (Fig. 6D and fig. S25, A and B; P < 0.05, repeated-measures multifactorial ANOVA). As with stimulus-evoked LTP, cAMP-LTP was equally impaired at both 2 weeks and 1 month after blast exposure, demonstrating the long-term nature of blast effects on both activity-dependent and chemically evoked synaptic plasticity (Fig. 6F; P < 0.05, repeated-measures multifactorial ANOVA).

**Single-blast exposure induces long-term behavioral deficits that are prevented by head immobilization during blast exposure**

We did not detect significant differences between single-blast and sham-blast mice in total distance, mean velocity, or central zone entries in open-field behavior testing (Fig. 7, A to C), indicating that blast exposure did not impair gross neurologically functioning with respect to locomotion, exploratory activity, and thigmotaxis (an indicator of murine anxiety assessed by movement close to the wall of the experimental apparatus). In contrast, when we tested acquisition and long-term retention of hippocampal-dependent spatial learning and memory deficits that are prevented by head fixation (immobilization) during blast exposure. (A to C) Open-field testing showed no effect of blast exposure on gross locomotor function, exploratory activity, or thigmotaxis as measured by total distance traveled (A), mean velocity (B), and number of central zone entries (C), respectively, in mice exposed to single blast (red bars, single blast, head free, n = 10; blue bars, single blast, head fixed, n = 10) or sham blast (black bars, sham blast, n = 20). (D to F) Barnes maze testing demonstrated significant impairments in hippocampal-dependent spatial learning acquisition measured by decreasing latency to find the escape box across 4 days of training (D) (two-way ANOVA, P = 0.020) and long-term memory assessed by escape box location recall assessed 24 hours after the last training session (E) (**P = 0.004, Student’s t test). Mice exposed to single blast (red squares, single blast, head free, n = 10) are compared to pooled sham-blast control mice (circles, sham blast, n = 20). Fixation (immobilization) of the head during blast exposure (blue squares, single blast, head fixed, n = 10) reversed blast-induced learning and memory deficits. Arrowhead in (E) represents 5% level predicted by chance selection of the escape box from among the 20-hole choices. (F) Representative Barnes maze tracks obtained on trials 1, 8, and 16 for mice exposed to a single blast (bottom row) compared to sham blast (top row).
learning and memory in the Barnes maze (Fig. 7, D to F), we observed that blast-exposed mice exhibited significantly longer escape latencies (Fig. 7D; \( P < 0.05 \), two-way ANOVA) and poorer memory retrieval 24 hours after the final training session (Fig. 7E; \( P < 0.05 \), Student's \( t \) test) compared to sham-blast control mice. These findings are consistent with persistent blast-related hippocampal dysfunction.

The results of kinematic analysis (Fig. 2, D to G) suggested that blast-induced head acceleration was a likely pathogenic mechanism by which blast exposure leads to TBI and neurobehavioral sequelae. To test this hypothesis, we compared hippocampal-dependent learning acquisition and memory retention in mice with and without head immobilization during single-blast exposure and in sham-blast control mice. Head immobilization during blast exposure eliminated blast-related impairments in hippocampal-dependent learning acquisition (Fig. 7D; \( P > 0.20 \), repeated-measures ANOVA with post hoc Scheffe test compared to sham-blast controls) and restored blast-related memory retention deficits to normal levels (Fig. 7E; \( P > 0.20 \), one-way ANOVA with post hoc Scheffe test), supporting the conclusion that head acceleration is necessary for behavioral learning impairments.

**DISCUSSION**

We analyzed a case series of postmortem human brains from U.S. military veterans with blast exposure and/or concussive injury and compared them to brains from young-adult athletes with histories of concussive injury and from normal controls of comparable ages without histories of blast exposure, concussive injury, or neurological disease. We uncovered evidence of CTE-linked tau neuropathology, including multifocal perivascular foci of neurofibrillary and glial tangles immunoreactive for phosphorylation-independent (Tau-46) and phosphorylation-dependent (CP-13) tau epitopes (20, 21), in the brains of blast-exposed and/or concussive-injured veterans. This blast-associated CTE-linked tau neuropathology was indistinguishable from the tau neuropathology, neuroinflammation, and neurodegeneration observed in the brains of young-adult athletes with histories of repeat concussive injury. Examination of brains from wild-type C57BL/6 mice 2 weeks after exposure to a single controlled blast also revealed histopathological, ultrastructural, and biochemical evidence of CTE-linked neuropathology, including tau protein–linked immunoreactivity, persistent perivascular pathology, cortical and hippocampal neurodegeneration, myelinated axonopathy, chronic neuroinflammation with widespread astrocytosis and microgliosis, and phosphorylated tau proteinopathy. Overall, our findings of persistent CTE-linked neuropathology in the brains of military veterans with blast exposure and/or concussive injury and young athletes with repeat concussive injury suggest that TBI induced by different insults under different conditions can trigger common pathogenic mechanisms leading to similar neuropathology and sequelae. Notably, within this small controlled case series, the effects of blast exposure, concussive injury, and mixed trauma (blast exposure and concussive injury) were indistinguishable.

Experimental results from our murine blast neurotrauma model provide evidence linking blast exposure with development of CTE-like tau neuropathology. Moreover, this blast-related neuropathology was associated with persistent neurophysiological and cognitive deficits that recapitulate clinical signs and symptoms reported in military veterans with blast-related TBI (2, 7, 9–15) and concussive-injured athletes diagnosed with CTE (20, 21). It is notable that exposure to a single blast in our mouse model was sufficient to induce early CTE-like neuropathology, slowed axonal conduction velocity, and defective stimulus- and cAMP-dependent LTP of synaptic transmission. Notably, these blast-related neurophysiological abnormalities were contemporaneous with somatodendritic alterations in hippocampal and cortical total tau and phosphorylated tau neuropathology and biochemistry, microvascular ultrastructural pathology, and impairment in hippocampal-dependent learning acquisition and memory retention.

Although blast-exposed C57BL/6 mice recapitulated key features of human CTE neuropathology, including cellular accumulation of phosphorylated tau protein and pre-tangle tau protein neuropathology, it is notable that mature NFTs were not detected in the cortex or hippocampus of blast-exposed mice. This apparent discordance with human CTE neuropathology may be explained by the early time points chosen for evaluation in our mouse studies or, alternatively, as a forme fruste resulting from resistance of wild-type murine tau protein to form neurotoxic aggregates in vivo. However, our results demonstrate blast-related immunohistochemical and biochemical abnormalities in tau hyperphosphorylation at the 2-week time point after single-blast exposure. Studies of triple-transgenic mice expressing human tau protein and human amyloid-\( \beta \) peptide have shown that controlled cortical impact injury leads to rapid accumulation of hyperphosphorylated tau within 24 hours after experimental injury (49, 50). These findings suggest that genotypic determinants may be critical factors that modulate temporal and phenotypic expression of TBI and late-emerging sequelae, including CTE.

ICP dynamics recorded during blast exposure revealed blast-induced pressure transients in the hippocampus that were coincident with and comparable in amplitude, waveform, and impulse to FFP measurements outside the cranium. This finding is consistent with the head acting as a lumped element for which the blast-induced external pressure differential equilibrates within \( \sim 100 \) \( \mu \)s. Measured blast pressure amplitudes in the brain were on the order of 100 kPa (~1 bar), a magnitude equivalent to water pressure at a depth of \( \sim 10 \) m. Although it is possible that high-frequency components (>100 kHz) could lead to localized focusing due to reverberation and constructive interference, the pressure amplitudes we measured were far below tissue damage thresholds. Tissue damage associated with clinical ultrasound requires negative acoustic pressures in excess of 1 MPa (51) that lead to excitation of cavitation bubbles. Thresholds for positive pressures are not well characterized but are likely to exceed 40 MPa because positive pressures commonly used in clinical shock wave lithotripsy are not associated with significant, if any, tissue damage (52). Thresholds for tissue damage from underwater sound require \( \sim 100 \) kPa (53) and result from many cycles of bubble growth and collapse over tens of seconds of continuous wave excitation. Tissue damage in this setting is due to the negative pressure rather than exposure to a single compression pulse. These considerations indicate that direct tissue damage resulting from transmission of the blast shock wave through the brain is unlikely. Our results indicate that ICP transients closely approximate FFP measurements in air (27, 54, 55). Moreover, blast wavefront transmission was identical when measured in the brain of intact living mice or isolated mouse heads severed at the cervical spine, suggesting that neither thoracic-mediated mechanisms nor vascular hemodynamic effects contributed significantly to ICP transients during blast exposure. Together, our findings point to the substantial inertial forces and oscillating acceleration-deceleration cycles imposed on the head by blast wind (bobblehead effect) as the primary biomechanical mechanism by which
blunt trauma and postmortem examination, potential contributions of confounding factors need to be considered. Further studies are needed to differentiate the relative contributions of these and possibly other pathogenic mechanisms.

The significance of the neurophysiological abnormalities in blast-exposed wild-type C57BL/6 mice is substantial. First, although blast exposure did not produce detectable long-term dysfunction in basal synaptic transmission, exposure to a single sublethal blast was sufficient to induce profound and persistent impairment of both activity- and cAMP-dependent LTP in hippocampal CA1 pyramidal neurons, candidate cellular mechanisms of long-term memory processing. The fact that both forms of LTP require dendritic protein synthesis (70, 71) and gene transcription (72) indicate that blast exposure may induce long-lasting damage to cellular signal transduction downstream of synaptic glutamate release. Mechanisms that may be altered by blast exposure include N-methyl-D-aspartate glutamate receptor activation, intracellular second messenger systems, gene expression, protein synthesis, and posttranslational modification. Our results also indicate that blast exposure can induce persistent axonal conduction defects that further impair cognitive processing and are consistent with recent findings from human studies (14, 15). These effects may be mediated by diffuse axonal injury, Wallerian degeneration, and or differential susceptibility of larger neurons to structural or functional axotomy. Damage to these and other brain structures, systems, and mechanisms may contribute to abnormalities in neurochemical homeostasis, cerebral metabolism, and neurophysiological functions associated with blast-related TBI (73). Our results suggest that blast exposure may hold comparable or even greater pathogenic potential than repetitive head injury associated with contact athletics (74–76).

Limitations of the human neuropathology reported here include the small number of available cases, the time interval between trauma and postmortem examination, potential contributions of confounding comorbidities and risk factors, and inherent limitations of neuropathological analysis to establish mechanistic causality. Clinicopathological correlation may be further complicated by genetic contributions (for example, APOE (apolipoprotein E) genotype (77)), history of previous head trauma, innate inflammatory responsivity, neuropsychiatric comorbidity, age and gender, and other factors with potential to modulate susceptibility and pathological expression of blast-related neurotrauma and sequelae. Furthermore, emerging evidence indicates that PTSD may represent an important overlapping comorbidity with potential to synergistically affect both the incidence and the severity of blast TBI and military deployment–related cognitive dysfunction (2, 7, 78–80). Limitations of our animal experiments include use of adult male C57BL/6 wild-type mice subjected to a single-blast exposure with post-exposure evaluation at time points only up to 1 month. Interpretation and generalizability of our animal experiments are further constrained by interspecies differences, including the significantly greater deformability of the murine skull, the relative instability of the murine cervical spine, and differential force loading on the head and neck in mice and humans.

Our results provide compelling evidence linking blast exposure to long-lasting brain injury. Specifically, our study suggests that blast injury may increase risk for later development of CTE and associated neurobehavioral sequelae. Indeed, the severity, persistence, and possible progression of the neuropathological abnormalities and neurophysiological deficits observed in our study indicate that blast exposure is a potent insult with enduring pathogenic potential and functional significance. The availability of a neuropathologically validated murine model with correspondence to human CTE is expected to open new avenues for investigation of mechanisms, biomarkers, and risk factors relevant to blast-related brain injury and facilitate development of urgently needed diagnostics, therapeutics, and prophylactic measures for blast neurotrauma and its aftermath.

MATERIALS AND METHODS

Human subjects

The brain and spinal cord of 12 human subjects (male military veterans, ages 22 to 45 years, mean 32.3 years, with histories of explosive blast and/or concussive injury 1 to 6 years before death, n = 4; male athletes with histories of repetitive concussive injury, including 3 amateur American football players and a professional wrestler, ages 17 to 27 years, mean 20.8 years, n = 4; male controls, ages 18 to 24 years, mean 20.5 years, without known blast exposure, trauma history, or neurologic disorder, n = 4) were procured through the Boston University Alzheimer’s Disease Center and Center for the Study of Traumatic Encephalopathy at Boston University School of Medicine. Blast exposure, trauma history, and neurological status at the time of death were determined through review of medical records and interviews with next of kin. Ethical permission to conduct this investigation was approved by Institutional Review Board at Boston University School of Medicine. The study conforms to institutional regulatory guidelines and principles of human subject protection in the Declaration of Helsinki.

Animal subjects

Adult wild-type C57BL/6 male mice (Charles River Laboratories) were group-housed at the Laboratory Animal Science Center, Boston University School of Medicine. All animal experiments used 12- to 16-week-old mice with 8 to 10 mice per group. Animal housing and experimental conditions were performed in accordance with the principles of human subject protection in the Declaration of Helsinki.
use were conducted in accordance with Association for Assessment and Accreditation of Laboratory Animal Care guidelines, in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and adherence to principles in the National Research Council Guide for the Care and Use of Laboratory Animals. All studies were approved by Institutional Animal Care and Use Committees at Boston University School of Medicine and New York Medical College.

Histopathological and electron microscopic analyses
Postmortem human brain and spinal cord were received as fresh tissue and as fixed tissue in formalin after processing by medical examiners. Neuropathological analysis followed established protocols at the Boston University Alzheimer’s Disease Center and included comprehensive examination for all neurodegenerative conditions (81). Paraffin-embedded sections from at least 15 brain regions were stained with Luxol fast blue, hematoxylin and eosin, and Bielschowsky silver stain. Mice were euthanized by CO2 asphyxiation and transcardially perfused with phosphate-buffered saline (PBS). Whole brains were prefixed in 10% neutral buffered formalin, block-sectioned into 2-mm coronal slabs, postfixed in 4% paraformaldehyde, paraffin-embedded, and serially sectioned at 10 μm. A battery of primary detection antibodies (table S1) was used for immunohistopathological analyses. Ultrastructural studies were conducted on fixed brain specimens embedded in Epon, sectioned at 60 nm, stained with uranyl acetate or lead citrate, and examined with a Tecnai-G2 Spirit BioTWIN electron microscope with an AMT 2K CCD camera.

Murine blast neurotrauma model system
A compressed gas-driven shock tube (fig. S3) was developed in collaboration with the Fraunhofer Center for Manufacturing Innovation at Boston University (Brookline, MA) and installed at the Neurotrauma Laboratory, Boston University School of Medicine. This instrument was used to deliver highly reproducible blast waves (Fig. 2A, figs. S3 to S7, and tables S2 and S3). Adult wild-type C57BL/6 male mice (2.5 months) were anesthetized with ketamine (75 mg/kg, intraperitoneally), xylazine (4.3 mg/kg, intraperitoneally), and buprenorphine (0.2 mg/kg, subcutaneously) and secured in the prone position in a thoracic-restraint system inside the shock tube (fig. S3). The head and neck were free to allow flexion, extension, and rotation of the cervical spine in the horizontal and sagittal planes of motion to model conditions relevant to military blast exposure. Maximum burst pressure compatible with 100% survival and no gross motor abnormalities was ascertained empirically (table S2). Experimental blast parameters (incident static pressure, 77 ± 2 kPag; blast overpressure rise time, 38 ± 3 μs; compressive phase duration, 4.8 ± 0.1 ms; shock wave velocity, 1.26 ± 0.04 Mach; calculated blast wind velocity, 150 m/s = 336 miles/hour; table S2) closely approximate explosive blast produced by detonation of 5.8 kg of TNT measured at a standoff distance of 5.5 m [ConWep analysis (44); table S3]. This blast exposure is within the range of typical IED detonations and standoff distances associated with military blast injury (45). Anesthetized mice were exposed to a single blast or sham blast, removed from the apparatus, monitored until recovery of gross locomotor function, and then transferred to their home cage.

Static and reflected FFP measurements
Assessment of static and reflected FFP was assessed by two piezoelectric pressure sensors (model HM102A15, PCB Piezotronics) placed in the shock tube at the same axial distance relative to the head of the animal subjects. A static pressure (side-on) sensor was flushed-mounted inside the shock tube. A second transducer was positioned with the detector facing into the shock wave in a reflected pressure (face-on) orientation. Pressure signals were processed with a PCB signal conditioner (model 482C05, PCB Piezotronics) and recorded at a frequency of 5 MHz with a digital oscilloscope (640Zi WaveRunner, LeCroy). Voltages were converted to pressure with calibration data provided by the manufacturer and processed with 2-kHz low-pass filtering.

ICP measurements
ICP measurements were conducted with a broad-bandwidth piezoelectric needle hydrophone (NP10-3, DAPCO Industries) with a 0.6-mm-diameter element sheathed in a stainless steel hypodermic needle. Pressure sensitivity was flat to within ±3 dB for frequencies ranging from 1 Hz to 170 kHz. The needle hydrophone was inserted into the hippocampus at −3.00 mm caudal to the bregma suture, +3.50 mm lateral to the sagittal suture, and +2.00 mm ventral to the skull surface. For ICP measurements, the head was immobilized to prevent displacement of the pressure sensor. Piezoelectric voltage signals were recorded by a digital oscilloscope (640Zi WaveRunner, LeCroy) and converted to pressure units with calibration data supplied by the manufacturer and processed with 20-kHz low-pass filtering. Post-acquisition processing was performed with Matlab 2009 (MathWorks).

High-speed videographic kinematic analysis
High-speed videography was conducted with a FASTCAM SA5 camera (Photron USA Inc.; courtesy of Tech Imaging) operated at 10-μs frame capture rate. Videographic records were reassembled with open-source ImageJ software and processed in Matlab (MathWorks). Angular position and motion of the head were assessed by tracking a reflective paint mark on the snout, calculated by assuming a central pivot point between the scapulae (fig. S3B), and processed with 500-Hz low-pass filtering (Fig. 2, D to G, fig. S3B, and video S1).

Hippocampal electrophysiology
Mice were decapitated under deep isoflurane anesthesia, and the brains were quickly removed, hemisected, and sectioned with a Leica model VT 1200S vibratome at 350 μm. Slices were fixed to a stage with cyanoacrylate adhesive and immersed in oxygenated artificial cerebrospinal fluid (126 mM NaCl, 3 mM KCl, 1.25 mM NaH2PO4, 1.3 mM MgCl2, 2.5 mM CaCl2, 26 mM NaHCO3, 10 mM glucose, saturated with 95% O2 and 5% CO2) at 32°C. Experimental drugs were bath applied in the perfusate at a rate of 3 ml/min. Axonal conduction velocity was assessed with a recording electrode placed in CA1 stratum alveus. Schaffer collateral–CA1 synaptic transmission and plasticity were assessed with a recording electrode in the CA1 stratum radiatum.

Hippocampal-dependent learning and memory
Open-field testing (Med-Associates) was used to assess gross locomotor function, exploratory activity, and thigmotaxis. Hippocampal-dependent learning acquisition and memory retention were evaluated in the Barnes maze (82). Spatial learning was assisted by visual cues in the environment that remained constant across test sessions. Movement was tracked and recorded electronically (Stoelting). Latency to find the escape box, trajectory velocity to the escape box, and total trajectory distance were assessed and recorded daily in four sessions conducted over 4 days. Memory retrieval was electronically assessed
by recording the number of nose pokes in blank holes as a percentage of total nose pokes recorded 24 hours after completion of the learning protocol.

Quantitative assessment of phosphorylated and total tau protein
Quantitative immunoblot analysis was conducted with left and right hemisected brains obtained from PBS-perfused mice 2 weeks after exposure to a single blast (n = 6 mice) or sham blast (n = 6 mice). Snap-frozen hemisected brain specimens were thawed, resuspended in 0.7 ml of protease-phosphatase inhibitor buffer, and homogenized as previously described (83). Protein concentrations were normalized and equal sample volumes were subjected to standard polyacrylamide gel electrophoresis in duplicate. Immunoblot detection used monoclonal antibody AT270 (Innogenetics) directed against tau protein phosphorylated at Thr181 (pT181), monoclonal antibody CP-13 (P. Davies, Albert Einstein College of Medicine) directed against tau protein phosphorylated at Ser202 (pS202) and Thr205, or monoclonal antibody Tau 5 (L. Binder, Northwestern University Medical School) directed against phosphorylation-independent tau protein. Triplicate densitometry measurements were analyzed with open-source ImageJ software. A commercial ELISA kit was used to quantify murine-specific tau protein phosphorylated at Ser199 (Invitrogen). Frozen brain samples were homogenized in eight volumes of 5 M guanidine-HCl and 50 mM tris (pH 8) followed by five passes in a glass Teflon homogenizer. Homogenates were mixed for 3 hours, diluted into PBS containing protease inhibitors, and centrifuged for 20 min at 16,000g. Supernatants were diluted and assayed in quadruplicate according to the manufacturer’s instructions.

Statistical analyses
Comparisons of axonal conduction velocity and LTP magnitude were conducted with repeated-measures multifactoral ANOVA with Bonferroni-Dunn post hoc correction. Longitudinal neurobehavioral data were analyzed by repeated-measures ANOVA. Memory retrieval was evaluated by ANOVA. Statistical significance was preset at P < 0.05.

SUPPLEMENTARY MATERIALS
www.sciencetranslationalmedicine.org/cgi/content/full/4/134/134ra60/DC1
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Table S1. Summary of antibodies used in this study.
Table S2. Murine blast neurotrauma model blast parameters.
Table S3. Shock tube blast compared to equivalent explosive blast.
Fig. S1. Phosphorylated tau axonopathy in a single axon from the brain of a 22-year-old male military veteran with exposure to a single improvised explosive device blast and persistent blast-related traumatic brain injury symptoms.
Fig. S2. Absence of CTE neuropathology in a representative postmortem human brain from Bonferroni-Dunn post hoc correction. Longitudinal neurobehavioral data were analyzed by repeated-measures ANOVA. Memory retrieval was evaluated by ANOVA. Statistical significance was preset at P < 0.05.

REFERENCES AND NOTES
Supplementary Materials for

Chronic Traumatic Encephalopathy in Blast-Exposed Military Veterans and a Blast Neurotrauma Mouse Model


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Fig. S11. Electron micrographic montage of the hippocampus CA1 field in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.
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Fig. S15. Perivascular ultrastructural pathology in the hippocampus CA1 stratum radiatum in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.
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Fig. S17. Myelin figure in the hippocampus CA1 stratum pyramidale in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.
Fig. S18. A microglial cell amidst myelinated axons in the hippocampus CA1 stratum alveus in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.
Fig. S19. Autophagy and mitophagy in the hippocampus CA1 field in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.
Fig. S20. Degenerating (“dark”) pyramidal neurons in the hippocampus CA1 stratum pyramidale in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.
Fig. S21. Degenerating (dark) pyramidal neurons in the hippocampus CA1 stratum pyramidale in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.
Fig. S22. Degenerating (dark) pyramidal neurons in the hippocampus CA1 stratum pyramidale in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.
Fig. S23. Electrode placements for axonal conduction velocity and synaptic plasticity experiments.
Fig. S24. Schaffer collateral–CA1 synaptic input-output relations illustrating the absence of long-term effects of blast exposure on baseline synaptic transmission.
Fig. S25. Blast-induced deficits in cAMP-induced long-term potentiation of synaptic transmission at Schaffer collateral–CA1 synapses are bilateral and persistent.
Fig. S26. Model of blast- and concussion-related TBI and sequelae, including CTE.

Video S1 legend

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencetranslationalmedicine.org/cgi/content/full/4/134/134ra60/DC1)

Video S1 (.avi format). Mouse head kinematics during exposure to a single shock tube blast.
MATERIALS & METHODS

Human Subjects. The brain and spinal cord of 12 human subjects (male military veterans, ages 22 to 45 years, mean 32.3 years, with histories of explosive blast and/or concussive injury 1 to 6 years before death, n = 4; male athletes with histories of repetitive concussive injury, including 3 amateur American football players and a professional wrestler, ages 17 to 27 years, mean 20.8 years, n = 4; male normal controls, ages 18 to 24 years, mean 20.5 years, without known blast exposure, trauma history, or neurological disease, n = 4) were procured through the Boston University Alzheimer’s Disease Center and Center for the Study of Traumatic Encephalopathy at Boston University School of Medicine. Blast exposure, trauma history, and neurological status at the time of death were determined through review of medical records and interviews with next of kin. Ethical permission to conduct this investigation was approved by Institutional Review Board at Boston University School of Medicine. The study conforms to institutional regulatory guidelines and principles of human subject protection in the Declaration of Helsinki.

Animal Subjects. Adult wildtype C57BL/6 male mice were purchased from Charles River Laboratories (Wilmington, MA) and group housed at the Laboratory Animal Science Center, Boston University School of Medicine, Boston, MA. All animal experiments utilized 2.5-month-old mice at the time of blast exposure and included 8 to 10 mice per group. Mice were provided with standard mouse chow and water ad libitum. Ambient temperature was controlled at 20-22 °C with 12-hour light-dark cycles. Animal housing and experimental use were conducted in accordance with Association for Assessment and Accreditation of Laboratory Animal Care guidelines, in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and adherence to principles in the National Research Council Guide for the Care and Use of Laboratory Animals. All studies were approved by Institutional Animal Care and Use Committees at Boston University School of Medicine and New York Medical College.

Histopathology. Processing of human brains followed established procedures and protocols at the Boston University Alzheimer’s Disease Center, Boston, MA, and included comprehensive neuropathological analysis of all neurodegenerative conditions as previously described (1). Human brain and spinal cord specimens were received as fixed tissue in formalin after processing by medical
examiners. Paraffin-embedded sections from at least 15 brain regions were stained with Luxol fast blue, hematoxylin and eosin, and Bielschowsky silver stain. Sections evaluated by immunohistochemistry utilized a battery of primary antibodies (table S1), chromogen visualization (Vectastain Elite ABC Kit, Vector Labs, Burlingame, CA), and cresyl violet counterstaining as previously described (2, 3). For histological experiments involving mice, animals were euthanized by CO₂ asphyxiation according to IACUC-approved protocol followed by transcardial gravity perfusion with phosphate-buffered saline (PBS, Sigma-Aldrich, St Louis, MO). Brains were rapidly removed from the calvarium and placed in 10% neutral buffered formalin for 2 hours, then transferred to PBS. Coronal slabs (2 mm) were obtained by block sectioning, fixed in 4% paraformaldehyde for 2 hours, embedded in a single paraffin block, and serially sectioned at 10 µm. Sections were processed for immunohistochemistry with a battery of primary antibodies (table S1) and visualized by Vectastain Elite ABC Kit (Vector Labs, Burlingame, CA). Slides were developed according to manufacturer's instructions for exactly the same incubation time and counterstained with hematoxylin. For double immunostained sections, tissue was blocked with avidin and biotin before primary antibody incubation and visualized with DAB and aminoethylcarbazole according to manufacturer's instructions (Vector Laboratories, Burlingame, CA, USA). Bielschowsky silver stain was performed using 20% AgNO₃ titrated with ammonia and developed with HNO₃ and citric acid and unbuffered formalin.

**Electron Microscopy.** Small pieces (1-2 mm cubes) of harvested brain were fixed in 2.5% glutaraldehyde with 2.5% paraformaldehyde in 0.1M sodium cacodylate buffer (pH 7.4) overnight at room temperature, washed in 0.1M cacodylate buffer, postfixed with 1% osmium tetroxide (OsO₄) with 1.5% potassium ferrocyanide (KFeCN₆) for 1 hour, then washed in water. The specimens were then incubated in 1% aqueous uranyl acetate for 1 hr, washed, and sequentially dehydrated in increasing grades of alcohol (10 min each in 50%, 70%, 90%, 100%, 100%). Samples were treated in propylene oxide for 1 hr and infiltrated overnight in a 1:1 mixture of propylene oxide and TAAB Epon (Marivac Canada Inc., St. Laurent, Canada) and polymerized at 60 °C for 48 hrs. Ultrathin sections (60 nm) were cut on a Reichert Ultracut-S microtome, placed on copper grids, stained with lead citrate or uranyl acetate, and examined using a Tecnai-G2 Spirit BioTWIN electron microscope. Images were acquired with an AMT 2K CCD camera.
Murine Blast Neurotrauma Model. A compressed gas-driven shock tube (25 cm diameter; 5.3 m tube length; fig. S2) developed in collaboration with the Fraunhofer Center for Manufacturing Innovation at Boston University, Boston, MA, and installed at the Murine Neurotrauma Laboratory, Boston University School of Medicine, Boston, MA was used to deliver highly-reproducible sublethal blast shock waves relevant to human blast injury (fig. S2-6; tables S2, S3). Adult wildtype C57BL/6 male mice (Charles River Laboratories, Wilmington, MA) at 2.5-months-of-age were anesthetized with ketamine (75 mg/kg, i.p.), xylazine (4.3 mg/kg, i.p.), and buprenorphine (0.2 mg/kg, s.c.), secured in the prone position with a wire mesh holder, and inserted into a custom-fabricated restraint system that protected the thorax. The assembly was then fixed to an internal frame inside the shock tube with the unprotected head positioned exactly 0.56 m from the exit of the shock tube and 4.06 m from the blast origin (fig. S2). In order to model conditions relevant to human blast exposure conditions, the head and neck were free to allow flexion, extension, and rotation of the cervical spine in the sagittal and horizontal planes of motion. We empirically determined the maximum burst pressure (303 ± 9 kPag) and corresponding blast parameters compatible with 100% survival with no gross motor abnormalities 24 hours following blast exposure (table S2). Anesthetized mice were exposed to a single sublethal shock tube blast (table S2) or sham blast, removed from the apparatus, and monitored until recovery of gross locomotor function and exploratory activity. Mice were then transferred to their home cage.

Blast Comparators. Experimental shock tube blast parameters (i.e., peak static pressure amplitude, duration, and impulse) used in this study closely approximated characteristics of explosive blast produced by detonation of 5.8 kg of 2,4,6-trinitrotoluene (TNT) or 4.5 kg of Composition C-4 explosive measured at a standoff distance of 5.53 m (table S3) analyzed using the Conventional Weapons Effects Program (ConWep) (44). For comparison, an improvised explosive device (IED) commonly encountered by U.S. military personnel utilizes a 120 mm mortar round equivalent to 4.53 kg of TNT (1st Infantry Division Soldier’s Handbook to Iraq, U.S. Army, at weblink: http://www.gwu.edu/~nsarchiv/IMG/soldiershandbookiraq.pdf accessed January 2, 2012 (L.E.G.). The blast exposure utilized in this study was comparable to experimental conditions in recent studies utilizing a shock tube (4, 5) or detonated explosives (6) to model moderate intensity blast exposure relevant to the military.
**Static and Reflected Free-Field Pressure Measurements.** Assessment of static (side-on) and reflected (face-on) free-field pressure (FFP) during blast exposure was assessed by two piezoelectric pressure sensors (Model HM102A15, PCB Piezotronics Inc., Depew, NY, USA) placed in the shock tube at the same axial distance as the head of the mouse. One sensor was flushed-mounted inside the shock tube and secured in a static pressure (side-on) orientation relative to the blast shock wave. The second transducer was positioned with the detector facing into the shock tube in a reflected pressure (face-on) orientation relative to the blast shock wave. With respect to the reflected pressure sensor, the measured pressure magnitude does not capture the total pressure (i.e., stagnation pressure) of the blast wave as a consequence of the small size and geometry of the sensor system relative to the blast wave produced by our shock tube system. However, the reflected pressure transducer was comparable in size to the mouse head and thus recorded relevant pressure incident to the head during blast exposure. Pressure signals in both orientations were processed through a PCB signal conditioner (Model 482C05, PCB Piezotronics Inc., Depew, NY, USA) and recorded at a frequency of 2 MHz using a digital oscilloscope (640Zi Waverunner; LeCroy, Chestnut Ridge, NY). Voltages were converted to pressure using calibration data.

**Intracranial Pressure Measurements.** Intracranial pressure (ICP) measurements were conducted with a broad-bandwidth piezoelectric needle hydrophone (NP10-3; DAPCO Industries Inc., Oak Creek, WI) with a 0.6 mm diameter active element sheathed in a standard #19 gauge hypodermic needle (length, 75 mm; o.d., 1 mm). Pressure transducer sensitivity was flat to within ±3 dB for frequencies ranging from 1 Hz to 170 kHz. The needle hydrophone was inserted into the hippocampus (-3.00 mm caudal to the bregma suture, +3.50 mm lateral to the sagittal suture, +2.00 mm ventral to skull surface according to the atlas of Franklin and Paxinos *The Mouse Brain in Stereotaxic Coordinates*, 3rd Ed., Elsevier Academic Press, Boston, 2008. For ICP measurements, the head was secured in place to prevent intracranial displacement during blast exposure. ICP piezoelectric voltage signals were recorded by a digital oscilloscope (640Zi Waverunner; LeCroy, Chestnut Ridge, NY) converted to pressure using calibration data derived from substitution experiments with calibrated transducers over a frequency range up to 2 MHz. Post-acquisition processing was performed with Matlab 2009 software (MathWorks, Natick, MA, USA).
High-Speed Videography and Kinematic Analysis. High-speed videography was conducted with a FASTCAM SA5 camera and software (Photron USA Inc., San Diego, CA) operated at a 10 µs frame capture rate (100 kHz). Initial post-acquisition analysis of individual frames was conducted using ImageJ software (NIH, Bethesda, MD). All subsequent processing was carried out in Matlab (MathWorks, Natick, MA). Angular rotation of the head was calculated by assuming a central pivot point between the scapulae (fig. S2B). Cartesian motion of the head was calculated by tracking a paintmarked nose spot.

Head Fixation. Head fixation was accomplished using two miniature nylon cable ties with minimal face-on cross-sectional area. Prior to immobilization, the head was securely positioned on a rigid bite bar fixed to the in-tube restraint. The head was immobilized by positioning one band across the rostral aspect of the skull proximal to the incisor. The second band was placed immediately posterior to the caudalmost aspect of the skull. Neither band obstructed the oncoming blast shock wave (see figure, right). Care was taken to avoid airway compromise. Thoracic protection was provided as described above. This immobilization procedure prohibited head displacement in all three Cartesian planes of motion during experimental blast.

Mouse Hippocampus Slice Electrophysiology. Mice were decapitated under deep isoflurane anesthesia and the brains quickly removed, hemisected, and blocked with a vibratome (DTK1000, Ted Pella, Co., Redding, CA) at a thickness of 350 µm. The tissue block was glued with cyanoacrylate adhesive to a stage immersed in ice-cold, oxygenated artificial cerebrospinal fluid (aCSF; NaCl, 126 mM; KCl, 3 mM; NaH₂PO₄, 1.25 mM; MgCl₂, 1.3 mM; CaCl₂, 2.5 mM; NaHCO₃, 26 mM; glucose, 10 mM; saturated with 95% O₂ and 5% CO₂) maintained at 2-4 °C, then placed in a conditioning chamber containing aCSF at room temperature for at least 1 hr before transfer to an interface chamber maintained at 32°C for recording. Slices were perfused with aCSF during experiments. Experimental drugs were bath applied in the perfusate. For studies of Schaffer Collateral-CA1 synaptic transmission and plasticity, low resistance recording electrodes were pulled
with a Flaming/Brown Micropipette puller (Model P-97, Sutter Instrument, Novato, CA, USA) using thin-walled borosilicate glass (1-2 MΩ with aCSF; A-M Systems, Sequim, WA), and inserted into the stratum radiatum of the hippocampus CA1 field to record field excitatory post-synaptic potentials (fEPSPs). A bipolar stainless steel stimulating electrode was placed in Schaffer collateral-commissural fibers the stratum radiatum, and current pulses were applied with stimulus intensity adjusted to evoke approximately 50% of maximal fEPSPs (50 pA to 100 pA; 100 µs duration) at 30 s intervals. Electrical stimulation was delivered by an ISO-Flex isolator controlled by a Master eight-pulse generator (AMPI, Jerusalem, Israel) triggered by a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA), and signals were digitized and recorded using the Multiclamp 700B. fEPSP slope was measured by linear interpolation from 20-80% of maximum negative deflection, and slopes confirmed to be stable ±10% for at least 15 min. Data were analyzed using Clampfit (Version 9, Molecular Devices, Sunnyvale, CA) on an IBM-compatible personal computer. Evoked fEPSPs (50% of maximum amplitude, 2-4 mV) were recorded in the apical dendritic field in stratum radiatum for a stable baseline period of at least 30 min and evoked by single square pulses (10-100 µA, 150 µs) applied at 30 s intervals from a bipolar stainless-steel stimulating electrode (FHC, Bowdoin, ME). The high-frequency stimulus (HFS) paradigm for induction of homosynaptic LTP consisted of three theta burst trains, each train consisting of 10 bursts of 5 pulses each with a burst frequency of 100Hz with interburst interval of 200 ms applied at 120 s intervals. For measurement of axonal conduction velocity, two extracellular recording electrodes were placed in CA1 stratum alveus approximately 200 µm apart, and a bipolar stimulating electrode placed 100 µm away from the nearest of the two recording electrodes to antidromically activate CA1 pyramidal neuron axons coursing through the stratum alveus. The latency differences of the peak negativity between the two recording electrodes and the spatial distance were used to calculate axonal conduction velocity for each slice.

Assessment of Hippocampal-Dependent Learning and Memory. Neurobehavioral assessment was performed using an open-field test and Barnes maze (Med-Associates, Inc., St. Albans, VT, USA). Open-field testing to assess baseline locomotor functioning (average velocity), exploratory activity (total distance), and thigmotaxis (number of central zone entries) was performed by placing each animal subject in the middle of a 42.5 cm x 42.5 cm open arena and monitoring movement for 10 min using a 3D infrared diode motion detector system (Any-Maze, Stoelting Co., Inc., Wood Dale, IL). Barnes maze evaluation (7) was conducted using a 20-box apparatus with 900 lux surface light
Animal subjects were familiarized with the test apparatus by placement on the platform and gentle guidance to the escape box. Training sessions were conducted across four training trials per day for four days. The order of testing of individual subjects was the same throughout daily sessions, but randomized across the four test days for a total of 16 trials. To initiate testing, a single mouse was placed in the start box in the middle of the maze and released. Test subjects were evaluated while locating a single escape box placed at a constant position. Spatial learning was assisted by distant visual cues that remained constant during across test sessions. Movement was tracked and recorded electronically. Latency to find the escape box, trajectory velocity to the escape box, and total trajectory distance was assessed and recorded daily. Memory retrieval was evaluated by replacing the escape box with a blank box 24 hours after the last training session. Memory retrieval was assessed by electronically recording the number of nose pokes into the blank box as a percentage of total nose pokes.

**Quantitative Assessment of Phosphorylated and Total Tau Protein.** For immunoblot analysis, left and right hemisected brain samples were obtained from PBS-perfused mice 2 weeks after exposure to a single shock tube blast \((n = 6 \text{ mice})\) or sham blast \((n = 6 \text{ mice})\). Snap frozen hemisected brain specimens were thawed and resuspended in 0.7 ml protease-phosphatase inhibitor buffer as previously described \((8)\). Equal volumes of homogenized samples were subjected to standard polyacrylamide gel electrophoresis in duplicate and immunoblotted with monoclonal antibody AT270 (Innogenetics Inc., Alpharetta, GA, USA) directed against tau protein phosphorylated at threonine-181 \((\text{pT181})\), monoclonal antibody CP-13 (Dr. Peter Davies, Albert Einstein College of Medicine, Manhasset, NY, USA) directed against tau protein phosphorylated at serine-202 \((\text{pS202})\), or monoclonal antibody Tau 5 (Dr. Lester Binder, Northwestern University Medical School, Chicago, IL, USA) directed at phosphorylation-independent tau protein (total tau). In order to compare the Tau 5 immunolabeling patterns between the experimental and control samples, triplicate densitometry measurements were conducted on each of the 3 tau isoform bands (maximum for each band) and summed. We used a commercial enzyme-linked immunosorbent assay (ELISA) kit to quantitate murine-specific tau protein phosphorylated at serine 199 (Invitrogen, Carlsbad, CA, USA). Frozen brain samples were homogenized in eight volumes of 5 M guanidine-HCl 50 mM Tris \((\text{pH} 8)\) followed by five passes in a glass teflon homogenizer. Homogenates were mixed for 3 hrs, diluted into PBS
containing protease inhibitors, and centrifuged for 20 min at 16,000 g. Supernatants were diluted and assayed in quadruplicate for phosphorylated tau according to the manufacturer’s instructions.

Statistical Analyses. Comparisons of axonal conduction velocity and LTP magnitude between sham-blast control mice and blast-exposed mice 14 and 28 days post-exposure were made using repeated-measures multi-factorial ANOVA with Bonferroni-Dunn post-hoc correction. Neurobehavioral assessment was conducted using an open-field test and Barnes maze (Med-Associates, St. Albans, VT). Longitudinal data were compared between blast-exposed mice and sham-blast controls using repeated measures ANOVA. Memory retrieval was evaluated by Student’s t-test for two-tailed data. Immunoblot densitometry and biochemical data were evaluated by two-tailed Student’s t-test. Levels of significance are indicated as follows: *, \( P < 0.05 \); **, \( P < 0.01 \); ***, \( P < 0.001 \). Statistical significance was pre-set at \( P < 0.05 \).

References


**Table S1. Summary of antibodies used in this study.**

<table>
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<tr>
<th>Protein Target</th>
<th>Antibody</th>
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<th>Type</th>
<th>Assay*</th>
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*IHC, immunohistochemistry; WB, western blot (protein immunoblot)*
Table S2. Murine blast neurotrauma model blast parameters.

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<th>Mean</th>
<th>SD</th>
<th>% RSD</th>
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<td>Rupture Pressure (kPag)</td>
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<td>3</td>
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<td>Incident Static Pressure (kPag)</td>
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<td>7</td>
<td>3</td>
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<td>Shock wave Velocity (Mach)</td>
<td>1.26</td>
<td>0.04</td>
<td>2</td>
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<tr>
<td>Blast Wind Velocity (m/s)</td>
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<td></td>
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<tr>
<td>Blast Wind Velocity (mph)</td>
<td>336*</td>
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*Calculated value based on empirically-determined pressure measurements.
<table>
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<th>Blast Parameter</th>
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<th>ConWep²</th>
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<tr>
<td>Blast System</td>
<td>Compressed Gas Shock Tube</td>
<td>ConWep Algorithm</td>
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<td>Blast Wave Initiation</td>
<td>Burst Pressure</td>
<td>C4 Explosive</td>
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<tr>
<td>TNT Equivalence (kg)</td>
<td>303 kPag</td>
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<td>Distance from Source (m)</td>
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<td>Static Incident Pressure (kPag)</td>
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<td>77</td>
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<tr>
<td>Positive Phase Duration (ms)</td>
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<tr>
<td>Incident Impulse (kPag•ms)</td>
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<td>112</td>
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<tr>
<td>Shockwave Velocity (Mach)</td>
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<tr>
<td>Peak Particle Velocity (m/s)</td>
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<td>Peak Blast Wind Velocity (mph)</td>
<td>336</td>
<td>321</td>
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¹ Blast is comparable to a commonly encountered improvised explosive device (IED) constructed of a 120 mm mortar round with blast equivalence of 4.53 kg of TNT (http://www.gwu.edu/~nsarchiv/IMG/soldiershandbookiraq.pdf).

Fig. S1. Phosphorylated tau axonopathy in a single axon from the brain of a 22-year-old male military veteran with exposure to a single improvised explosive device blast and persistent blast-related traumatic brain injury symptoms. Micrographic montage demonstrating a CP13-immunoreactive axon with beaded (black arrows) and lentiform (white arrows) varicosities along a ~4 cm length in the external capsule. Calibration bar, 50 µm. Clinical details can be found in Results (Case 3).
Fig. S2. Absence of CTE neuropathology in a representative postmortem human brain from 21-year-old male control subject without known history of blast exposure or concussive injury. (A) Absence of specific CP-13 immunostaining for phosphorylated tau protein (pS202/pT205) in the dorsolateral prefrontal cortex. Magnification, X20. (B) Absence of specific AT8 immunostaining for phosphorylated tau protein (pS202/pT205) in the dorsolateral prefrontal cortex. Magnification, X10. (C) Absence of specific LN3 immunostaining for MHC class II-positive microglia in the subcortical frontal white matter. Magnification, X10. Sections were counterstained with cresyl violet.
Fig. S3. Schematic and geometry of the murine blast neurotrauma shock tube system. 
(A) Schematic of the purpose-designed shock tube blast neurotrauma system used in this study. Pressurized gas is delivered into the closed system of the pre-burst compression chamber. Abrupt rupture of a mylar membrane diaphragm separating the compression and expansion chambers initiates a blast shock wave front that traverses the long axis of the 4.5 m shock tube at supersonic velocity (Mach 1.26 ± 0.04; see Supplemental Table S2). (B) Geometry of blast-induced head motion. Anesthetized mice were secured in a thoracic-protective restraint system positioned inside the shock tube exactly 0.56 m from the open exit of the expansion chamber. High-speed videography enabled precise tracking of a single point on the head in the indicated projected planes of motion. The projected path and kinematics of the head during blast exposure was determined from frame-capture images at a capture rate of 100,000 fps. To translate from the recorded projected head rotation path (X,Y), a motion radius (R) was determined using a pivot point between the scapulae and an endpoint at the snout. The rotational angle of the head (θ) was calculated trigonometrically. (C) Murine blast neurotrauma system was developed in collaboration with the Fraunhofer Center for Manufacturing Innovation at Boston University, Brookline, MA, and operated at the Neurotrauma Laboratory, Boston University School of Medicine, Boston, MA.
Fig. S4. Reproducibility of shock tube blast static and reflected pressure.

(A) Reproducibility of shock tube blast wave pressure waveforms assessed with pressure transducer positioned in the reflected (face-on) orientation relative to the direction of the oncoming shock wave. (B) Same shock tube blast waves assessed with pressure transducer positioned in the incident static (side-on) orientation. Note that the static component does not capture dynamic pressure associated with particle motion. The signal at 30 ms (arrow) detected in both orientations was identified as a small reflected wave originating outside the shock tube. Peak pressure was determined by linearly extrapolating the decay of the curve to shock arrival time. Note that the initial pressure spike represents an artifact associated with diffraction at the pressure transducer. In the case shown, the peak static overpressure was 80 kPag with a diffraction artifact spike ~120 kPag. Pressure data was processed with 20 kHz low-pass filtering.
Fig. S5. Peak reflected and static incident pressure as a function of shock tube burst pressure. Reflected (face-on) and static incident (side-on) pressure demonstrate linear proportionality (i.e., peak pressure as a function of rupture pressure) over ranges relevant to human blast neurotrauma.
Fig. S6. Shock wave velocity (Mach) regression analysis.
Arrival time of the shock wave as a function of the position of the static (side-on) free-field pressure transducer in the shock tube. The pressure transducer was flush mounted inside the shock tube. The slope of the linear regression was 2.32 µs/mm ($R^2 = 1.00$). The corresponding shock wave velocity yielded a calculated Mach number of 1.26.
Fig. S7. X-T wave diagram demonstrating positional and temporal features of the blast shock wave. Blast shock wave front (blue line), shock wave tail (red line), and release wave corresponding to the trailing edge of the compression phase (green line) were calculated according to gas dynamic equations (Liepman & Roshko, *Elements of Gas Dynamics*, Wiley & Sons, New York, 1957). Interactions between counter-propagating waves in the compression section have been ignored. Wave transmission is shown from the blast origin (x = 0) at the interface between the compression and expansion chambers of the shock tube. Mice were positioned 0.56 m from the open exit of the shock tube. Note that near the exit of the shock tube, the release wave has almost caught up with the shock wavefront in agreement with measured waveform at a distance of 4.06 m. The predicted waveform is based on theoretical considerations and the timing of the shock wave at 4.06 m. These data are in good agreement with the amplitude, duration, impulse, and shape of the blast waveform measured experimentally (Fig. 2).
Fig. S8. Unperfused C57BL/6 mouse brain 2 weeks after single shock tube blast exposure. Representative unperfused brain from adult male wildtype C57BL/6 mice sacrificed two weeks after exposure to a single shock tube blast did not exhibit gross brain pathology, contusion, necrosis, hematoma, petechial hemorrhage, or focal tissue damage. Dorsal (A), ventral (B), and lateral (C) surfaces of a representative freshly dissected unperfused brain.
Fig. S9. Neuropathology in the CA3 field and dentate gyrus in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.

(A) Semi-thick sections of the hippocampus in a C57BL/6 mouse brain two weeks after control exposure to sham blast. Normal histological structure in the hippocampal CA1 and CA3 fields and dentate gyrus. (B,C) Toluidine blue-stained semi-thick section of the hippocampus and dentate gyrus in a C57BL/6 mouse brain two weeks after exposure to a single shock tube blast. In addition to neuropathology in the CA1 field (Fig. 4), the CA3 field and dentate gyrus also demonstrate evidence of extensive neuronal damage, including local neuronal pyknosis (black arrows, B,C), chromatolysis (white arrows, B,C) and dropout (asterisk, C).
Fig. S10. Decreased choline acetyltransferase (ChAT) immunoreactivity in the brainstem and neuronal dropout in the cerebellum of C57BL/6 mice 2 weeks after exposure to a single shock tube blast. (A, B) Luxol fast blue/hematoxylin and eosin staining shows cervical spinal cords well-populated with intact motor neurons (arrows) in mice exposed to sham blast (A) or single blast (B). (C, E) Immunohistochemical staining for ChAT in sham blast mice shows robust staining of motor neurons of the cervical spinal cord (C) as well as motor neurons in the nucleus of cranial nerve XII (E). (D, F) In contrast, ChAT immunostaining is markedly decreased in cervical spinal cord (D) and CN XII motor neurons (F) two weeks after single blast exposure. (G) Bielschowsky silver stain reveals intact cerebellar Purkinje cells (arrows, inset) associated with basket cell axons in sham blast mice. (H) Focal loss of cerebellar Purkinje cells and presence of empty baskets (asterisk, inset) in blast-exposed mice. Bar, 100 μm.
Fig. S11. Electron micrographic montage of the hippocampus CA1 field in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.

EM montage of the CA1 field *stratum radiatum* shows an enlarged field of the same perivascular profile presented in Fig. 4L. A pale, hydropic astrocyte (A), astrocytic process (Ap), and pathologically swollen astrocytic end-feet (Af) in the vicinity of an irregularly shaped capillary (cap) with a thickened, tortuous basal lamina (black arrows). An endothelial cell (E) with an abnormally contoured multilobed nucleus is located near a perivascular pericyte (P). A process containing lipofuscin granules (lf) is also evident. Bar, 2 µm.
Fig. S12. High-magnification electron micrographs of the hippocampus CA1 field in a C57BL/6 mouse brain 2 weeks after single-blast exposure. These EM micrographs show selected enlarged fields of the same hydropic perivascular profile presented in Fig. 4L and fig. S10. (A) Hydropic perivascular region of the CA1 field demonstrating an edematous astrocytic process (Ap) surrounding an irregularly shaped capillary (cap). An abnormal endothelial cell (E) with a multilobed nucleus is located near a pericyte (P). Lipofuscin granules (lf) are also evident. Black box corresponds to high-magnification micrograph in (B). White box corresponds to high-magnification micrograph in (C). Bar, 2 µm. (B) High-magnification EM micrograph showing lipofuscin granules (lf) and degenerating mitochondria (numbered 1 to 5). A capillary (cap) with a grossly thickened, tortuous basal lamina (black arrows) and adjacent pericyte (P) are also evident. Bar, 500 nm. (C) High-magnification EM micrograph showing a perivascular astrocytic process (Ap), abnormal mitochondria (numbers 1-6), and lipofuscin granules (lf). A grossly thickened basal lamina (black arrow) is also evident. Bar, 500 nm.
Fig. S13. Perivascular ultrastructural pathology in the hippocampus CA1 stratum radiatum in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast. Perivascular astrocyte (A) with edematous end-feet (Af) containing numerous dilated vacuoles (vac). Note the endothelial cell (E) with an irregularly contoured nucleus and grossly thickened basal lamina (black arrows). The capillary lumen is not patent ("string vessel"). Bar, 2 µm.
Fig. S14. Perivascular ultrastructural pathology in the hippocampus CA1 stratum radiatum in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast. A swollen astrocytic end-foot (Af) surrounds an endothelial cell (E) with an irregularly contoured nucleus and adjacent pericyte (P). A thickened basal lamina (arrows) and electron-dense inclusion granule (i) are also evident. Dysmorphic myelinated axons (asterisks) are present in the surrounding neuropil (asterisks). Bar, 2 µm.
Fig. S15. Perivascular ultrastructural pathology in the hippocampus CA1 stratum radiatum in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast. Hydropic astrocytic end-foot (Af) containing numerous vacuoles (vac) and a swollen mitochondrion (m) is associated with a thickened, tortuous basal lamina (black arrows) of an adjacent capillary (cap). Two dendritic spines (d), a dystrophic myelinated axon (white asterisk), and a tight junction (white arrowhead) are also evident in this micrograph. Bar, 500 nm.
Fig. S16. Perivascular ultrastructural pathology in the hippocampus CA1 stratum radiatum in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast. Hydropic astrocytic end-feet (Af) surrounding a pericyte (P), endothelial cell (E), and thickened capillary basal lamina (white arrow). Note that the capillary lumen is not patent, an ultrastructural feature that corresponds to string vessels observable by conventional light microscopy. A dystrophic myelinated axon (asterisk) is also evident. Bar, 2 μm.
Fig. S17. Myelin figure in the hippocampus CA1 stratum pyramidal in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast. An edematous astrocytic end-foot (Af) with swollen mitochondria (1-5) and a myelin figure (asterisk). Note the abnormally thickened basal lamina (black arrows) of the adjacent capillary (cap). Bar, 500 nm.
Fig. S18. A microglial cell amidst myelinated axons in the hippocampus CA1 stratum alveus in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast. A microglial cell (M) is present a field of myelinated nerve fibers in the hippocampus of a blast-exposed mouse. Note the electron dense nucleus and dark cytoplasm that are characteristic features of microglial cells. Bar, 500 nm.
Fig. S19. Autophagy and mitophagy in the hippocampus CA1 field in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast.

(A) Presumptive degenerating myelinated nerve fiber (black asterisk) in an astrocytic process in the hippocampal stratum alveus. Bar, 500 nm. (B) Astrocytic processes with presumptive multilamellar body (black asterisk), an autophagosomic vesicle variant. Numerous degenerating mitochondria are also evident in this profile (1-6). Bar, 500 nm. (C) Perivascular astrocyte in the stratum pyramidale exhibiting a hydropic process (Ap) with numerous vacuoles (vac) and swollen mitochondria (1,2). Note the lumen of a nearby capillary (cap). Bar, 500 nm.
Fig. S20. Degenerating (“dark”) pyramidal neurons in the hippocampus CA1 stratum pyramidale in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast. (A) “Dark” neurons (N₁, N₂) and adjacent capillary (cap) and endothelial cell (E) in a blast-exposed mouse hippocampus (Fig. 4J). Black box outlines enlarged region in (B) below. (B) Degenerating neurons (N₁, N₂) with electron-dense (“dark”) cytoplasm and irregularly shaped nuclear envelopes (white arrows). A nearby capillary (cap) and endothelial cell (E) are surrounded by grossly swollen astrocytic end-feet (Af) containing dilated vacuoles (vac). A normal-appearing neuron (N₃) is present in this micrograph. Bar, 2 μm.
Fig. S21. Degenerating (dark) pyramidal neurons in the hippocampus CA1 stratum pyramidale in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast. Degenerating pyramidal neuron (Nx) is characteristically electron-dense ("dark") and exhibits a convoluted nuclear envelope (white arrows). Vacuoles (vac) and degenerating mitochondria (numbers 1-4) are also present. An adjacent hydropic astrocytic process (Ap) is also evident in this micrograph. Bar, 500 nm.
Fig. S22. Degenerating (dark) pyramidal neurons in the hippocampus CA1 stratum pyramidale in a C57BL/6 mouse brain 2 weeks after exposure to a single shock tube blast. A degenerating pyramidal neuron (Nx) exhibits electron-dense (“dark”) cytoplasm and comparably electron-dense nucleus with an irregularly contoured nuclear envelope (white arrows). “Dark” neurons correspond to the pyknotic pyramidal neurons observed in adjacent toluidine blue-stained semi-thick section (Fig. 4H). Two neighboring pyramidal neurons (N1, N2) demonstrate relatively normal ultrastructure. Bar, 2 μm.
Fig. S23. Electrode placements for axonal conduction velocity and synaptic plasticity experiments. (A) Schematic of the hippocampal slice preparation illustrating electrophysiological arrangement for evaluating axonal conduction velocity in the \textit{stratum alveus}, the hippocampal CA1 axonal output pathway. The positioning of a stimulating electrode and two recording electrodes in \textit{stratum alveus} of field CA1 are shown relative to local Schaffer collateral-CA1 synaptic circuitry. Recordings of compound action potentials from CA1 pyramidal neurons were used to calculate axonal conduction velocity in the \textit{stratum alveus}. The time difference between peak negativities at the two recording sites illustrated by each arrow in the CA1 axonal output pathway and distance between the electrodes was used to calculate conduction velocity. (B) Schematic of the hippocampal slice preparation illustrating positioning of stimulation and recording electrodes in \textit{stratum radiatum} of field CA1 to record Schaffer collateral-evoked field excitatory postsynaptic potentials (fEPSPs) to measure stimulus-evoked and chemically-evoked cAMP-dependent long-term potentiation (LTP) of Schaffer collateral-CA1 synaptic transmission. See Methods for details.
Fig. S24. Schaffer collateral-CA1 synaptic input-output relations illustrating the absence of long-term effects of blast exposure on baseline synaptic transmission. Hippocampal slices were prepared from mice exposed to a single blast (○) compared to control sham-blast (●) four weeks after experimental exposure. Normalized peak fEPSP slope amplitudes are plotted versus Schaffer collateral stimulus intensity. The curves demonstrate that a given intensity of synaptic stimulation elicited the same magnitude response in hippocampal slices from blast-exposed mice compared to sham-blast controls.
Fig. S25. Blast-induced deficits in cAMP-induced long-term potentiation of synaptic transmission at Schaffer collateral-CA1 synapses are bilateral and persistent. (A) Time course of cyclic AMP-induced LTP evoked by bath application of the adenylate cyclase activator forskolin (50μM) plus the type II phosphodiesterase inhibitor rolipram (10μM) (FOR+ROL; solid bar) in hippocampal slices from the right hemisphere from mice exposed to a single shock tube blast two weeks (■, n=6) or four weeks (●, n=9) before sacrifice compared to sham-blast control mice (○, n=10). (B) Time course of cyclic AMP-induced LTP evoked by bath application of the adenylate cyclase activator forskolin (50μM) plus the type II phosphodiesterase inhibitor rolipram (10μM) (FOR+ROL; solid bar) in hippocampal slices from the left hemisphere from mice exposed to a single sublethal blast two weeks (■, n=6) or four weeks (●, n=6) before sacrifice compared to sham-blast control mice (○, n=9). Each fEPSP point = mean ± S.E.M..
Inertial Force Oscillation

Bobblehead effect

Blast Wind Oscillation

Inertial Force Oscillation

coup-contrecoup injury

Physical Impact

Inertial Force Oscillation

coup-contrecoup injury

Angular Acceleration

Elastic Recoil

Fig. S26. Model of blast- and concussion-related TBI and sequelae, including CTE.

Goldstein LE, et al., Supplemental Material
Video S1. Mouse head kinematics during exposure to a single shock tube blast.
Single frame from high-speed videographic kinetograph shows the parametric plot of nose position (top left) during blast exposure as a function of time. Nose position was measured in two directions in which the x-axis is parallel to the axis of the shock tube and the y-axis is perpendicular to ground. High-speed videographic record of the blast pressure waveform (bottom left) shows a plot of the coincident free-field pressure dynamics as a function of time. On the right, the radial kinematics, position, velocity and acceleration of blast-induced head movement in both the horizontal (blue) and sagittal (red) planes are shown as a function of time. Static pressure data was processed with 2 kHz low-pass filtering. Angular position data was processed with 500 Hz low-pass filtering. The complete Supplemental Video S1 is available as a downloadable avi-formatted video file.
APOEε4 Status and Traumatic Brain Injury on the Gridiron or the Battlefield

A FEW WEEKS AGO, FORMER SAN DIEGO CHARGER JUNIOR SEAU DIED OF AN APPARENTLY SELF-INFLICTED GUNSHOT WOUND TO THE CHEST. This news immediately brought to mind a similar suicide in February, 2011—that of former Chicago Bears football player Dave Duerson, who left a note indicating that he had chosen a method of suicide that preserved his brain so it could be examined for signs of chronic traumatic encephalopathy (CTE). In CTE, the brain contains deposits consisting of neurofibrillary tangles of aberrantly phosphorylated tau protein similar to those found in the brains of Alzheimer’s disease (AD) patients, and appears identical to the pathology of boxers, previously known as dementia pugilistica. As of this writing, we don’t yet know whether Seau’s brain shows CTE, but there is accumulating evidence that traumatic brain injury (TBI) from repeated concussions and sub-concussive injuries suffered in high-impact sports like American football, ice hockey, and professional wrestling can lead to CTE; the dementia is clinically somewhat similar to AD (1, 2). In 2010, a recent college graduate and collegiate football player committed suicide and proved to have CTE at autopsy. His was the youngest documented case of CTE, providing highly suggestive evidence that certain children and adolescents are at risk for developing CTE because of their exposure to high-impact sports. CTE has also been identified in military veterans of the Iraq and Afghanistan wars, a result apparently of their repeated exposure to blast injuries from improvised explosive devices (IEDs) (2, 3).

Some of the blame for dementia can also be placed on genetics. Carriers of one or two copies of a particular allele for apolipoprotein E (APOE) are more likely to get AD. Carrying a single APOE ε4 allele increases by three-fold a person’s relative risk for developing AD in late life. Two APOE ε4 alleles increase the relative risk for AD more than 10-fold. In fact, 50% of late-onset AD patients have at least one APOE ε4 allele. Based on our own experience studying CTE-like pathology arising from TBI in boxers with dementia (4), we predict that the risk for neurological disability from sports-associated and blast-associated CTE is also increased by the presence of one or two alleles of APOE ε4. Indeed, when a person with AD heterozygous for the APOE ε4 allele suffers TBI, the effect on relative risk for dementia is synergistic and increases by 10-fold or more (5).

The idea that potentially avoidable repetitive TBI could contribute to the eventual development of dementia is an unsettling thought for physicians—and for some in the media. A recent public debate (“Ban Football Now!” Intelligence Debates) and an article in a January New Yorker (“Does Football Have a Future?”) speculate about the eventual demise of football, in part because of TBI risks. Nonetheless, there has been little discussion about possible public health benefits of APOE genotyping of high school athletes who intend to participate in impact sports, or of prospective military personnel. Is APOE genotyping feasible and could it help to avert dementia in susceptible persons exposed to TBI through sports or battlefield service?

To help address this question, we performed an informal email poll of experts in AD, TBI, and CTE to estimate their attitudes and practices. Forty-five (95%) responded, voting 2:1 against using our current knowledge of APOE genotyping to guide decisions about permitting adolescents to play high school or college sports. Interestingly, the response was an even stronger 3:1 “nay” vote when it came to the prospect of genotyping prospective armed services enlistees. None of the experts polled had ever used this information in their own lives. However, nearly half said that they would use APOE genotyping in making sports play decisions for their own children if there had been incidental documentation of APOE ε4 alleles in the family (for example, if a grandparent with AD was known to be an APOE ε4 carrier). We noted with interest this dissonance between willingness to use APOE genotype information that was determined for other reasons but reluctance toward APOE genotyping explicitly for making a sports/military exposure decision.
The consensus was clear: Most of the experts we consulted believe that it is premature to introduce APOE genotyping into schools or the military now. They generally feel that we need much better data on which to base a decision. But what type of information would allow us to evaluate properly the utility of APOE ε4 status for decisions about exposing children, adolescents, or adults to the risks of TBI in sports or in the military?

We know that APOEε4 alleles are overrepresented among boxers coming to medical attention because of clinical evidence of CTE (4). However, we lack several key pieces of quantitative information upon which to base recommendations to young athletes or military personnel. First, since there is no systematic registry, screening, or genotyping of at-risk individuals, we cannot estimate the prevalence of CTE, the proportion of CTE patients who have APOEε4 alleles, or the relative risk of sports or military exposure to carriers and non-carriers of APOEε4 alleles. Most existing data on APOE ε4 and dementia risk are from cross-sectional studies of mid- to late-life AD cohorts and age-equivalent healthy controls, and so these results cannot be assumed to apply to the subjects at risk for CTE, whose exposure may begin during adolescence. Also, for CTE, we do not know the natural course of the disease. The variable but usually long latency between initial exposure to TBI and the appearance of clinical dementia is a major challenge in establishing the etiologies of CTE. Second, we have no prospective, population-based natural history data that would reveal whether APOE genotyping at an early age is predictive of the eventual development of CTE. Such studies would need to assess subject ethnicity because APOE ε4-mediated relative risk for AD in African Americans and Hispanics may be somewhat attenuated, and this probably applies to CTE as well.

One approach to collecting the necessary data would be to set up a network of TBI/CTE Research Centers modeled on the national network of AD Research Centers with brain banks, clinical, neuropathological, educational, and data programs, as well as basic and clinical research projects. This would enable a critical experiment: A cohort of subjects would be recruited, including high-risk adolescents exposed to sports TBI and representing all three APOE genotypes (ε2, ε3 and ε4). Various ethnicities would be represented across all APOE genotypes, since, as mentioned above, African and Hispanic heritage can modify the relative risk of APOE ε4 alleles. Subjects would maintain lifelong concussion diaries, and, to the extent available, they would wear electronic monitoring “smart helmets” to estimate the forces, vectors, and torque acting upon their brains. These measures, taking into account genetic susceptibility together with the nature, severity, and number of TBIs, and in the context of surveillance for clinical and biomarker evidence for neurodegeneration that might herald CTE, could form the basis for predictive mathematical models that could then be tested for their reliability in identifying those at highest risk for CTE. The length of the latency period between TBI and onset of CTE is measured in decades, so the acquisition of these data will constitute a large, long, and expensive effort. If APOE ε4 allele carrier athletes are found to be more susceptible to CTE after a typical course of sports-inflicted TBI, we will want to seriously consider APOE genotyping of prospective soldiers or athletes. Knowledge of their child’s APOE genotype may change the decision made by parents to sign the informed consent and release required for their children to register for high impact sports.

There are several cohorts already being assembled that might provide some relevant prospective longitudinal data on TBI. For example, the proposed National Institute of Child Health and Development Vanguard Study will document the major life events and environmental exposures of 100,000 children until their 21st birthdays. Chronicling the head and sports injuries of these children would yield valuable prospective, longitudinal data. Of interest also would be evaluation and follow-up of members of the National Football League Hall of Fame or prospective studies of professional football players. TBI-related questions are already being integrated into the U.S. Department of Defense-Alzheimer’s Disease Neuroimaging Initiative (DoD-ADNI). Under this new initiative, military personnel and veterans at high risk for exposure to IEDs will provide DNA for genetic research (including APOE genotyping) and will undergo serial assessments of cognitive status, brain structure, and a panel of imaging and body fluid biomarkers.

Ethical and psychological issues complicate any solution to the problem. There is the very real concern about the effect of genotype information on family members and on personal employability and insurability. Although, the federal Genetic Information Nondisclosure Act (GINA) and state-level efforts were established to shield patients from discrimination according to genetic information, GINA has not been tested in court. In practice,
GINA only protects if genotype information is kept separate from the main medical record, which may not happen routinely. In addition, recruiting high school–age subjects for APOE genotyping and follow-up could be controversial as parental informed consent would be required, necessitating pre-test genetic counseling for adolescents and their parents. Although APOE genotypes would not be disclosed during the study, the purposes of the study would need to be explained carefully during the informed consent process. In the event that unintentional disclosures were to occur, this counseling would provide families with a firm basis for understanding the potential consequences.

In spite of the obstacles to considering APOE genotyping for high schools and the military, it is, without doubt, a worthwhile challenge. In the United States alone, there are an estimated 1.1 million high school football players. The prevalence of APOE ε4 homozygosity and heterozygosity (in Caucasian populations) are 2% and 20%, respectively. Therefore, the APOE genotyping issues discussed here apply to somewhere between 22,000 and 220,000 athletes. The RAND Corporation estimates at 285,000 the total number of Iraq and Afghanistan veterans with TBI diagnoses. Adding to the emotional cost to the patient and family of years of slow cognitive decline, culminating in profound dementia and death, each person with dementia represents an approximately $1 million expense to his or her family and to insurers. CTE and AD are likely to be preventable illnesses, but serious prospects for meaningful prophylaxis are at least a decade away. Half of the population over age 85 is demented now; by 2050, the annual cost of caring for dementia patients will exceed $1 trillion. Therefore, if lifestyle modifications for APOE ε4 carriers—such as avoiding high-impact sports or opting for military careers that do not put the brain at risk—can reduce dementia prevalence in 2050 by even 1%, we would gain an annual savings of $10 billion in costs of care—and immeasurable savings in terms of human suffering.

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