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Review

Traumatic Brain Injury and the Neuronal Microenvironment: A Potential Role for Neuropathological Mechanotransduction

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Traumatic brain injury (TBI) is linked to several pathologies for which there is a lack of understanding of disease mechanisms and therapeutic strategies. To elucidate injury mechanisms, it is important to consider how physical forces are transmitted and transduced across all spatial scales of the brain. Although the mechanical response of the brain is typically characterized by its material properties and biological structure, cellular mechanotransduction mechanisms also exist. Such mechanisms can affect physiological processes by responding to exogenous mechanical forces directed through sub-cellular components, such as extracellular matrix and cell adhesion molecules, to mechanosensitive intracellular structures that regulate mechanochemical signaling pathways. We suggest that cellular mechanotransduction may be an important mechanism underlying the initiation of cell and sub-cellular injuries ultimately responsible for the diffuse pathological damage and clinical symptoms observed in TBI, thereby providing potential therapeutic opportunities not previously explored in TBI.

Introduction

A blow to the head may potentiate traumatic brain injury (TBI), estimated to affect 1.7 million people annually in the United States (Faul et al., 2010) and 235 per 100,000 people annually in Europe (Tagliaferri et al., 2006). TBI is categorized as mild, moderate, or severe based on clinical symptoms often in combination with imaging and postmortem histology. Pathological lesions consisting of contusion, hemorrhage, and edema are traditional clinical indicators of head injury. As such, traumatic cell death in regions directly adjacent to sites of trauma is often viewed as the primary neurological damage (reviewed by Johnson et al., 2013).

In addition to cell death, a complex array of microscale pathologies including diffuse axonal injury (DAI), microvascular damage, and diffuse neuronal injury can also develop throughout the brain parenchyma following trauma and contribute to the ensuing morbidity. Clinical symptoms linked to these injuries include loss of consciousness, dizziness, and headache, as well as deficits in attention, memory, and motor skills (reviewed by Alexander, 1995). Even though clinical symptoms may improve with time, the microscale damage may persist and potentially contribute to an increased likelihood of future neurodegenerative disease (reviewed by Johnson et al., 2013; McKee et al., 2009). Although the risk associated with diffuse damage is now recognized, a comprehensive understanding of the mechanical and biological processes that initiate this injury remains elusive.

Difficulties in identifying the events that initiate diffuse pathologies are due in part to the complexity of characterizing forces distributed through structures ranging in size from sub-cellular microcompartments to the brain (reviewed by Cloots et al., 2013). Understanding the biomechanical events that initiate brain injuries has typically relied on determining how neural tissue responds to rapidly applied loads through both mathematical modeling and experimental techniques (reviewed by Cernak and Noble-Haeusslein, 2010). In order to understand disease pathogenesis, there has been a tendency to focus on forces that disrupt the integrity of the networked architecture of the brain (Kilinc et al., 2008; Tang-Schomer et al., 2010). However, attempts to establish a causal link between a mechanical insult and the ensuing diffuse pathology observed in TBI has been met with limited success.

In this review, we will briefly discuss the diffuse neuropathological damage associated with TBI and then focus on the multi-scale biomechanics that may explain diffuse patterns of injury in the brain microenvironment. The importance of cellular mechanotransduction, or the ability of cells to convert mechanical forces into biological signals, has been widely established in many organs, tissues, and cells across multiple species (reviewed by Ingber, 2003a). Cell-cell and cell-matrix interactions have been shown to influence both physiological and pathophysiological processes (reviewed by Ingber, 2003b), suggesting that they play an important role in brain injury and remodeling after TBI. We argue that to improve the mechanistic understanding of diffuse brain damage, it may be necessary to consider how mechanical forces below the threshold for mechanical failure influence cellular physiology in the brain.

Neuropathology and Biomechanics of TBI

Assessing and Diagnosing Brain Injury. Diagnosis of TBI typically relies on a neurological assessment followed by imaging to detect pathology. Povlishock and Katz suggested a distinct classification between focal and diffuse injuries (Figure 1A) predicated upon both clinical and basic science perspectives, with the acknowledgment of some overlap in pathobiology and

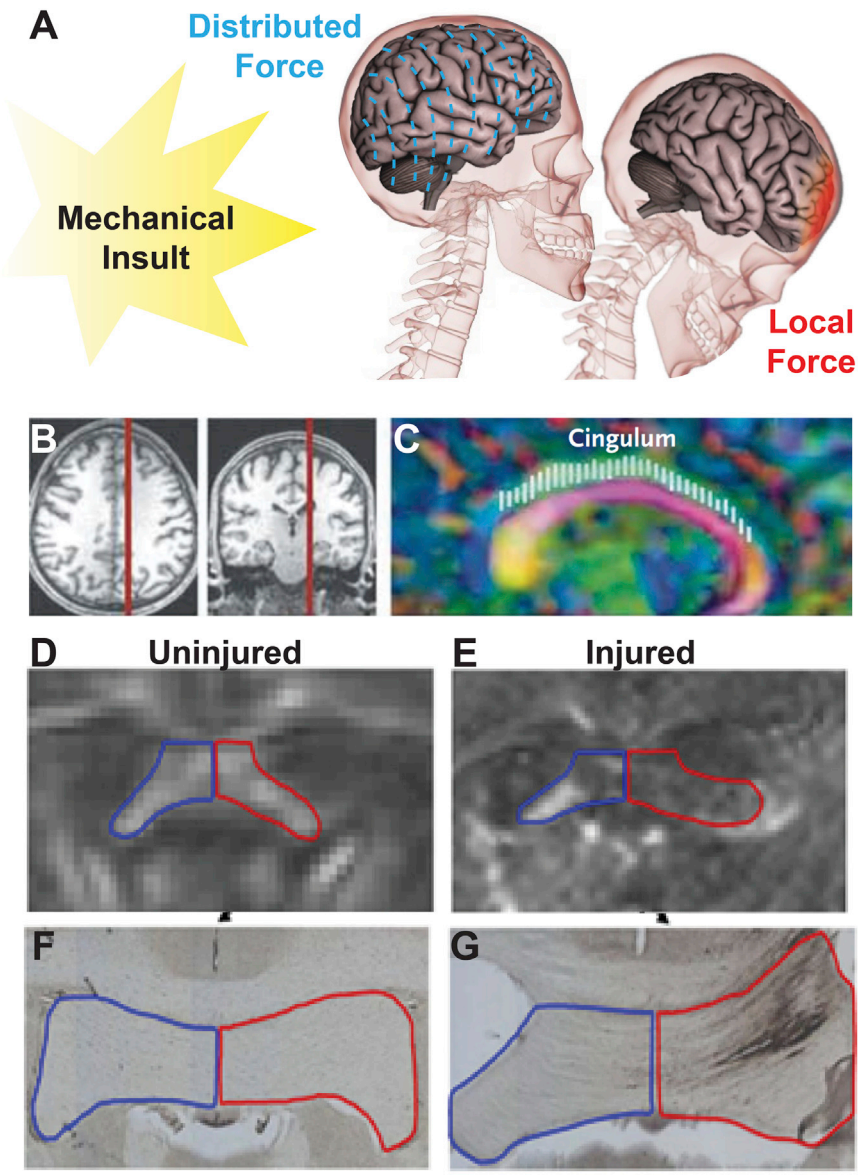

Figure 1. Traumatic Mechanical Loading Damages the Brain

(A) A mechanical insult to the head can generate both localized and distributed forces throughout the brain. Localized forces are typically associated with deformation due to impact of the brain against the skull. Distributed forces are associated with inertial effects due to rapid acceleration or increased pressure transients caused by explosive blast.

(B) MRI has been utilized to generate images of the brain that describe the gross anatomy and which sometimes indicate lesions associated with mechanical trauma.

(C) DTI, a type of MRI, has proven especially useful in identifying diffuse pathology associated with mild trauma that is not indicated by conventional MRI. The extent of damage is quantified by changes in relative anisotropy of diffusion, which have been shown predominately in large axonal bundles. Red, green, and blue indicate the principal directions of diffusion, with red denoting right to left, green anterior to posterior, and blue dorsal to ventral. The anatomical location is indicated by the red line in (B).

(D and E) Experimental models of trauma comparing changes in relative anisotropy in both (D) uninjured and (E) injured brain regions illustrate the effectiveness of DTI in identifying tissue damage. In this example, an overall decrease in relative anisotropy is indicated in the injured tissue by a decrease in the grayscale image intensity.

(F) A lack of change in relative anisotropy in uninjured brain regions was shown to correlate with (F) a lack of detectible pathology using immunohistochemistry.

(G) In contrast, changes in relative anisotropy in injured brain regions did correlate with (G) positively stained regions (dark areas) of amyloid precursor protein (evidence of axonal injury), indicating that DTI can detect TBI related pathology in patients.

(B) and (C) are adapted from Mac Donald et al. (2011). (D) and (G) are adapted from Mac Donald et al. (2007) with permission.

clinical outcome (reviewed by Povlishock and Katz, 2005). Focal injuries typically present contusion and hemorrhage located near the site of impact. Diffuse injuries exhibit complex distributions comprised of diffuse neuronal injury, petechial white matter hemorrhage, and DAIs that occur at multiple sites throughout the brain. While pathological lesions consisting of contusion and hematoma formation associated with focal injures are readily distinguishable by computerized tomography (CT) and traditional magnetic resonance imaging (MRI) scans, DAI and other subtle hemorrhages are not. For example, abnormalities were detected by CT scan in only 5%–30% of patients exhibiting clinical symptoms consistent with mild TBI (mTBI) (Borg et al., 2004), and the extent of pathology detected by CT scan and MRI did not correlate with the clinical outcomes (Lee et al., 2008). Therefore, identifying diffuse pathology following head injury has historically relied on postmortem histological analysis. DAI is readily detected in histological slices by the presence of punctate, swollen axons (Strich, 1956). While the existence of this hallmark morphology of DAI has long been known, the inability to measure the extent of diffuse pathology in patients demonstrates a deficiency in diagnostic methods that may contribute to the debate about the etiology of TBI. Subsequent studies further correlated reductions in diffusion anisotropy with neuropsychological dysfunction in longitudinal studies (Bendlin et al., 2008) while also showing that diffusion tensor imaging (DTI) abnormalities can be used to predict cognitive outcome (Gu et al., 2013). DTI measures the diffusion of water molecules, which is influenced by biological structures such as axons. Therefore, DTI can detect white matter tracts because diffusion occurs faster along the predominant fiber direction and more slowly in perpendicular directions. The anisotropy of water diffusion has been shown to be reduced after axonal injury, (Figures 1B and 1C), which was later shown to correlate with regions of axonal damage from histological analysis (Figures 1D–1G) (Mac Donald et al., 2007). While

incorporation of DTI as a diagnostic technique has improved the ability to identify some forms of diffuse brain damage, many aspects of the underlying pathology and pathophysiology remain unclear.

Regional Pathology Associated with Diffuse Injury. While traumatic cell death attributed to focal injuries was for many years viewed as the primary contributor to morbidity following TBI, it is now understood that pathophysiological conditions may ensue even without widespread cell death (Farkas and Povlishock, 2007). As such, we will briefly cover a few findings that illustrate the complexity of injury distribution at the cellular level and direct the reader to published reports on the complex pathophysiological processes that are known to occur following TBI.

The distribution of injury within the brain following TBI is complex. Diffuse patterns of metabolic change throughout the brains of human patients have been detected following trauma using positron emission tomography (PET) (Bergsneider et al., 2000). These abnormal activity patterns have been shown to initiate immediately and persist up to weeks following the initial insult. Furthermore, these changes were identified in patients diagnosed with mTBI based on neurological assessment where neither focal pathology nor, in some cases, diffuse pathology was detected.

Glutamate release combined with a loss of ionic homeostasis may manifest in mitochondrial dysfunction (reviewed by Giza and Hovda, 2001) as well as other general cellular dysfunctions. For example, an acute phase of neuroexcitation was shown to occur in the brain immediately following impact (Alessandri et al., 1999; Yoshino et al., 1992). This event has been linked to the indiscriminant release of neurotransmitters, such as glutamate, that activate excitatory neurons (Alessandri et al., 1999; Yoshino et al., 1992). Furthermore, progressive degeneration linked to this energy crisis may cause deafferentiation, or a loss of synaptic terminals (Erb and Povlishock, 1991), affecting regulation of neuronal activity patterns that are crucial to brain function. Interestingly, patients with mTBI were shown to exhibit decreased connectivity between brain regions, as measured by magnetoencephalography (Zouridakis et al., 2012), supporting the notion that diffuse damage may contribute significantly to the morbidity associated with TBI.

Identification of diffuse damage at multiple sites throughout the brain has revealed non-uniform distributions of injury, suggesting that certain tissue and cellular structures may be more vulnerable than others. Blumbergs et al. (1995) compared the extent of DAI occurring in multiple brain regions by analyzing histological slices from patients diagnosed with either mild or severe TBI. Following mTBI, no evidence of DAI was detected in the cerebellum, one half of the patients exhibited injury in the brainstem, and all patients exhibited injury in the cerebral hemispheres. Following severe TBI, patients exhibited evidence of DAI in all examined regions. White matter within the cerebral cortex and large axonal tracts within the cerebral hemispheres, such as the corpus callosum and fornices, were especially susceptible to injury. These vulnerabilities were further suggested by findings in patients in which MRI was used to detect a greater number of lesions located in the cerebral hemispheres compared to the brainstem, which also correlated with injury severity (Jenkins et al., 1986). A preferential decrease in diffusion anisotropy has been measured in structures such as the corpus callosum, internal capsule, and centrum semiovale (Inglese et al., 2005), confirming the susceptibility of large axonal bundles to damage. DAI lesions have also been shown to localize to the interface of cerebral white and gray matter (Smith et al., 1997). Therefore, diffuse damage appears to differentially affect tissue regions and structures within the brain.

Heterogeneity at the cellular level exists within damaged regions. Clusters of injured axons are observed at multiple locations distributed throughout the tissue commonly referred to as a multi-focal distribution (reviewed by Smith and Meaney, 2000). Furthermore, injured axons are typically dispersed among neighboring uninjured axons within these regions (Blumbergs et al., 1995), indicating differences in susceptibility among neighboring axons within a cluster. Variability in axonal vulnerability is also suggested by reports indicating differential injury responses in myelinated versus small-caliber, non-myelinated axons (Reeves et al., 2005). Even within myelinated axons, DAI has been shown to occur preferentially at the nodes of Ranvier (Reeves et al., 2010), the periodic regions of exposed axon between regions of myelination, further stressing the complexity of injury vulnerability.

Additional heterogeneity in injury distribution is observed in relation to the vasculature. Evidence of diffuse damage in the vasculature has been suggested by micro-hemorrhage of small vessels, which often occurs diffusely within the white matter similar to that of DAI (Blumbergs et al., 1995). Furthermore, tau-immunoreactive neurofibrillary and astrocytic tangles associated with chronic traumatic encephalopathy (CTE) have been shown to exhibit perivascular localization (McKee et al., 2009), suggesting a role for the vasculature in the injury process. In cases of severe blast TBI, constriction of large vessels, termed vasospasm, can occur within 48 hr of injury (Armonda et al., 2006; Ling et al., 2009). Interestingly, vasospasm is not typically associated with non-penetrating, diffuse TBI indicating that the vasculature itself may be more susceptible to mechanical trauma than previously understood.

Recent reports have shown that diffuse damage can occur even after mild insults with no previously identifiable neuropathology (McKee et al., 2013; Omalu et al., 2010; Stern et al., 2011). Concussion and repetitive sub-concussive impacts are now linked to progressive neurodegeneration diagnosed as CTE (McKee et al., 2013). This disease was first reported in boxing as *dementia pugilistica* where it was characterized clinically by declining mental capacity, lack of coordination, and behavioral problems (Corsellis et al., 1973). McKee et al. (2009) showed that professional athletes who suffered repetitive mTBI developed diffuse, microscale injury patterns characterized by postmortem identification of neurofibrillary and glial tangles, hyperphosphorylated tau, axonal degeneration, and immunoreactive microglia. Moreover, blast combat casualties from the wars in Afghanistan and Iraq exhibit multiple forms of tissue damage (Goldstein et al., 2012), including cerebral vasospasm (Armonda et al., 2006) and traumatic axonal injury (Mac Donald et al., 2011). The diversity of neuropathology combined with the heterogeneity in injury distribution suggests an extensive pathological remodeling of the cellular microenvironment of the brain, the causes of which need to be elucidated in order to prevent TBI. Given that

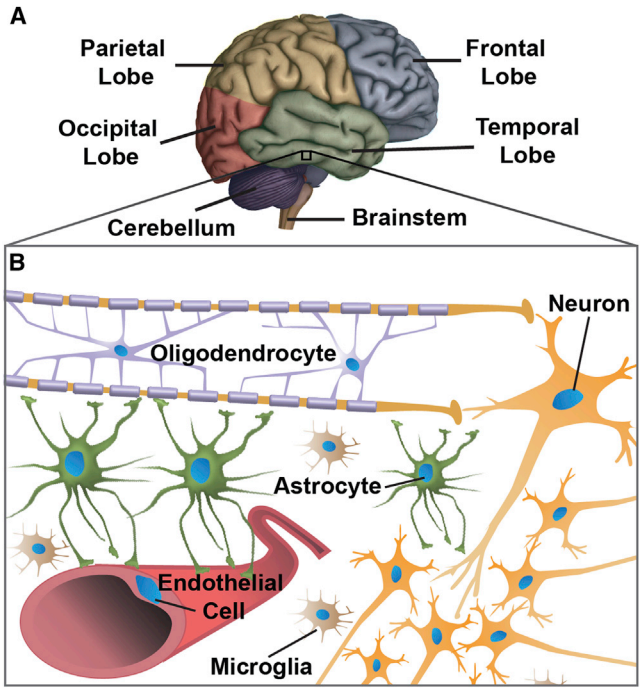

Figure 2. Structural Organization of the Brain
Understanding the occurrence of diffuse pathology at the cellular level is complicated by the intricate cell and tissue structures that span approximately four orders of magnitude in spatial scale to form the human brain.
(A) Large brain regions such as the cerebral hemispheres (comprised of four lobes), the cerebellum, and the brainstem are well defined by anatomical features at the mm (10^{-3} m) to cm (10^{-2} m) length scale.
(B) Within each tissue structure, there is an underlying multicellular composition comprised of neurons, glia, and vascular cells that vary in size at a spatial scale measured in μm (10^{-6} m). Many of these cells are highly polarized and together form intricate multi-cellular structures. Differences in material properties between brain regions are attributed to differences in cell population demographics, network architectures, and other micro-architectural attributes that facilitate functional uniqueness.

mechanical forces are a primary contributor to the etiology of TBI, it is reasonable to assume that understanding the biomechanics of injury may elucidate complex injury mechanisms and explain the diverse pathophysiology associated with TBI.

Multiscale Biomechanics and Mechanisms of Diffuse TBI

Head Trauma Causes Mechanical Loading of the Brain. Many studies have explored the ability of trauma caused by sudden impact, acceleration, and explosive blast to induce mechanical loading of the brain (Bauman et al., 2009; Ommaya et al., 1971, 2002). Localized forces are typically associated with impact injuries where the brain deforms against the skull, while distributed loading is typically attributed to both inertial forces due to rapid head acceleration (Ommaya et al., 2002) as well as pressure transients due to explosive blast waves (Bauman et al., 2009). Although these loading patterns are not mutually exclusive, in this review we will focus on the effects of diffuse loading patterns and the complexity of the cellular damage and injury mechanisms associated with them.

TBI biomechanics have been studied over many decades and can provide some explanation for diffuse injury distributions. Reports have identified important trauma characteristics such as impact (e.g., the head either strikes a stationary object or is struck by a moving object) versus impulsive (e.g., the head moves as a result of motion imparted to some other part of the body) loading (reviewed by Goldsmith and Plunkett, 2004) and translational versus rotational acceleration (reviewed by Holbourn, 1944; Ommaya et al., 2002) in generating injurious forces throughout the brain. Ommaya and Gennarelli (1974) posited that head rotation results in an injury distribution consistent with the degree of inertial forces generated by centripetal acceleration. This suggests that the severity of tissue damage is dependent upon the distance from the point of rotation, typically the neck. While purely mechanical explanations provide some insight into injury patterns, they currently cannot fully explain the subsequent distribution of diffuse tissue, cellular, and subcellular damage associated with TBI. This is likely due to the fact that several orders of spatial magnitude separate the relevant anatomies, suggesting a comprehensive understanding of TBI biomechanics will require analyses that link trauma forces at the level of the head to neuronal microcompartments.

Multiscale Composition and Material Properties of the Brain Complicate Injury Biomechanics. Biomechanics of TBI are complicated by the brain's variation in composition, structure, and range of material properties. Although the brain is a relatively soft tissue, it is encased by multiple layers of tissue and fluid that provide protection from mechanical forces against deformation. Tissue stiffness is described by the elastic modulus measured in units of Pascals (Pa = N/m^2), which is the resistance to deformation, measured by applying a force to a defined area of material and calculating the resulting change in length. These protective layers include the skin (~1.0 MPa), the skull (~8.0 GPa), the dura matter (31.5 MPa), the pia matter (11.5 MPa), and the cerebral spinal fluid (Zhang et al., 2001). Together, these layers provide a relatively rigid structure that protects the softer underlying brain tissue from normal environmental factors, such as mechanical forces.

The brain tissue has distinct regional anatomies such as the cerebral hemispheres, the cerebellum, and the brainstem (Figure 2A), which are historically defined by anatomical features and functional properties (Kandel et al., 2000). Early studies on whole human brain tissue reported differences in material properties with shear stiffness values between 0.6 and 1.1 kPa (Fallenstein et al., 1969). Subsequent studies highlighted the variation in material properties due to age, region, and sample preparation (Prange and Margulies, 2002), reporting stiffness values between 0.7–33 kPa (Thibault and Margulies, 1998). Together, these reports indicate that the brain is relatively soft and susceptible to deformation compared to other biological tissues. Moreover, whole brain tissue exhibits viscoelastic behavior, with loss modulus ranging between 2.8–81.4 kPa (Shuck and Advani, 1972). This indicates that the amount of deformation that will occur is dependent upon the rate at which a force is applied. A large variation in material properties of brain tissue is depicted by elasticity maps of rodent brains that illustrate regional variations in stiffness ranging between 2–25 kPa (Macé et al., 2011). Heterogeneity in material properties is important because it indicates that different regions may deform uniquely when experiencing the same mechanical loading.

Therefore, understanding force transmission through the brain requires knowledge of the microenvironments that give rise to its heterogeneous material properties.

The large variation in material properties between brain regions may be attributed to differences in cell population demographics, network architectures, and other micro-architectural attributes that facilitate functional uniqueness. The adult human brain consists of a diverse cell population of approximately 100 billion neurons and at least as many non-neuronal cells (Azevedo et al., 2009). Neuronal, glial, and vascular cells are the primary constituents of the brain parenchyma and together form intricate cellular and multicellular structures (Figure 2B). For example, while the typical neuronal cell body is 10–50 μm in diameter, neuronal processes extend up to 500 μm (dendrites) or several centimeters to a meter (axons) away while remaining only 0.2–20 μm in diameter (Alberts, 1994). Besides neurons, other cell types in the brain also exhibit polarized morphologies that form complex multi-cellular structures, such as oligodendrocytes; myelinating cells, which provide an axonal wrapping that forms periodic regions of exposed axon; nodes of Ranvier, which are important for electrical conduction; and astrocytes, interacting with axons and dendrites to form synapses that are critical for transmitting signals between neurons. The cerebral vasculature is formed by multicellular structures within the brain with vessels existing over a large range of diameters: 1–3 mm for large arteries and veins (Fahrig et al., 1999), 10–60 μm for small arterioles and venules, and 4–8 μm for capillaries (Zlokovic, 2008). While larger vessels near the surface of the brain are sparse, smaller vessels within the parenchyma have been reported at high densities ranging from 25 per mm^2 in the corpus callosum to 150 per mm^2 in the hippocampus (Cavaglia et al., 2001). These cell populations and micro-tissue architectures are the underlying basis for why different regions of the brain respond uniquely to mechanical loading and injury.

Heterogeneity in material properties is also important because of the potential for shear forces to occur at the interface between regions with different shear stiffness. These interfaces exist between prominent structures such as cerebral white and gray matter (Smith et al., 1997), which were measured in humans to have shear stiffnesses of 13.6 kPa and 5.22 kPa, respectively (Kruse et al., 2008). Additionally, the stiffness of human arteries was reported to be ~20 MPa and veins to be ~3 MPa (Monson et al., 2003), two to four orders of magnitude larger than bulk brain tissue. Although the largest arteries and veins course superficially in the brain, smaller arterioles, venules, and capillaries permeate the brain parenchyma and account for ~2.5% of the total volume (Nowinski et al., 2013), contributing to regional material heterogeneity.

Within these micro-architectures, the cells themselves have varying material properties. For example, astrocytes may deform to a greater extent than neurons, since elasticity measurements have shown neuronal somas to vary between 480 Pa and 970 Pa (Bernal et al., 2007; Dennerll et al., 1989) and astrocyte somas to vary between 300 Pa and 520 Pa (Lu et al., 2006). Differences may also exist between sub-cellular regions, as soma stiffness was reported to be ~500 Pa (Lu et al., 2006) while axons were reported to be ~12 kPa (reviewed by Bernal et al., 2007; Dennerll et al., 1989). Evidence of "delayed elasticity" has been observed in some axons following rapid stretch, in which a return to their initial length occurs over timescales much larger than the initial stretch (Smith et al., 1999). Therefore, traditional mechanical metrics such as elasticity may not fully capture the behavior of these biological structures.

Linking Biomechanics across Spatial Scales Using In Silico Models. The combination of mathematical modeling and new experimental techniques has provided insight as to how forces applied to the brain are transmitted to the cellular level. Finite element models can incorporate the material properties of the head to predict stress and strain generation within the brain tissue in response to mechanical trauma. By including cellular anisotropy, multiscale models have recapitulated the effect of cellular structures, such as the microvasculature and axonal orientation, on localized cellular strain and have begun to incorporate these effects over the spatial scales of TBI (Chatelin et al., 2011; Cloots et al., 2013). These models have suggested that vessels can create micro-scale heterogeneities that increase localized stress or strain generation near the vessel, potentially explaining the susceptibility of these structures to injury (Cloots et al., 2011). For example, micro-scale inclusions were predicted to increase localized cellular strain levels by 60% compared to the tissue level. Furthermore, the same modeling effort suggested that vessels may force abrupt changes in axonal orientation that can affect localized strain distribution along the axon. Mathematical predictions of micro-scale variations in strain have been verified by high-speed optical measurements of tissue deformation that indicate inhomogeneity in strain fields in local regions containing both white and gray matter (Lauret et al., 2009). Therefore, gross tissue deformation may not completely describe the distribution of forces exerted on the underlying cellular structures or the variability in cellular injury within a region of the brain.

Biomechanics of Injury at the Cellular Level. Both in vivo and in vitro models of the mechanical insults commonly considered to injure brain tissue are used to study the potential injury mechanisms and secondary injury processes of TBI. As the brain is comprised of many cell types and multicellular structures, a multifaceted cellular level injury is likely to occur. As such, here we focus on cellular injury mechanisms directly linked to mechanical forces and direct the reader to additional reviews for a more thorough presentation of cellular level injury (reviewed by Povlishock and Jenkins, 1995; Povlishock and Katz, 2005).

There is growing evidence that brain injury disrupts the blood brain barrier (BBB). Mechanical injury of tight junctions regulating barrier function are believed to cause increased paracellular permeability. Disruption of the BBB after injury has been suggested to potentiate the loss of neurons and altered brain function (e.g., impaired consciousness, memory, and motor ability) and to affect the time course and the extent of neuronal repair (reviewed by Chodobski et al., 2011).

Most studies on TBI to date have focused on neuronal injury. The presence of punctate swollen axons is a hallmark morphology associated with DAI in vivo and can be reproduced using in vitro models (Figure 3). While numerous studies have focused on the initiation of axonal injury, the sequence of events linking initial trauma to specific pathological conditions remains poorly understood. Transient membrane tearing, termed

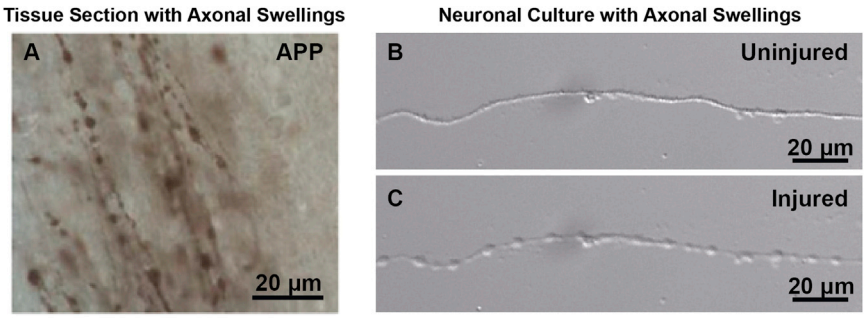

Figure 3. DAI Is Characterized by Axonal Swelling

Identification of DAI following head injury is readily detected by the presence of punctate, swollen axons.

(A) This hallmark morphology can be identified in histological tissue slices stained with markers such as amyloid precursor protein (APP) that depict the punctate nature of swollen axons.

(B) Similar changes in morphology can be recapitulated in vitro when neurons are subjected to mechanical trauma.

(A) is adapted from Mac Donald et al. (2007) with permission.

mechanoporation, has been suggested as a possible mechanism of axonal injury (Kilinc et al., 2008). Mechanoporation is believed to occur in response to a rapid increase in axonal strain, which has been shown using in vitro stretch models of axonal injury. In these experiments, a 30% uni-axial strain applied at a rate of 10 s^{-1} resulted in brief tearing of the membrane that is observed through the cellular uptake of a membrane impermeable marker (Geddes et al., 2003). It is proposed that transient membrane tearing causes a loss in cellular homeostasis that initiates a cascade of secondary events that ultimately result in DAI.

Previous studies have suggested that cytoskeletal abnormalities occur due to impaired axonal transport, causing an accumulation of vesicles and organelles leading to axonal swelling (Gennarelli et al., 1982; Povlishock, 1992). This disruption is thought to result from the action of cysteine proteases, such as calpains and caspases that degrade the cytoskeleton (CSK) (Büki et al., 1999). The hypothesized involvement of protease activation in CSK disruption is suggested by improved injury outcomes in rats that were administered protease inhibitors prior to or following experimental brain injury (Saatman et al., 1996). Pathological activation of proteases has been linked to an influx of ions, such as Ca^{2+} and Na^{+}, that occurs due to mechanoporation (Geddes-Klein et al., 2006; Kilinc et al., 2008). As such, one therapeutic candidate, poloxamer 188, has shown the potential to treat DAI by promoting membrane resealing. Although poloxamer 188 has exhibited neuroprotective effects following TBI in animal models, it has yet to be tested in humans (Serbest et al., 2006).

While membrane poration has been linked to aspects of the secondary injury cascade associated with DAI in experimental models, its role as the sole injury mechanism is not without controversy. Evidence of CSK breakdown, as indicated by calpain-mediated proteolysis (Farkas et al., 2006; Wolf et al., 2001), as well as impaired axonal transport (Stone et al., 2004), has been reported to occur without evidence of membrane poration, challenging the membrane poration hypothesis. Additional injury markers such as neurofilament compaction have also been shown to occur in different cell populations than impaired axonal transport, implicating the existence of multiple injury mechanisms (DiLeonardi et al., 2009). Additional injury mechanisms, such as traumatic mechanical failure of microtubules (Tang-Schomer et al., 2010), impaired sodium channel function (Iwata et al., 2004), and mechanical activation of NMDA receptors (Tang-Schomer et al., 2010) have also been linked to the secondary injury cascades associated with DAI. So while a variety of injury mechanisms have been identified when considering DAI, the majority of these reports are exploring the effects of mechanical forces at the extreme of the spectrum of forces associated with nonlethal brain injury. If one considers mechanical forces just greater than those routinely exerted in the brain microenvironment, the pathological activation of physiological signaling pathways may represent additional mechanisms of injury that are more amenable to pharmacological intervention. Cells have specialized protein networks, the CSK, that maintain their structural integrity while serving as a substrate for biochemical reactions and propagating information, encoded as mechanical forces, from the extracellular environment to the intracellular space. These mechanical networks are the basis of cellular mechanotransduction and have been the subject of intense study the last quarter century in non-neuronal cells.

Mechanobiology and the Cellular Microenvironment of the Brain

The Cytoskeletal and Extracellular Matrix Networks Maintain Neuronal Network Architectures. In the brain, two massively parallel protein networks, one in the extracellular space and the other in the intracellular space, maintain the structural integrity of cell networks and cells themselves. In the intracellular space, the CSK consists of multiple filamentous polymers including actin, microtubules, and neurofilaments that form a protein polymer network continuous with extracellular structures, such as the extracellular matrix (ECM), or neighboring cells, through specialized adhesion proteins located in the cell membrane (Figure 4). Actin filaments are 5–9 nm in diameter and, with cross-linking proteins, bear tensile loads within the cell (Burridge, 1981). Microtubules are ~25 nm in diameter and form rigid structures with stiffness measurements of ~100 MPa that can bear compressive loads (Kis et al., 2002). Intermediate filaments are ~10 nm in diameter and have shown the ability to resist tensile forces by stretching up to 3.5-fold (Kreplak et al., 2005). The networked interaction of these tensile and compressive load bearing elements has been theorized to form a tensegrity structure that, in addition to providing mechanical support, may provide the architectural basis of cellular mechanotransduction (Ingber, 1997). The tensegrity theory of cellular architecture offers an explanation for how cells sense and respond to exogenous forces at the molecular level, as well as how these interactions can scale to affect cell behavior (Ingber, 2003a).

The specialized transmembrane structures that maintain mechanical continuity between the intracellular and extracellular

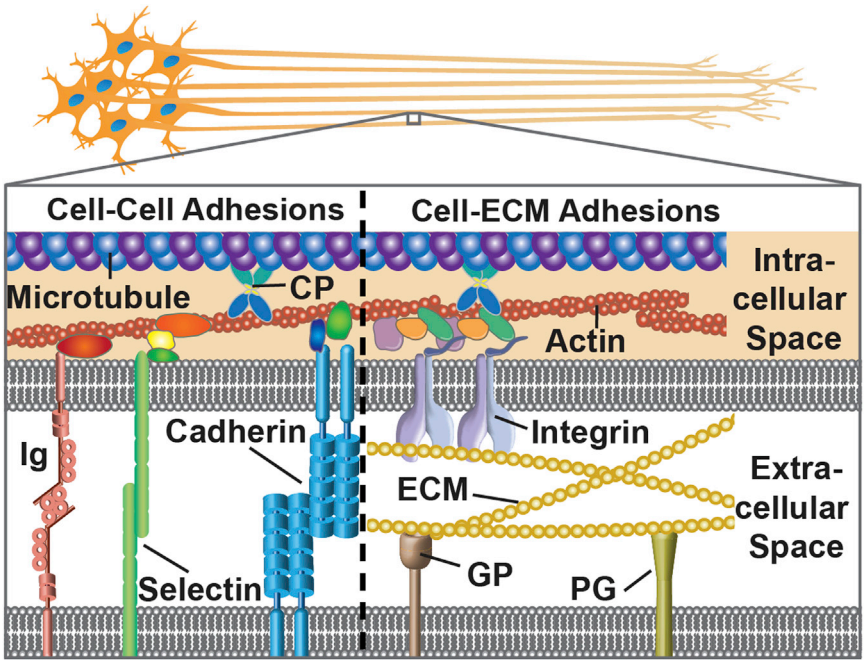

Figure 4. Sub-Cellular Structural Elements Provide Mechanical Support for the Cell
All cell types contain sub-cellular components that dictate cellular architecture by providing mechanical structure. Such structure is formed by interconnected networks of molecules in both the intracellular and extracellular space. Intracellular structures include CSK components such as actin, microtubules, neurofilaments (intermediate filaments), and crosslinking proteins (CPs). Extracellular structures include ECM as well as neighboring cells. These structures are physically connected through transmembrane receptors called CAMs that include IgCAM (Ig), selectin, cadherin, integrin, and other GPs and PGs. The resulting networks bear loads and distribute forces throughout the cell and across the cell membrane to the extracellular space and neighboring cells.

spaces are referred to as cell adhesion molecules (CAMs). This class of proteins includes integrin, cadherin, immunoglobulin, selectin, and proteoglycan (PG) superfamilies. CAMs exhibit binding specificity to both intracellular and extracellular components, some of which are listed in Table 1. For example, tenascin binds integrins but not L1 IgCAMs (Liao et al., 2008; Milner and Campbell, 2002), and chondroitin sulfate PGs (CSPGs) bind L1 IgCAMs but not integrins (Main et al., 1992; Walsh and Doherty, 1997). Furthermore, intracellular components, such as the focal adhesion complex, bind to integrins but not to cadherins (DeMali et al., 2003; Gumbiner and McCrea, 1993). These proteins are important because they bear the responsibility of transmitting mechanical forces into and out of the cells.

Cytoskeletal proteins and CAMs have been studied as biomarkers of TBI. Serum concentrations of intermediate filaments, microtubule-associated proteins, and actin binding elements have shown the potential to discriminate injury in human patients (Yokobori et al., 2013). Furthermore, decreased serum concentration levels of ICAM and VCAM have been reported following mild trauma (Berger et al., 2009). While identifying these components may provide a promising diagnostic strategy, a comprehensive understanding of their ability to maintain network architecture during mechanical trauma is far from complete.

The extracellular space constitutes approximately one-fifth of its total volume of the brain (Syková and Nicholson, 2008) and is structurally stabilized by the ECM network composed of glycosaminoglycans (GAGs), PGs, and glycoproteins (GPs) (Figures 5A–5C; Table 2). Table 2 summarizes the extent of known ECM localization reported in the literature for both non-human and human brains, indicating that while less is known about the human distribution, brain ECM is ubiquitously expressed and differentially distributed. Hyaluronic acid (HA), CSPGs, tenascin-R (TnR), and link proteins together form the primary extracellular scaffolding structure within the brain. Although an extensive analysis of the regional expression levels of individual ECM molecules has only been performed in the mouse brain (Costa et al., 2007), several reports support the existence of differential regional expression in multiple species including humans (Brückner et al., 2008). The most prevalent component of the brain's ECM is HA, a GAG formed by a linear polymer of disaccharides (Meyer et al., 1951). HA is secreted into the extracellular space by membrane-bound enzymes called hyaluronan synthases (HASs) that may localize to neurons (Carulli et al., 2006). CSPGs are comprised of a protein core to which variable numbers of linear, unbranched chondroitin sulfate GAG chains are covalently bonded. Aggrecan, brevican, neurocan, and versican are prominent CSPGs, commonly referred to as lecticans, that vary in size between ~90 kDa (brevican) up to ~400 kDa (versican) (Zimmermann and Dours-Zimmermann, 2008). The cellular source of CSPGs is controversial but likely includes both neurons and glial cells (Carulli et al., 2006). Tenascin-R is a trimeric, modular GP found primarily in the nervous system (Tucker and Chiquet-Ehrismann, 2009) and has been shown to be synthesized by both neurons and glial cells (Carulli et al., 2006). Link proteins are a group of small ~38–43 kDa proteins shown to be synthesized by both neurons and glial cells (Carulli et al., 2006). Additional PGs, such as heparin sulfate PGs (HSPGs), and GPs, such as reelin, also exist in the brain ECM. Distinct compositions of these components exist within the brain, forming specific cellular microenvironments that contribute to structural and functional diversity. In addition to the importance of the ECM for brain physiology, its role in pathology is gaining attention. ECM changes have been observed in brain injury (Asher et al., 2001) and various diseases, including Alzheimer's disease (Bonneh-Barkay and Wiley, 2009), schizophrenia (Berretta, 2012), autism (Mercier et al., 2012), multiple sclerosis (Bonneh-Barkay and Wiley, 2009), and gliomas (Hu et al., 2008), supporting the notion that the cellular microenvironment is an influential component of both normal and disease states.

Table 1. CAMs and Binding Partners Expressed in the CNS

Receptor Family	Sub-Types Expressed in the CNS	Intracellular CSK Binding	Extracellular Binding	Associated Signaling Pathways	References
Integrin	α(1,5,6,v); β(1,3)	FAC → Actin; α-actinin → Actin	ECM proteins (Laminin, Tenascin Reelin) CAM (Ig, Cadherin	Rho-family GTPases; PTK, PTP	([1], [2], [3], [4], [5])
Cadherin	N-Cadherin; E-Cadherin	Catenin → Actin; Desmoplakin → IF	Cadherin, integrin, ECM (Reelin)	Rho-family GTPases; PTK; Wnt signaling; Src-family kinase	([6], [7], [8], [9], [10], [11], [12], [13], [14], [15], [16])
IgCAM	L1, NCAM	Ankyrin→spectrin → Actin; Ezrin → Actin	IgCAM, Integrin, HSPG, CSPG	PTK, PTP, MAPK; Src-family kinase	([9], [10], [11], [12], [13], [14], [15], [16])
Selectin		α –actinin → Actin	Selectin; PSGL-1	MAPK, c-Src	([10])
Additional Glycoproteins and PGs	CD44; Neurexin; Neuroligin	ERM → Actin; Ankyrin → Actin	HA, laminin; Neuroligin, Neurexin	PTK (p195HER2, c-src); Rho-family GTPases	([16], [17], [18], [19])

HA: hyaluronan; CSPG: chondroitin sulfate proteoglycan; HSPG: heparin sulfate proteoglycan; FAC: focal adhesion complex; IF: intermediate filament; PTK: protein tyrosine kinase; PTP: protein tyrosine phosphatase; MAPK: mitogen-activated protein kinase.

[1]Hynes, 1992.
[2]Hynes, 1999.
[3]Liu et al., 2000.
[4]Juliano, 2002.
[5]DeMali, Wennerberg et al., 2003.
[6]Gumbiner and McCrea, 1993.
[7]Nelson, 2004.
[8]Benson et al., 2000.
[9]Angst et al., 2001.
[10]Juliano, 2002.
[11]Wheelock and Johnson, 2003.
[12]Kiryushko et al., 2004.
[13]Nelson and Nusse, 2004.
[14]Gumbiner, 1993.
[15]Gumbiner 1993.
[16]Benson, Schnapp et al., 2000.
[17]Underhill, 1992.
[18]Goodison, 1999.
[19]Turley et al., 2002.

The CSK, cell-cell adhesion molecules, integrins, and the ECM form a continuous massively parallel structural network through the intracellular and extracellular spaces. This network, actually a system of redundant and embedded networks, allow mechanical signals that encode information to be conditioned, propagated and selectively responded to by cells. The CSK endows a cell with its own signal biases and processing capabilities. Thus, each cell's CSK network and connectivity to the ECM represents a unique response capability to mechanical perturbations and insults.

Mechanisms of Conversion of Mechanical Signals to Biochemical Signals. Cellular mechanotransduction, or the ability of cells to convert mechanical forces into chemical signals, is a widely established phenomenon that has been linked to the regulation of diverse cellular physiological processes (Huang et al., 2004), including morphogenesis, growth, and survival (DeMali et al., 2003; Mammoto et al., 2008). Mechanotransduction pathways are influenced by interactions between intracellular structures, such as the CSK, and extracellular structures, such as the ECM or neighboring cells, through CAMs in the cell membrane. Mechanical coupling of the intracellular and extracellular space allows cells to "sense" their local mechanical environment through physical interactions directed through these structures (Janmey and McCulloch, 2007), and mechanosensitive properties have been demonstrated in many cell types (Hall, 1998). Many conditions including stroke, migraine, and cerebral edema have been linked to mechanisms of cellular mechanotransduction (Ingber, 2003a), indicating the potential for these pathways to contribute to disease and to, in turn, be exploited for therapeutic purposes.

Semi-static properties of the cellular microenvironment, such as ECM stiffness (Discher et al., 2005), and dynamic conditions, such as a change in cellular force distribution (Mammoto et al., 2008), can affect development, differentiation, disease, and regeneration by influencing gene expression and signaling pathways (Larsen et al., 2006; Wang et al., 2009). Redistribution of forces within a cell can be initiated by intracellular events, such as alterations in the CSK due to remodeling of microtubules (Bayless and Davis, 2004), and also by extracellular events, such as an exogenous force directed through CAMs (Wang

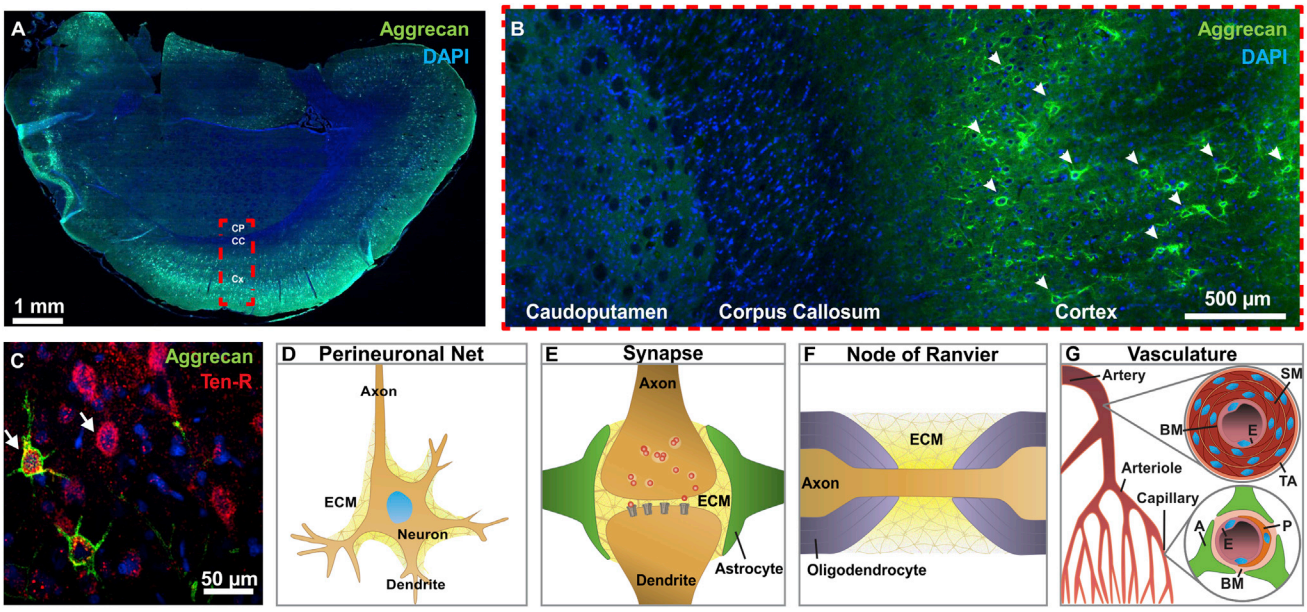

Figure 5. Unique Cellular Microenvironments Exist within the Brain

(A) Immunohistochemistry of coronal sections of adult rat brain reveal differences in the ECM expression throughout distinct brain regions, with high aggrecan signals in the cortex.

(B) A close-up image shows clear differences in the number of aggrecan-containing PNNs (arrow heads) within distinct brain regions, with higher numbers of aggrecan-positive PNNs in the cortex (Cx) compared to the corpus callosum (CC) and the caudoputamen (CP).

(C) Dual labeling suggests that the ECM composition varies locally and that different ECM proteins are differentially concentrated near different cell bodies and processes (arrows).

(D) The PNN is a particular formation of ECM that engulfs the soma and proximal dendrites of neurons. Neuronal structure may be influenced by the PNN through interactions with CAMs, which together regulate neuronal connectivity.

(E) Synapses are formed between neuronal axons and dendrites but are also influenced by supporting ECM and astrocytes. These components are structurally connected through binding of CAMs that influence formation and remodeling of the synapse.

(F) Myelinated axons consist of multiple layers of oligodendrocyte processes that wrap around the axon with distinct spacing referred to as nodes of Ranvier (NOR). ECM within the NOR may influence the clustering of ion channels important for axonal signal propagation.

(G) The extensive vasculature within the brain varies in size from larger arteries and veins down to small capillaries, each of which has a unique structure. ECM comprised of collagen, laminin, and HSPGs surround the inner layer of endothelial cells (En) and also engulf pericytes (P), forming a basement membrane (BM) for cell attachment. In larger vessels, such as arteries and arterioles, a middle layer referred to as the tunica media is formed by multiple layers of ECM and smooth muscle (SM) cells as well as an outer layer called the tunica adventitia (TA). The external layer of ECM promotes adhesion of neural cells, such as terminal nerve fibers and astrocyte endfeet (A), which contribute to numerous vasculature functions including regulating cerebral blood flow and the BBB.

et al., 1993). For example, changes in the relative force balance between the intracellular and extracellular space have been linked to acute CSK remodeling (Tzima, 2006) through the activation of Rho signaling pathways (Zhao et al., 2007). An important mechanochemical control mechanism is hypothesized to be force-induced enzymatic activation of protein kinases. For example, a single mechanical perturbation has been shown to immediately activate Src kinase in both endothelial and smooth muscle cells (Na et al., 2008; Seong et al., 2011), suggesting that an acute insult may be sufficient to influence biological signaling pathways. Moreover, this activation can occur at locations distant to the stimulus site and over time scales faster than diffusion or active transport, suggesting mechanical propagation through the CSK network. Force transmission through the CSK has been previously postulated to explain how mechanotransduction may alter signaling pathways in the nucleus that regulate gene expression (Wang et al., 2009), indicating that an acute force may influence cellular processes over several temporal scales. While extensive studies have focused on mechanotransduction in non-neuronal cells, relatively few have explored the concept in the mature brain.

Mechanical Forces Influence Normal Brain Function. Although the brain is typically considered as a protected organ, mechanical interactions at the cellular and sub-cellular level are critical components of normal biological function. Endogenous forces regulate cellular function in the brain (reviewed by Tyler, 2012). Specifically, mechanical interactions between sub-cellular structures such as the cell membrane, CSK, ECM, CAMs, and ion channels have been shown to influence diverse neural functions including ion channel activity (Arnadóttir and Chalfie, 2010), synaptic vesicle clustering (Siechen et al., 2009), neurotransmitter release (Chen and Grinnell, 1995), and axonal growth cone dynamics (Smith, 1988). Signaling pathways associated with mechanotransduction have also been linked to important neuronal functions. For example, integrin binding forms a mechanical linkage at the synapse and activation of integrin-mediated signaling pathways is a critical step in the formation and regulation of synapse morphology and maturation (Bernard-Trifilo et al., 2005). Moreover, RhoA activation has been shown to influence synaptic plasticity (Yasuda and Murakoshi, 2011) potentially by affecting the structural stability of dendritic spines through CSK remodeling. Integrin-mediated Rho-associated

Table 2. Regional ECM Expression in the Developed CNS

	Non-Human	Human	Reference (N,H)
Cerebral Hemisphere			
Cerebral Cortex	HA, A, B, N, V, P, LP, Tn-R, Tn-C, R, HSPG	HA, CSPG, A, B	([1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16])
Amygdala	HA, A, N, B, V, P, LP, Tn-R, Tn-C, L, HSPG	CSPG	([14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30])
Hippocampus	HA, A, N, B, V, P, LP, Tn-R, Tn-C, R, HSPG	HA, A, B, R	([3, 4, 8, 9, 10, 16, 17, 19, 20, 22, 23, 24, 26, 27, 31, 32, 33, 34])
Thalamus	HA, A, N, B, V, P, LP, Tn-R, Tn-C, HSPG	HA, CSPG, A	([6, 7, 8, 9, 14, 16, 19, 24])
Basal Ganglia	HA, CSPG, A, HSPG	CSPG	([7, 10, 35])
Hypothalamus	HA, CSPG, N, V, LP, Tn-C, HSPG	CSPG	([7, 14, 21, 25, 36, 37])
Cerebellum	HA, A, B, LP, Tn-C, R, HSPG	HA	([3, 7, 9, 10, 22, 23, 27, 33, 38, 39, 40])
Brainstem	HA, A, N, B, V, P, LP, Tn-R, Tn-C, HSPG	CSPG	([6, 7, 8, 9, 19, 24, 41])
Spinal Cord			
Gray Matter	HA, CSPG, A, HSPG	HA, Tn-R, A, B, N, V	([7, 42])
White Matter	HA, CSPG, A, HSPG	HA, Tn-R	([7, 42])

HA: hyaluronan; CSPG: chondroitin sulfate proteoglycan; A: aggrecan; B: brevican; N: neurocan; V: versican; HSPG: heparin sulfate proteoglycan; LP: link protein; TnC: tenascin-C; TnR: tenascin-R; R: reelin.

[1]Crossin and Edelman, 1986.
[2]Crossin et al., 1986.
[3]Bartsch et al., 1993.
[4]Dorries et al., 1993.
[5]Gates et al., 1995.
[6]Bruckner et al., 1996.
[7]Costa et al., 2007.
[8]Zimmermann and Dours-Zimmermann, 2008.
[9]Morawski et al., 2012.
[10]Yashura et al., 1994.
[11]Seeger et al., 1996.
[12]Bruckner et al., 1999.
[13]Virgintino et al., 2009.
[14]Morawski et al., 2010.
[15]Pantazopoulos et al., 2010.
[16]Lendvai et al., 2013.
[17]Gates et al., 1995.
[18]Miller et al., 1995.
[19]Bertolotto et al., 1996.
[20]Ferhat et al., 1996.
[21]Meyer-Puttlitz et al., 1996.
[22]Impagnatiello et al., 1998.
[23]Costa et al., 2001.
[24]Vitellaro-Zuccarello et al., 2001.
[25]Popp et al., 2003.
[26]Costa et al., 2007.
[27]Maloku et al., 2010.
[28]Mercier and Arikawa-Hirasawa, 2012.
[29]Morwaski et al., 2012.
[30]Pantazopoulos et al., 2008.
[31]Bertolotto et al., 1986.
[32]Bertolotto et al., 1986.
[33]Sekeljic and Andjus, 2012.
[34]Frotscher, 2010.
[35]Bruckner et al., 2008.
[36]Miller et al., 1995.
[37]Andersen et al., 2003.
[38]Prieto et al., 1990.
[39]Yamada et al., 1994.
[40]Yamada et al., 1997.
[41]Schmidt et al., 2010.
[42]Jager et al., 2013.

kinase (ROCK) signaling also affects the potential for remyelination in damaged white matter (O'Meara et al., 2011), indicating the broad influence of these pathways in neural function. Although a comprehensive understanding eludes the field, the role of mechanics in regulating inter- and intra-neuronal homeostasis is commonly accepted.

Distinct Cellular Microenvironments Contain Sub-Cellular Structures that Influence Physiological Processes in the Brain. Diverse cell types and ECM molecules compose distinct microenvironments and functions within the brain. Organized ECM structures called perineuronal nets (PNNs) (Figures 5C and 5D) localize to the soma and perisomatic extensions of neurons in multiple brain regions (Brückner et al., 2008; Pizzorusso et al., 2002). PNNs preferentially ensheath highly active neurons (Morris and Henderson, 2000), suggesting they may function as a buffer for cations important in action potential generation (Härtig et al., 1999). PNNs may also limit plasticity as they are upregulated at the end of critical periods in development (Pizzorusso et al., 2002) and stabilize dendritic spine formation (Kwok et al., 2011), potentially by influencing binding of CAMs. PNNs and the brain ECM are believed to be distributed unevenly throughout the brain (Figures 5A and 5B). Moreover, this uneven distribution likely occurs at the cellular level, where neighboring cells are enveloped by different compositions of ECM proteins (Figure 5C). Distinct ECM architectures stabilize synapses, thereby regulating development and plasticity (Figure 5D) (Dityatev and Schachner, 2003). At the synapse, the clustering of postsynaptic receptors is regulated by the ECM, which limits their diffusion within the membrane (Frischknecht et al., 2009). This is accomplished through interactions with CAMs such as integrins and NCAMs (Dityatev and Schachner, 2006) and transmembrane channels such as voltage-dependent calcium channels (Kochlamazashvili et al., 2010). Traditional adhesions like integrins (Chavis and Westbrook, 2001) and cadherins (Uchida et al., 1996) as well as neural-specific adhesions like neuroligin, neurexin (Südhof, 2008), and SynCAM (Biederer et al., 2002) affect synaptic connectivity through signaling pathways that directly influence synapse formation and plasticity by regulating CSK organization (Dityatev and Schachner, 2006; Kochlamazashvili et al., 2010). Specific CAMs such as Caspr2, contactin, and NrCAM are also important in organizing the myelin structure that forms around axons in the brain (Poliak and Peles, 2003). Organized ECM scaffolds occupy the space between myelin wrappings, called Nodes of Ranvier (Figure 5E) (Bekku et al., 2009) and may promote clustering of Na^+ channels that is necessary for signal propagation in the axon (Susuki and Rasband, 2008). Finally, an organized ECM scaffold exists around the vasculature in the brain and influences cellular attachment and organization of the multicellular vascular structures (Figure 5F).

ECM comprised of collagen, laminin, and HSPGs surrounds the inner layer of endothelial cells and also engulfs pericytes forming a structure referred to as the tunica intima (Golding, 2002). In larger vessels, such as arteries and arterioles, a middle layer referred to as the tunica media is formed by multiple layers of ECM and smooth muscle cells as well as an outer layer called the tunica adventitia (Iadecola and Nedergaard, 2007). The external layer of ECM promotes adhesion of neural cells, such as terminal nerve fibers and astrocyte endfeet, which contribute to numerous vasculature functions including regulating cerebral blood flow and forming the BBB (Abbott et al., 2010). Thus, distinct cellular microenvironments influence both cellular structures and functions important to normal brain physiology. Thus, comparing the differences in the architectures of the neuronal and vascular networks in the brain indicates the difficulty in modeling how forces associated with injury propagate through the tissue with centimeter-scale features yet are processed by all cells at the nanometer scale.

Pathological Mechanotransduction and TBI

Pathological Mechanotransduction in DAI. The role of pathological cellular mechanotransduction in the brain remains unclear; however, several reports suggest it may be a contributor to the etiology of TBI. In an in vitro study, rapid deformation of 3D collagen gels resulted in a reduction in embedded neuronal viability when collagen concentration was increased, suggesting a potential influence of cell-matrix interactions on injury (Cullen et al., 2007). The CSK and membrane-associated proteins such as integrins have been previously hypothesized to influence the axonal morphology associated with DAI in peripheral nerve fibers as well (Ochs et al., 1997). Other studies propose that neurite retraction exhibits a strain-dependent response that is regulated by stretch sensitive activity (Franze et al., 2009), and mechanically initiated sodium influx has been shown to occur in neurons subjected to rapid stretch in vitro (Wolf et al., 2001). Interestingly, integrins have been linked to these stretch-activated channels in sensory neurons and other cell types (Dabiri et al., 2012; Lin et al., 2009; Wang et al., 2005). This evidence for the involvement of cellular mechanochemical coupling before transient membrane rupture is emerging.

The wars in Afghanistan and Iraq offered another interesting insight into the potential role of cellular mechanotransduction in brain injury. War casualties exposed to blast, often without an initial diagnosis of TBI, were later diagnosed with symptoms that were suggestive of cerebrovascular stroke. In these cases, a cerebral vasospasm was observed, often without subarachnoid hemorrhage, weeks after exposure to blast (Ling et al., 2009). This clinical observation led us to hypothesize that blast forces propagating through the cerebrovasculature stimulated integrins and subsequently activated Rho, triggering vascular smooth muscle contraction. Using 2D engineered human vascular tissue on a fibronectin-coated, flexible thin film mounted on a customized, high-velocity tissue stretcher designed to mimic blast-like forces in the brain, we showed that vascular smooth muscle cells subjected to a single, rapid stretch exhibited increased contractility, followed by a phenotypic switch from the contractile to the proliferative, state (Alford et al., 2011). Previously, reports have detailed how vascular hypercontraction is characterized by cell proliferation (Borel et al., 2003) and ECM remodeling (Humphrey et al., 2007; Zhang and Macdonald, 2006). In our study, and with the help of computational models, we showed that the phenotypic switch, and subsequent cell proliferation, is a maladaptive measure to reduce the stress in the vascular wall. We went on to show that the increased contractility was mitigated by immediate treatment with a ROCK inhibitor, providing additional evidence for the involvement of Rho-signaling following stretch-induced injury. Prophylactic treatment of the engineered vascular tissues with

a ROCK inhibitor prior to simulated blast prevented the phenotypic switch. This is important, because it demonstrates that injury forces, exerted on the vascular tissue via ECM-integrin interactions, activates Rho and potentiates cellular contraction.

If neurons contract with significant force, this results in the retraction of neurites. There are numerous studies that Rho activation is a hallmark of neural tissue injury. This is important because of Rho activation's role in cellular contraction. Intrinsic activation of Rho signaling, increased localization of RhoA with F-actin-rich swellings in primary hippocampal neurons, and upregulation of RhoA in both rats subjected to fluid percussion brain injury and in stretch injured organotypic hippocampal slices was observed (Di Pietro et al., 2010; Dubreuil et al., 2006; Garland et al., 2012). Given our results with our in vitro model of cerebrovasospasm combined with the evidence suggesting a multifaceted injury mechanism, we reasoned that (1) some forms of TBI may be an integrin signaling disease and (2) signaling pathways downstream of integrin signaling may represent therapeutic opportunities.

To test our hypotheses, we developed in vitro models to control the ECM and cell adhesions through which injurious forces were delivered to neurons (Hemphill et al., 2011). Using a magnetic tweezer model, we showed that an injury force, administered via an integrin-bound magnetic bead bound to a neuron, triggers focal swelling through extended neurites and their retraction, a global injury, reminiscent of clinically observed DAI. When magnetic beads were bound to neurons by non-specific interactions with the cell membrane, rapid application of the magnetic field designed to mechanically manipulate the bead elicited injury only local to the delivery site (local injury). This result suggests that integrins may be mediating the distribution of injury forces throughout the neuron by propagating forces through the CSK.

Using an in vitro uniaxial stretch similar to our studies in vascular muscle tissue, we showed that the ECM protein that the neurons are bound to on a substrate can influence the probability of neuronal injury, as indicated by focal swelling. Additionally, we showed that inhibition of ROCK, a downstream effector of Rho-GTPases, reduced the incidence of axonal injury, suggesting that these pathways may be activated in neurons following an acute mechanical stimulus (Hemphill et al., 2011). All told, our results suggest that injury-induced, integrin-mediated activation of Rho potentiates focal swelling and axonal retraction and that downstream Rho signaling pathways may represent a therapeutic opportunity.

Additional work with an experimental model of injury to the optic nerve also demonstrated that CSK injury, accompanied by Rho accumulation in focal swellings, suggests that Rho signaling is important in DAI (Di Pietro et al., 2010; Dubreuil et al., 2006; Garland et al., 2012). CSK remodeling has been shown to occur through integrin-mediated activation of Rho signaling proteins that influence microtubule stability and actin dynamics (Jaffe and Hall, 2005; Palazzo et al., 2004), providing a potential link to the microtubule breakdown and transport disruption observed in DAI. Membrane receptors, such as HAS, CD44, RHAMM, Layilin, and GPI-linked brevican may also provide conduits into the cell as they bind directly to HA and may thus serve as a physical link to the brain ECM (Frischknecht and Seidenbecher, 2008). In fact, reports have shown that GPI-linked proteins can transmit extracellular forces to the CSK (Wang et al., 1995). Therefore, in addition to the traditional cell-ECM linkages, brain-specific CAMs may provide additional diversity in influencing both the mechanical linkages and biological signaling pathways regulated by factors in the cellular microenvironment. Much of the biological signaling that links acute mechanical stimulation to cellular changes within the brain remains to be discerned. However, the potential for mechanotransduction to regulate diverse cellular functions including CSK remodeling and gene expression may provide promising targets for future studies.

Conclusion

Brain injury is a complex, mechanobiology problem whose spatial dimensions range from the nanometer-scale integrins in the neuronal membrane to the centimeter-scale material properties of the brain and skull. Temporally, from the nanosecond-scale conformational changes in mechanically loaded proteins to the years over which neurodegenerative diseases such as Alzheimer's and Parkinson's disease emerge, TBI represents a formidable challenge in both the way it is studied and clinically treated.

There is potential promise because of the last two decades worth of work in cellular mechanotransduction, illustrating how cells are designed and built to transduce mechanical forces into chemical cascades. In vitro experimental techniques can be registered with clinical observations afforded by advanced medical imaging capabilities, allowing tissue-engineered models of the brain that are amenable to the granular studies required of drug discovery work. Furthermore, known cellular mechanotransduction signaling pathways may guide research questions relevant to the pathology of TBI.

In this paper, we argue that the ECM, CAMs, and CSK represent a pathway worthy of study to reveal the mechanisms of brain injury and potential therapeutic opportunities. Future studies designed to elucidate the brain-specific structures involved in cellular mechanotransduction as well as their associated signaling pathways will improve our ability to understand how the brain responds to exogenous mechanical forces, such as those experienced during TBI. Key aspects to consider include (1) identifying the sub-cellular components involved in mechanotransduction within the brain, such as localized ECM and associated CAM expression; (2) understanding whether heterogeneities in the distribution of mechanosensitive components can explain regional vulnerabilities to TBI; and (3) understanding if these mechanosensitive components can result in activation of signaling pathways that ultimately lead to the secondary injury associated with TBI.

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REFERENCES

Abbott, N.J., Patabendige, A.A.K., Dolman, D.E.M., Yusof, S.R., and Begley, D.J. (2010). Structure and function of the blood-brain barrier. Neurobiol. Dis. *37*, 13–25.

Alberts, B. (1994). Molecular Biology of the Cell, Third Edition. (New York: Garland Pub.).

Alessandri, B., Doppenberg, E., Zauner, A., Woodward, J., Choi, S., and Bullock, R. (1999). Evidence for time-dependent glutamate-mediated glycolysis in head-injured patients: a microdialysis study. Acta Neurochir. Supplemental (Wien) *75*, 25–28.

Alexander, M.P. (1995). Mild traumatic brain injury: pathophysiology, natural history, and clinical management. Neurology *45*, 1253–1260.

Alford, P.W., Dabiri, B.E., Goss, J.A., Hemphill, M.A., Brigham, M.D., and Parker, K.K. (2011). Blast-induced phenotypic switching in cerebral vasospasm. Proc. Natl. Acad. Sci. USA *108*, 12705–12710.

Armonda, R.A., Bell, R.S., Vo, A.H., Ling, G., DeGraba, T.J., Crandall, B., Ecklund, J., and Campbell, W.W. (2006). Wartime traumatic cerebral vasospasm: recent review of combat casualties. Neurosurgery *59*, 1215–1225, discussion 1225.

Arnadóttir, J., and Chalfie, M. (2010). Eukaryotic mechanosensitive channels. Annu. Rev. Biophys. *39*, 111–137.

Asher, R.A., Morgenstern, D.A., Moon, L.D., and Fawcett, J.W. (2001). Chondroitin sulphate proteoglycans: inhibitory components of the glial scar. Prog. Brain Res. *132*, 611–619.

Azevedo, F.A., Carvalho, L.R., Grinberg, L.T., Farfel, J.M., Ferretti, R.E., Leite, R.E., Jacob Filho, W., Lent, R., and Herculano-Houzel, S. (2009). Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J. Comp. Neurol. *513*, 532–541.

Bauman, R.A., Ling, G., Tong, L., Januszkiewicz, A., Agoston, D., Delanerolle, N., Kim, Y., Ritzel, D., Bell, R., Ecklund, J., et al. (2009). An introductory characterization of a combat-casualty-care relevant swine model of closed head injury resulting from exposure to explosive blast. J. Neurotrauma *26*, 841–860.

Bayless, K.J., and Davis, G.E. (2004). Microtubule depolymerization rapidly collapses capillary tube networks in vitro and angiogenic vessels in vivo through the small GTPase Rho. J. Biol. Chem. *279*, 11686–11695.

Bekku, Y., Rauch, U., Ninomiya, Y., and Oohashi, T. (2009). Brevican distinctively assembles extracellular components at the large diameter nodes of Ranvier in the CNS. J. Neurochem. *108*, 1266–1276.

Bendlin, B.B., Ries, M.L., Lazar, M., Alexander, A.L., Dempsey, R.J., Rowley, H.A., Sherman, J.E., and Johnson, S.C. (2008). Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging. Neuroimage *42*, 503–514.

Berger, R.P., Ta'asan, S., Rand, A., Lokshin, A., and Kochanek, P. (2009). Multiplex assessment of serum biomarker concentrations in well-appearing children with inflicted traumatic brain injury. Pediatr. Res. *65*, 97–102.

Bergsneider, M., Hovda, D.A., Lee, S.M., Kelly, D.F., McArthur, D.L., Vespa, P.M., Lee, J.H., Huang, S.C., Martin, N.A., Phelps, M.E., and Becker, D.P. (2000). Dissociation of cerebral glucose metabolism and level of consciousness during the period of metabolic depression following human traumatic brain injury. J. Neurotrauma *17*, 389–401.

Bernal, R., Pullarkat, P.A., and Melo, F. (2007). Mechanical properties of axons. Phys. Rev. Lett. *99*, 018301.

Bernard-Trifilo, J.A., Kramár, E.A., Torp, R., Lin, C.Y., Pineda, E.A., Lynch, G., and Gall, C.M. (2005). Integrin signaling cascades are operational in adult hippocampal synapses and modulate NMDA receptor physiology. J. Neurochem. *93*, 834–849.

Berretta, S. (2012). Extracellular matrix abnormalities in schizophrenia. Neuropharmacology *62*, 1584–1597.

Biederer, T., Sara, Y., Mozhayeva, M., Atasoy, D., Liu, X., Kavalali, E.T., and Südhof, T.C.; SynCAM (2002). SynCAM, a synaptic adhesion molecule that drives synapse assembly. Science *297*, 1525–1531.

Blumbergs, P.C., Scott, G., Manavis, J., Wainwright, H., Simpson, D.A., and McLean, A.J. (1995). Topography of axonal injury as defined by amyloid precursor protein and the sector scoring method in mild and severe closed head injury. J. Neurotrauma *12*, 565–572.

Bonneh-Barkay, D., and Wiley, C.A. (2009). Brain extracellular matrix in neurodegeneration. Brain Pathol. *19*, 573–585.

Borel, C.O., McKee, A., Parra, A., Haglund, M.M., Solan, A., Prabhakar, V., Sheng, H., Warner, D.S., and Niklason, L. (2003). Possible role for vascular cell proliferation in cerebral vasospasm after subarachnoid hemorrhage. Stroke *34*, 427–433.

Borg, J., Holm, L., Cassidy, J.D., Peloso, P.M., Carroll, L.J., von Holst, H., and Ericson, K.; WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury (2004). Diagnostic procedures in mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J. Rehabil. Med. *36* (*Suppl*), 61–75.

Brückner, G., Morawski, M., and Arendt, T. (2008). Aggrecan-based extracellular matrix is an integral part of the human basal ganglia circuit. Neuroscience *151*, 489–504.

Büki, A., Siman, R., Trojanowski, J.Q., and Povlishock, J.T. (1999). The role of calpain-mediated spectrin proteolysis in traumatically induced axonal injury. J. Neuropathol. Exp. Neurol. *58*, 365–375.

Burridge, K. (1981). Are stress fibres contractile? Nature *294*, 691–692.

Carulli, D., Rhodes, K.E., Brown, D.J., Bonnert, T.P., Pollack, S.J., Oliver, K., Strata, P., and Fawcett, J.W. (2006). Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components. J. Comp. Neurol. *494*, 559–577.

Cavaglia, M., Dombrowski, S.M., Drazba, J., Vasanji, A., Bokesch, P.M., and Janigro, D. (2001). Regional variation in brain capillary density and vascular response to ischemia. Brain Res. *910*, 81–93.

Cernak, I., and Noble-Haeusslein, L.J. (2010). Traumatic brain injury: an overview of pathobiology with emphasis on military populations. J. Cereb. Blood Flow Metab. *30*, 255–266.

Chatelin, S., Deck, C., Renard, F., Kremer, S., Heinrich, C., Armspach, J.P., and Willinger, R. (2011). Computation of axonal elongation in head trauma finite element simulation. J. Mech. Behav. Biomed. Mater. *4*, 1905–1919.

Chavis, P., and Westbrook, G. (2001). Integrins mediate functional pre- and postsynaptic maturation at a hippocampal synapse. Nature *411*, 317–321.

Chen, B.M., and Grinnell, A.D. (1995). Integrins and modulation of transmitter release from motor nerve terminals by stretch. Science *269*, 1578–1580.

Chodobski, A., Zink, B.J., and Szmydynger-Chodobska, J. (2011). Blood-brain barrier pathophysiology in traumatic brain injury. Transl. Stroke. Res. *2*, 492–516.

Cloots, R.J., van Dommelen, J.A., Nyberg, T., Kleiven, S., and Geers, M.G. (2011). Micromechanics of diffuse axonal injury: influence of axonal orientation and anisotropy. Biomech. Model. Mechanobiol. *10*, 413–422.

Cloots, R.J., van Dommelen, J.A., Kleiven, S., and Geers, M.G. (2013). Multi-scale mechanics of traumatic brain injury: predicting axonal strains from head loads. Biomech. Model. Mechanobiol. *12*, 137–150.

Corsellis, J.A., Bruton, C.J., and Freeman-Browne, D. (1973). The aftermath of boxing. Psychol. Med. *3*, 270–303.

Costa, C., Tortosa, R., Domènech, A., Vidal, E., Pumarola, M., and Bassols, A. (2007). Mapping of aggrecan, hyaluronic acid, heparan sulphate proteoglycans and aquaporin 4 in the central nervous system of the mouse. J. Chem. Neuroanat. *33*, 111–123.

Cullen, D.K., Lessing, M.C., and LaPlaca, M.C. (2007). Collagen-dependent neurite outgrowth and response to dynamic deformation in three-dimensional neuronal cultures. Ann. Biomed. Eng. *35*, 835–846.

Dabiri, B.E., Lee, H., and Parker, K.K. (2012). A potential role for integrin signaling in mechanoelectrical feedback. Prog. Biophys. Mol. Biol. *110*, 196–203.

DeMali, K.A., Wennerberg, K., and Burridge, K. (2003). Integrin signaling to the actin cytoskeleton. Curr. Opin. Cell Biol. *15*, 572–582.

Dennerll, T.J., Lamoureux, P., Buxbaum, R.E., and Heidemann, S.R. (1989). The cytomechanics of axonal elongation and retraction. J. Cell Biol. *109*, 3073–3083.

Di Pietro, V., Amin, D., Pernagallo, S., Lazzarino, G., Tavazzi, B., Vagnozzi, R., Pringle, A., and Belli, A. (2010). Transcriptomics of traumatic brain injury: gene expression and molecular pathways of different grades of insult in a rat organotypic hippocampal culture model. J. Neurotrauma *27*, 349–359.

DiLeonardi, A.M., Huh, J.W., and Raghupathi, R. (2009). Impaired axonal transport and neurofilament compaction occur in separate populations of injured axons following diffuse brain injury in the immature rat. Brain Res. *1263*, 174–182.

Discher, D.E., Janmey, P., and Wang, Y.L. (2005). Tissue cells feel and respond to the stiffness of their substrate. Science *310*, 1139–1143.

Dityatev, A., and Schachner, M. (2003). Extracellular matrix molecules and synaptic plasticity. Nat. Rev. Neurosci. *4*, 456–468.

Dityatev, A., and Schachner, M. (2006). The extracellular matrix and synapses. Cell Tissue Res. *326*, 647–654.

Dubreuil, C.I., Marklund, N., Deschamps, K., McIntosh, T.K., and McKerracher, L. (2006). Activation of Rho after traumatic brain injury and seizure in rats. Exp. Neurol. *198*, 361–369.

Erb, D.E., and Povlishock, J.T. (1991). Neuroplasticity following traumatic brain injury: a study of GABAergic terminal loss and recovery in the cat dorsal lateral vestibular nucleus. Exp. Brain Res. *83*, 253–267.

Fahrig, R., Nikolov, H., Fox, A.J., and Holdsworth, D.W. (1999). A three-dimensional cerebrovascular flow phantom. Med. Phys. *26*, 1589–1599.

Fallenstein, G.T., Hulce, V.D., and Melvin, J.W. (1969). Dynamic mechanical properties of human brain tissue. J. Biomech. *2*, 217–226.

Farkas, O., and Povlishock, J.T. (2007). Cellular and subcellular change evoked by diffuse traumatic brain injury: a complex web of change extending far beyond focal damage. Prog. Brain Res. *161*, 43–59.

Farkas, O., Lifshitz, J., and Povlishock, J.T. (2006). Mechanoporation induced by diffuse traumatic brain injury: an irreversible or reversible response to injury? J. Neurosci. *26*, 3130–3140.

Faul, M., Xu, L., Wald, M., and Coronado, V. (2010). Traumatic brain injury in the United States: Emergency department visits, hospitalizations and deaths 2002–2006. (Atlanta, GA: Centers for Disease Control and Prevention, National Center for Injury Prevention and Control).

Franze, K., Gerdelmann, J., Weick, M., Betz, T., Pawlizak, S., Lakadamyali, M., Bayer, J., Rillich, K., Gögler, M., Lu, Y.B., et al. (2009). Neurite branch retraction is caused by a threshold-dependent mechanical impact. Biophys. J. *97*, 1883–1890.

Frischknecht, R., and Seidenbecher, C.I. (2008). The crosstalk of hyaluronan-based extracellular matrix and synapses. Neuron Glia Biol. *4*, 249–257.

Frischknecht, R., Heine, M., Perrais, D., Seidenbecher, C.I., Choquet, D., and Gundelfinger, E.D. (2009). Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity. Nat. Neurosci. *12*, 897–904.

Garland, P., Broom, L.J., Quraishe, S., Dalton, P.D., Skipp, P., Newman, T.A., and Perry, V.H. (2012). Soluble axoplasm enriched from injured CNS axons reveals the early modulation of the actin cytoskeleton. PLoS ONE *7*, e47552.

Geddes, D.M., Cargill, R.S., 2nd, and LaPlaca, M.C. (2003). Mechanical stretch to neurons results in a strain rate and magnitude-dependent increase in plasma membrane permeability. J. Neurotrauma *20*, 1039–1049.

Geddes-Klein, D.M., Serbest, G., Mesfin, M.N., Cohen, A.S., and Meaney, D.F. (2006). Pharmacologically induced calcium oscillations protect neurons from increases in cytosolic calcium after trauma. J. Neurochem. *97*, 462–474.

Gennarelli, T.A., Thibault, L.E., Adams, J.H., Graham, D.I., Thompson, C.J., and Marcincin, R.P. (1982). Diffuse axonal injury and traumatic coma in the primate. Ann. Neurol. *12*, 564–574.

Giza, C.C., and Hovda, D.A. (2001). The Neurometabolic Cascade of Concussion. J. Athl. Train. *36*, 228–235.

Golding, E.M. (2002). Sequelae following traumatic brain injury. The cerebrovascular perspective. Brain Res. Brain Res. Rev. *38*, 377–388.

Goldsmith, W., and Plunkett, J. (2004). A biomechanical analysis of the causes of traumatic brain injury in infants and children. Am. J. Forensic Med. Pathol. *25*, 89–100.

Goldstein, L.E., Fisher, A.M., Tagge, C.A., Zhang, X.L., Velisek, L., Sullivan, J.A., Upreti, C., Kracht, J.M., Ericsson, M., Wojnarowicz, M.W., et al. (2012). Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. Sci. Transl. Med. *4*, http://dx.doi.org/10.1126/scitranslmed.3003716.

Gu, L., Li, J., Feng, D.F., Cheng, E.T., Li, D.C., Yang, X.Q., and Wang, B.C. (2013). Detection of white matter lesions in the acute stage of diffuse axonal injury predicts long-term cognitive impairments: a clinical diffusion tensor imaging study. J. Trauma Acute Care Surg. *74*, 242–247.

Gumbiner, B.M., and McCrea, P.D. (1993). Catenins as mediators of the cytoplasmic functions of cadherins. J. Cell Sci. Suppl. *17*, 155–158.

Hall, A. (1998). Rho GTPases and the actin cytoskeleton. Science *279*, 509–514.

Härtig, W., Derouiche, A., Welt, K., Brauer, K., Grosche, J., Mäder, M., Reichenbach, A., and Brückner, G. (1999). Cortical neurons immunoreactive for the potassium channel Kv3.1b subunit are predominantly surrounded by perineuronal nets presumed as a buffering system for cations. Brain Res. *842*, 15–29.

Hemphill, M.A., Dabiri, B.E., Gabriele, S., Kerscher, L., Franck, C., Goss, J.A., Alford, P.W., and Parker, K.K. (2011). A possible role for integrin signaling in diffuse axonal injury. PLoS ONE *6*, e22899.

Holbourn, A.H.S. (1944). Mechanics of Head Injuries. Lancet *243*, 483.

Hu, B., Kong, L.L., Matthews, R.T., and Viapiano, M.S. (2008). The proteoglycan brevican binds to fibronectin after proteolytic cleavage and promotes glioma cell motility. J. Biol. Chem. *283*, 24848–24859.

Huang, H., Kamm, R.D., and Lee, R.T. (2004). Cell mechanics and mechanotransduction: pathways, probes, and physiology. Am. J. Physiol. Cell Physiol. *287*, C1–C11.

Humphrey, J.D., Baek, S., and Niklason, L.E. (2007). Biochemomechanics of cerebral vasospasm and its resolution: I. A new hypothesis and theoretical framework. Ann. Biomed. Eng. *35*, 1485–1497.

Iadecola, C., and Nedergaard, M. (2007). Glial regulation of the cerebral microvasculature. Nat. Neurosci. *10*, 1369–1376.

Ingber, D.E. (1997). Tensegrity: the architectural basis of cellular mechanotransduction. Annu. Rev. Physiol. *59*, 575–599.

Ingber, D.E. (2003a). Tensegrity I. Cell structure and hierarchical systems biology. J. Cell Sci. *116*, 1157–1173.

Ingber, D.E. (2003b). Mechanobiology and diseases of mechanotransduction. Ann. Med. *35*, 564–577.

Inglese, M., Makani, S., Johnson, G., Cohen, B.A., Silver, J.A., Gonen, O., and Grossman, R.I. (2005). Diffuse axonal injury in mild traumatic brain injury: a diffusion tensor imaging study. J. Neurosurg. *103*, 298–303.

Iwata, A., Stys, P.K., Wolf, J.A., Chen, X.H., Taylor, A.G., Meaney, D.F., and Smith, D.H. (2004). Traumatic axonal injury induces proteolytic cleavage of the voltage-gated sodium channels modulated by tetrodotoxin and protease inhibitors. J. Neurosci. *24*, 4605–4613.

Jaffe, A.B., and Hall, A. (2005). Rho GTPases: biochemistry and biology. Annu. Rev. Cell Dev. Biol. *21*, 247–269.

Janmey, P.A., and McCulloch, C.A. (2007). Cell mechanics: integrating cell responses to mechanical stimuli. Annu. Rev. Biomed. Eng. *9*, 1–34.

Jenkins, A., Teasdale, G., Hadley, M.D., Macpherson, P., and Rowan, J.O. (1986). Brain lesions detected by magnetic resonance imaging in mild and severe head injuries. Lancet *2*, 445–446.

Johnson, V.E., Stewart, W., and Smith, D.H. (2013). Axonal pathology in traumatic brain injury. Exp. Neurol. *246*, 35–43.

Kandel, E.R., Schwartz, J.H., and Jessell, T.M. (2000). Principles of Neural Science, Fourth Edition. (New York: McGraw-Hill, Health Professions Division).

Kilinc, D., Gallo, G., and Barbee, K.A. (2008). Mechanically-induced membrane poration causes axonal beading and localized cytoskeletal damage. Exp. Neurol. *212*, 422–430.

Kis, A., Kasas, S., Babić, B., Kulik, A.J., Benoît, W., Briggs, G.A., Schönenberger, C., Catsicas, S., and Forró, L. (2002). Nanomechanics of microtubules. Phys. Rev. Lett. *89*, 248101.

Kochlamazashvili, G., Henneberger, C., Bukalo, O., Dvoretskova, E., Senkov, O., Lievens, P.M.J., Westenbroek, R., Engel, A.K., Catterall, W.A., Rusakov, D.A., et al. (2010). The extracellular matrix molecule hyaluronic acid regulates hippocampal synaptic plasticity by modulating postsynaptic L-type Ca(2+) channels. Neuron *67*, 116–128.

Kreplak, L., Bär, H., Leterrier, J.F., Herrmann, H., and Aebi, U. (2005). Exploring the mechanical behavior of single intermediate filaments. J. Mol. Biol. *354*, 569–577.

Kruse, S.A., Rose, G.H., Glaser, K.J., Manduca, A., Felmlee, J.P., Jack, C.R., Jr., and Ehman, R.L. (2008). Magnetic resonance elastography of the brain. Neuroimage *39*, 231–237.

Kwok, J.C., Dick, G., Wang, D., and Fawcett, J.W. (2011). Extracellular matrix and perineuronal nets in CNS repair. Dev. Neurobiol. *71*, 1073–1089.

Larsen, M., Artym, V.V., Green, J.A., and Yamada, K.M. (2006). The matrix reorganized: extracellular matrix remodeling and integrin signaling. Curr. Opin. Cell Biol. *18*, 463–471.

Lauret, C., Hrapko, M., van Dommelen, J.A., Peters, G.W., and Wismans, J.S. (2009). Optical characterization of acceleration-induced strain fields in inhomogeneous brain slices. Med. Eng. Phys. *31*, 392–399.

Lee, H., Wintermark, M., Gean, A.D., Ghajar, J., Manley, G.T., and Mukherjee, P. (2008). Focal lesions in acute mild traumatic brain injury and neurocognitive outcome: CT versus 3T MRI. J. Neurotrauma *25*, 1049–1056.

Liao, H., Huang, W., Schachner, M., Guan, Y., Guo, J., Yan, J., Qin, J., Bai, X., and Zhang, L. (2008). Beta 1 integrin-mediated effects of tenascin-R domains EGFL and FN6-8 on neural stem/progenitor cell proliferation and differentiation in vitro. J. Biol. Chem. *283*, 27927–27936.

Lin, Y.W., Cheng, C.M., Leduc, P.R., and Chen, C.C. (2009). Understanding sensory nerve mechanotransduction through localized elastomeric matrix control. PLoS ONE *4*, e4293.

Ling, G., Bandak, F., Armonda, R., Grant, G., and Ecklund, J. (2009). Explosive blast neurotrauma. J. Neurotrauma *26*, 815–825.

Lu, Y.B., Franze, K., Seifert, G., Steinhäuser, C., Kirchhoff, F., Wolburg, H., Guck, J., Janmey, P., Wei, E.Q., Käs, J., and Reichenbach, A. (2006). Viscoelastic properties of individual glial cells and neurons in the CNS. Proc. Natl. Acad. Sci. USA *103*, 17759–17764.

Mac Donald, C.L., Dikranian, K., Song, S.K., Bayly, P.V., Holtzman, D.M., and Brody, D.L. (2007). Detection of traumatic axonal injury with diffusion tensor imaging in a mouse model of traumatic brain injury. Exp. Neurol. *205*, 116–131.

Mac Donald, C.L., Johnson, A.M., Cooper, D., Nelson, E.C., Werner, N.J., Shimony, J.S., Snyder, A.Z., Raichle, M.E., Witherow, J.R., Fang, R., et al. (2011). Detection of blast-related traumatic brain injury in U.S. military personnel. N. Engl. J. Med. *364*, 2091–2100.

Macé, E., Cohen, I., Montaldo, G., Miles, R., Fink, M., and Tanter, M. (2011). In vivo mapping of brain elasticity in small animals using shear wave imaging. IEEE Trans. Med. Imaging *30*, 550–558.

Main, A.L., Harvey, T.S., Baron, M., Boyd, J., and Campbell, I.D. (1992). The three-dimensional structure of the tenth type III module of fibronectin: an insight into RGD-mediated interactions. Cell *71*, 671–678.

Mammoto, A., Mammoto, T., and Ingber, D.E. (2008). Rho signaling and mechanical control of vascular development. Curr. Opin. Hematol. *15*, 228–234.

McKee, A.C., Cantu, R.C., Nowinski, C.J., Hedley-Whyte, E.T., Gavett, B.E., Budson, A.E., Santini, V.E., Lee, H.S., Kubilus, C.A., and Stern, R.A. (2009). Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. J. Neuropathol. Exp. Neurol. *68*, 709–735.

McKee, A.C., Stern, R.A., Nowinski, C.J., Stein, T.D., Alvarez, V.E., Daneshvar, D.H., Lee, H.S., Wojtowicz, S.M., Hall, G., Baugh, C.M., et al. (2013). The spectrum of disease in chronic traumatic encephalopathy. Brain *136*, 43–64.

Mercier, F., Kwon, Y.C., and Douet, V. (2012). Hippocampus/amygdala alterations, loss of heparan sulfates, fractones and ventricle wall reduction in adult BTBR T+ tf/J mice, animal model for autism. Neurosci. Lett. *506*, 208–213.

Meyer, K., Linker, A., and Rapport, M.M. (1951). The production of monosaccharides from hyaluronic acid by beta-glucuronidase. J. Biol. Chem. *192*, 275–281.

Milner, R., and Campbell, I.L. (2002). The integrin family of cell adhesion molecules has multiple functions within the CNS. J. Neurosci. Res. *69*, 286–291.

Monson, K.L., Goldsmith, W., Barbaro, N.M., and Manley, G.T. (2003). Axial mechanical properties of fresh human cerebral blood vessels. J. Biomech. Eng. *125*, 288–294.

Morris, N.P., and Henderson, Z. (2000). Perineuronal nets ensheath fast spiking, parvalbumin-immunoreactive neurons in the medial septum/diagonal band complex. Eur. J. Neurosci. *12*, 828–838.

Na, S., Collin, O., Chowdhury, F., Tay, B., Ouyang, M.X., Wang, Y.X., and Wang, N. (2008). Rapid signal transduction in living cells is a unique feature of mechanotransduction. Proc. Natl. Acad. Sci. USA *105*, 6626–6631.

Nowinski, W.L., Puspitasari, F., Volkau, I., Orrison, W.W., Jr., and Knopp, M.V. (2013). Quantification of the human cerebrovasculature: a 7Tesla and 320-row CT in vivo study. J. Comput. Assist. Tomogr. *37*, 117–122.

O'Meara, R.W., Michalski, J.P., and Kothary, R. (2011). Integrin signaling in oligodendrocytes and its importance in CNS myelination. J. Signal Transduct. *2011*, 354091.

Ochs, S., Pourmand, R., Jersild, R.A., Jr., and Friedman, R.N. (1997). The origin and nature of beading: a reversible transformation of the shape of nerve fibers. Prog. Neurobiol. *52*, 391–426.

Omalu, B.I., Hamilton, R.L., Kamboh, M.I., DeKosky, S.T., and Bailes, J. (2010). Chronic traumatic encephalopathy (CTE) in a National Football League Player: Case report and emerging medicolegal practice questions. J. Forensic Nurs. *6*, 40–46.

Ommaya, A.K., and Gennarelli, T.A. (1974). Cerebral concussion and traumatic unconsciousness. Correlation of experimental and clinical observations of blunt head injuries. Brain *97*, 633–654.

Ommaya, A.K., Grubb, R.L., Jr., and Naumann, R.A. (1971). Coup and contrecoup injury: observations on the mechanics of visible brain injuries in the rhesus monkey. J. Neurosurg. *35*, 503–516.

Ommaya, A.K., Goldsmith, W., and Thibault, L. (2002). Biomechanics and neuropathology of adult and paediatric head injury. Br. J. Neurosurg. *16*, 220–242.

Palazzo, A.F., Eng, C.H., Schlaepfer, D.D., Marcantonio, E.E., and Gundersen, G.G. (2004). Localized stabilization of microtubules by integrin- and FAK-facilitated Rho signaling. Science *303*, 836–839.

Pizzorusso, T., Medini, P., Berardi, N., Chierzi, S., Fawcett, J.W., and Maffei, L. (2002). Reactivation of ocular dominance plasticity in the adult visual cortex. Science *298*, 1248–1251.

Poliak, S., and Peles, E. (2003). The local differentiation of myelinated axons at nodes of Ranvier. Nat. Rev. Neurosci. *4*, 968–980.

Povlishock, J.T. (1992). Traumatically induced axonal injury: pathogenesis and pathobiological implications. Brain Pathol. *2*, 1–12.

Povlishock, J.T., and Jenkins, L.W. (1995). Are the pathobiological changes evoked by traumatic brain injury immediate and irreversible? Brain Pathol. *5*, 415–426.

Povlishock, J.T., and Katz, D.I. (2005). Update of neuropathology and neurological recovery after traumatic brain injury. J. Head Trauma Rehabil. *20*, 76–94.

Prange, M.T., and Margulies, S.S. (2002). Regional, directional, and age-dependent properties of the brain undergoing large deformation. J. Biomech. Eng. *124*, 244–252.

Reeves, T.M., Phillips, L.L., and Povlishock, J.T. (2005). Myelinated and unmyelinated axons of the corpus callosum differ in vulnerability and functional recovery following traumatic brain injury. Exp. Neurol. *196*, 126–137.

Reeves, T.M., Greer, J.E., Vanderveer, A.S., and Phillips, L.L. (2010). Proteolysis of submembrane cytoskeletal proteins ankyrin-G and αII-spectrin following diffuse brain injury: a role in white matter vulnerability at Nodes of Ranvier. Brain Pathol. *20*, 1055–1068.

Saatman, K.E., Murai, H., Bartus, R.T., Smith, D.H., Hayward, N.J., Perri, B.R., and McIntosh, T.K. (1996). Calpain inhibitor AK295 attenuates motor and cognitive deficits following experimental brain injury in the rat. Proc. Natl. Acad. Sci. USA *93*, 3428–3433.

Seong, J., Lu, S.Y., and Wang, Y.X. (2011). Live Cell Imaging of Src/FAK Signaling by FRET. Cell Mol. Bioeng. *2*, 138–147.

Serbest, G., Horwitz, J., Jost, M., and Barbee, K.A. (2006). Mechanisms of cell death and neuroprotection by poloxamer 188 after mechanical trauma. FASEB J. *20*, 308–310.

Shuck, L.Z., and Advani, S.H. (1972). Rheological Response of Human Brain-Tissue in Shear. J. Basic Eng-T Asme. *94*, 905–911.

Siechen, S., Yang, S., Chiba, A., and Saif, T. (2009). Mechanical tension contributes to clustering of neurotransmitter vesicles at presynaptic terminals. Proc. Natl. Acad. Sci. USA *106*, 12611–12616.

Smith, S.J. (1988). Neuronal cytomechanics: the actin-based motility of growth cones. Science *242*, 708–715.

Smith, D.H., and Meaney, D.F. (2000). Axonal damage in traumatic brain injury. Neuroscientist *6*, 483–495.

Smith, D.H., Chen, X.H., Xu, B.N., McIntosh, T.K., Gennarelli, T.A., and Meaney, D.F. (1997). Characterization of diffuse axonal pathology and selective hippocampal damage following inertial brain trauma in the pig. J. Neuropathol. Exp. Neurol. *56*, 822–834.

Smith, D.H., Wolf, J.A., Lusardi, T.A., Lee, V.M., and Meaney, D.F. (1999). High tolerance and delayed elastic response of cultured axons to dynamic stretch injury. J. Neurosci. *19*, 4263–4269.

Stern, R.A., Riley, D.O., Daneshvar, D.H., Nowinski, C.J., Cantu, R.C., and McKee, A.C. (2011). Long-term consequences of repetitive brain trauma: chronic traumatic encephalopathy. PM R *3*, S460–S467.

Stone, J.R., Okonkwo, D.O., Dialo, A.O., Rubin, D.G., Mutlu, L.K., Povlishock, J.T., and Helm, G.A. (2004). Impaired axonal transport and altered axolemmal permeability occur in distinct populations of damaged axons following traumatic brain injury. Exp. Neurol. *190*, 59–69.

Strich, S.J. (1956). Diffuse degeneration of the cerebral white matter in severe dementia following head injury. J. Neurol. Neurosurg. Psychiatry *19*, 163–185.

Südhof, T.C. (2008). Neuroligins and neurexins link synaptic function to cognitive disease. Nature *455*, 903–911.

Susuki, K., and Rasband, M.N. (2008). Molecular mechanisms of node of Ranvier formation. Curr. Opin. Cell Biol. *20*, 616–623.

Syková, E., and Nicholson, C. (2008). Diffusion in brain extracellular space. Physiol. Rev. *88*, 1277–1340.

Tagliaferri, F., Compagnone, C., Korsic, M., Servadei, F., and Kraus, J. (2006). A systematic review of brain injury epidemiology in Europe. Acta Neurochir. (Wien) *148*, 255–268, discussion 268.

Tang-Schomer, M.D., Patel, A.R., Baas, P.W., and Smith, D.H. (2010). Mechanical breaking of microtubules in axons during dynamic stretch injury underlies delayed elasticity, microtubule disassembly, and axon degeneration. FASEB J. *24*, 1401–1410.

Thibault, K.L., and Margulies, S.S. (1998). Age-dependent material properties of the porcine cerebrum: effect on pediatric inertial head injury criteria. J. Biomech. *31*, 1119–1126.

Tucker, R.P., and Chiquet-Ehrismann, R. (2009). The regulation of tenascin expression by tissue microenvironments. Biochim. Biophys. Acta *1793*, 888–892.

Tyler, W.J. (2012). The mechanobiology of brain function. Nat. Rev. Neurosci. *13*, 867–878.

Tzima, E. (2006). Role of small GTPases in endothelial cytoskeletal dynamics and the shear stress response. Circ. Res. *98*, 176–185.

Uchida, N., Honjo, Y., Johnson, K.R., Wheelock, M.J., and Takeichi, M. (1996). The catenin/cadherin adhesion system is localized in synaptic junctions bordering transmitter release zones. J. Cell Biol. *135*, 767–779.

Walsh, F.S., and Doherty, P. (1997). Neural cell adhesion molecules of the immunoglobulin superfamily: role in axon growth and guidance. Annu. Rev. Cell Dev. Biol. *13*, 425–456.

Wang, N., Butler, J.P., and Ingber, D.E. (1993). Mechanotransduction across the cell surface and through the cytoskeleton. Science *260*, 1124–1127.

Wang, N., Planus, E., Pouchelet, M., Fredberg, J.J., and Barlovatz-Meimon, G. (1995). Urokinase receptor mediates mechanical force transfer across the cell surface. Am. J. Physiol. *268*, C1062–C1066.

Wang, Y., Botvinick, E.L., Zhao, Y., Berns, M.W., Usami, S., Tsien, R.Y., and Chien, S. (2005). Visualizing the mechanical activation of Src. Nature *434*, 1040–1045.

Wang, N., Tytell, J.D., and Ingber, D.E. (2009). Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. Nat. Rev. Mol. Cell Biol. *10*, 75–82.

Wolf, J.A., Stys, P.K., Lusardi, T., Meaney, D., and Smith, D.H. (2001). Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. J. Neurosci. *21*, 1923–1930.

Yasuda, R., and Murakoshi, H. (2011). The mechanisms underlying the spatial spreading of signaling activity. Curr. Opin. Neurobiol. *21*, 313–321.

Yokobori, S., Hosein, K., Burks, S., Sharma, I., Gajavelli, S., and Bullock, R. (2013). Biomarkers for the clinical differential diagnosis in traumatic brain injury—a systematic review. CNS Neurosci. Ther. *19*, 556–565.

Yoshino, A., Hovda, D.A., Katayama, Y., Kawamata, T., and Becker, D.P. (1992). Hippocampal CA3 lesion prevents postconcussive metabolic dysfunction in CA1. J. Cereb. Blood Flow Metab. *12*, 996–1006.

Zhang, Z.D., and Macdonald, R.L. (2006). Contribution of the remodeling response to cerebral vasospasm. Neurol. Res. *28*, 713–720.

Zhang, L.Y., Yang, K.H., and King, A.I. (2001). Comparison of brain responses between frontal and lateral impacts by finite element modeling. J. Neurotrauma *18*, 21–30.

Zhao, X.H., Laschinger, C., Arora, P., Szászi, K., Kapus, A., and McCulloch, C.A. (2007). Force activates smooth muscle alpha-actin promoter activity through the Rho signaling pathway. J. Cell Sci. *120*, 1801–1809.

Zimmermann, D.R., and Dours-Zimmermann, M.T. (2008). Extracellular matrix of the central nervous system: from neglect to challenge. Histochem. Cell Biol. *130*, 635–653.

Zlokovic, B.V. (2008). The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron *57*, 178–201.

Zouridakis, G., Patidar, U., Situ, N., Rezaie, R., Castillo, E.M., Levin, H.S., and Papanicolaou, A.C. (2012). Functional connectivity changes in mild traumatic brain injury assessed using magnetoencephalography. J. Mech. Med. Biol. *12*.