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CHAPTER 2

CNS injury biomechanics and experimental models

M.C. LaPlaca*, C.M. Simon, G.R. Prado and D.K. Cullen

Neural Injury Biomechanics and Repair Laboratory, Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, 313 Ferst Dr., Atlanta, GA 30332-0535, USA

Abstract: Traumatic brain injury (TBI) and traumatic spinal cord injury (SCI) are acquired when an external physical insult causes damage to the central nervous system (CNS). Functional disabilities resulting from CNS trauma are dependent upon the mode, severity, and anatomical location of the mechanical impact as well as the mechanical properties of the tissue. Although the biomechanical insult is the initiating factor in the pathophysiology of CNS trauma, the anatomical loading distribution and the resulting cellular responses are currently not well understood. For example, the primary response phase includes events such as increased membrane permeability to ions and other molecules, which may initiate complex signaling cascades that account for the prolonged damage and dysfunction. Correlation of insult parameters with cellular changes and subsequent deficits may lead to refined tolerance criteria and facilitate the development of improved protective gear. In addition, advancements in the understanding of injury biomechanics are essential for the development and interpretation of experimental studies at both the in vitro and in vivo levels and may lead to the development of new treatment approaches by determining injury mechanisms across the temporal spectrum of the injury response. Here we discuss basic concepts relevant to the biomechanics of CNS trauma, injury models used to experimentally simulate TBI and SCI, and novel multilevel approaches for improving the current understanding of primary damage mechanisms.

Keywords: traumatic brain injury; traumatic spiral cord injury; neurotrauma; biomechanics; membrane permeability; finite element analysis; injury tolerance criteria

Introduction

Traumatic brain injury (TBI) and spinal cord injury (SCI) result in a range of deficits depending on the insult severity and the anatomical region(s) affected. In traumatic central nervous injury (CNS) injury, a mechanical impact (caused by motor vehicle accidents, gunshot wounds, blows to the head or spine, etc.) induces a mechanical response at the cell and tissue level that ultimately causes a pathophysiological injury response (as shown in Fig. 1). In the acute phase of injury, primary damage occurs as a direct result of a mechanical input that has exceeded structural limits of cells and tissue. Primary damage is characterized by nonspecific cell loss as well as sublethal injury, which activates a cascade of secondary responses leading to prolonged cell death, network dysfunction, and system level changes (Fig. 2). Although the mechanical impact is the initiating event in traumatic CNS injury, the relationship between biomechanical inputs and the downstream pathological effects are not well understood. Investigation of relevant loading parameters and the resulting cell and tissue responses in a variety of model systems is imperative for deciphering injury-induced pathophysiological mechanisms and developing experimental models that hold fidelity to the human clinical situation.

*Corresponding author.
E-mail: michelle.laplaca@bme.gatech.edu

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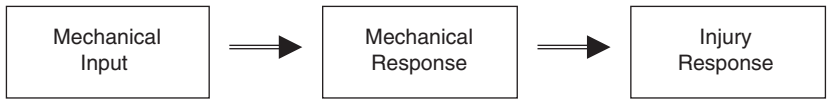


Fig. 1. Steps in CNS trauma. Traumatic brain and spinal cord injuries result from mechanical loading to the tissue. Pathophysiological events are initiated by the mechanical tissue response to impact.

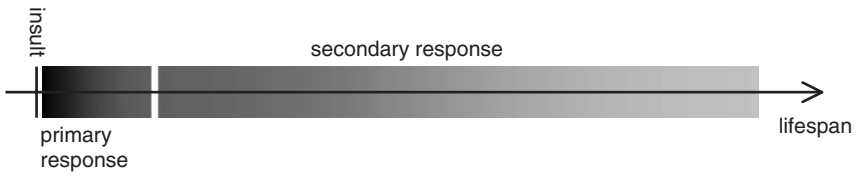


Fig. 2. Temporal aspects of injury. Mechanical loading causes an acute primary phase followed by a prolonged secondary phase. The primary response is characterized by nonspecific cell loss, which initiates a cascade of complex secondary events such as inflammation, excitotoxicity, and neurodegeneration.

A notable application of the study of biomechanics in CNS trauma is the determination of accurate tissue tolerances. Tissue tolerances are defined as the point at which structural and/or physiological failure occurs. An improved understanding of injury biomechanics and the resulting brain and spinal cord responses will ultimately facilitate the development of improved protective gear (e.g., helmets and seat belts). Determination of tolerance criteria requires information about the forces and deformations that lead to failure, but the mechanical parameters (i.e., magnitude and rate of force and deformation) are only partially understood. Tissue response and tolerance criteria for humans are largely based on cadaveric studies, but may not accurately represent the properties of living tissue. Basic cell and animal studies, in which a defined mechanical insult can be applied to live cells in culture or in an intact animal, have an advantage for the determination of tissue tolerance and may lead to the refinement of human tolerance criteria. These tolerance criteria must be model-independent and represent inherent system properties.

We will discuss basic biomechanical concepts as they relate to traumatic brain and spinal cord injuries and present experimental models that have been developed and characterized in an attempt to mimic the forces and deformations occurring in human CNS trauma. The mechanisms by which the mechanical response to a traumatic insult leads to dysfunction are complex, yet can be simplified using controlled cellular injury models that account for deformation magnitude and rate. Biomechanically relevant in vitro TBI models, used in combination with animal studies and computer simulations, may lead to improved cellular and tissue injury tolerance criteria as well as a more complete understanding of the relationship between the biomechanical input and pathophysiological changes. This multilevel approach will be discussed with respect to selection of experimental models, development of mechanistically driven treatment strategies, and future research priorities.

Basic biomechanics

Biomechanics is the study of forces and physical responses in stationary (static) and moving (dynamic) biological systems. A system (in the case of traumatic CNS injury — the brain or spinal cord) reacts in a specific way when a force, or load, is placed on it. These external loads may result in initial damage or lead to delayed damage. The point at which loading causes tissue damage is the threshold (or the tolerance) of the system and is dependent on the type and duration of the load. The basic terms, or descriptors, that biomechanicians use to describe applied loads are *force* and *stress* and the resulting responses are *deformations* and *strains*.

Force is defined as the action of one body (a physical entity in the system, such as a windshield) on another (as a result of an impact), which will cause acceleration of the second body (e.g., the head) unless acted upon by an equal and opposite action counteracting the effect of the first body. The unit is a Newton (N); 1 N is the force that will give 1 kg an acceleration of $1\,m/s^2$ (English unit is pound-force, lbf). When forces are generated in tissue, deformation may ensue depending on the *material properties* and the *nature of the force* itself. *Deformation* is defined as the change in shape of a body undergoing a force. A rigid body, for example, would experience extremely small deformations, while biological tissue (usually referred to as deformable or nonrigid) can often undergo substantially large deformations.

Stress is another term frequently used in biomechanical analysis and refers to the distribution of force relative to the area on which it acts. Normal stresses (designated by the Greek letter sigma (σ)) act perpendicular to the surface, while shear stresses (designated by the Greek letter tau (τ)) act tangential to the surface. The unit is the Pascal (Pa); $1\,Pa = 1\,N/m^2$. A given force acting on a small surface produces greater stress than the same force acting over a larger surface. In other words, the amount of mechanical stress created by a force is dependent on the size of the area over which the force is applied. The resulting *strain* that occurs relates the deformed state of the body to the undeformed state and is unitless. Extensional strain is the change in length divided by the original length (designated by the Greek letter epsilon) ($\varepsilon = \Delta l/l_o$) and can be further classified as being in *tension* (positive strain) or *compression* (negative strain). Extensional strain results from stresses generated from linear (or translational) loads. Shear strain, often resulting from rotational loads, is also the change in length divided by the original length (designated by the Greek letter gamma) ($\gamma = \Delta l/l_o$). Brain tissue is thought to be more sensitive to shear strain than extensional strain (Holbourn, 1943). Therefore loading that involves rotation of the head has been thought to result in more severe injuries, although this assumption has recently been questioned (King et al., 2003). The relationships between stress and strain are referred to as *constitutive relationships* and the resulting equations are used to define behavior of the tissue (or the mechanical response).

The basic mechanics terms defined above are valuable in describing the conditions that lead to injuries, although several factors surround biomechanical analysis of damage prediction. Mechanical conditions can be referred to as the *insult parameters* and the result as the *injury* (Fig. 1). Two broad categories of insults can be defined as *static* and *dynamic* loading, with dynamic loading being the most common. The *mechanical response* to insult is the tissue deformation or strain and will initiate the ensuing pathological events. The insult parameters and the mechanical response will dictate the types of injury (*focal* and/or *diffuse*). We will consider the categories of insults, the mechanical response to traumatic insult, the types of injuries produced, as well as two overlapping *response phases* (*primary* and *secondary*) in light of the biomechanical fidelity of experimental models used to simulate these conditions.

Traumatic mechanical insults

Loads are described as *direct* (e.g., physical contact between the head and another object) or *indirect* (e.g., as the result of motion of the head). In indirect loading, acceleration of the second body (e.g., the head) can act analogously to applied forces. Loads can be *translational* (linear), *rotational*, or *angular* (a combination of translational and rotational). The type of force and the direction, or plane, of loading, will also affect the resulting mechanical response in the tissue. The extent and severity of deformation increases with increasing force, and this relationship is *nonlinear*. In other words, the increase in tissue damage may be greater than the proportional increase in force. *Static loading* is a very slowly applied direct load. Usually there are no deficits until there is substantial tissue deformation. These loading conditions are relatively rare and often occur in human entrapment situations (e.g., earthquakes). *Dynamic loading*, on the other hand, can occur quite

rapidly (under 1 s, often <50 ms) and is the most common cause of TBI and SCI. Dynamic loading can further be broken down into *impact loading* (direct loading where an impact occurs with an object hitting the head or the head hitting an object) or *impulsive loading* (indirect loading where no contact occurs). Impact loading can be either focal or diffuse, depending on the magnitude of the force and area of impact. Although pure impact would involve contact with no head movement, impact loading is usually a combination of contact forces — from the impact itself — and inertial forces — from the motion of the head and the brain within the skull. It is important to consider the size, mass, and hardness of the impacting object as well as the surface area and velocity at which contact occurs. For example, impact with smaller objects (i.e., <2 in. in diameter) results in high local stress concentrations and therefore is associated with a greater risk for more local and severe damage and is more likely to result in tissue penetration. Impulsive loading is due to inertial forces alone and leads to diffuse brain injuries. Models of impulsive loading include angular acceleration of the head, yet many of the models utilized for impact loading are designed to deliver a rapid bulk insult that has inertial components. Ultimately, the response is dictated by the mechanical response of the tissue or cells.

Loads, in particular rotational inputs to the brain, however, do not linearly scale between humans and animal, as the mass of the brain is much smaller. In fact, to produce an equivalent rotational load in a rodent brain as in a human brain the angular acceleration would need to be approximately two orders of magnitude higher. This anatomical complexity introduces difficulty in directly linking pathological consequences to the biomechanical input. In addition to these constraints in animal modeling, the regional stresses and strains have yet to be well characterized. Future investigations to determine the relationships between biomechanical parameters and cellular responses will require a detailed spatial characterization of local cellular stresses and strains in animal models of CNS trauma.

Mechanical response to traumatic insult

A traumatic insult to brain or spinal cord will lead to a mechanical response of the tissue that is dependent on the mode, severity, and anatomical location of the impact as well as the mechanical properties of the tissue. The mechanical properties of a tissue vary from individual to individual, as well as with age and previous injuries or disease (Prange and Margulies, 2002). In addition, cellular orientation and tissue composition varies among anatomical regions of the brain and spinal cord, creating nonuniform (or heterogeneous) mechanical properties that directly affect structural and functional tolerances as well as the load distribution throughout the tissue upon mechanical loading.

Because of the properties of soft tissues, like brain and spinal cord, both the *rate* and the *duration* of the insult will also influence the response. Loads that are applied quickly may incur more damage due to the material properties of CNS tissue. When loads are applied at a high rate, the tissue cannot absorb (or reduce) the force fast enough and can fail both structurally and functionally. In contrast, slowly applied loads give the tissue ''time'' to reduce the force and generally result in less damage. For short durations of force, much of the effects of the force are reduced. As the duration of force increases, less reduction occurs and therefore less force is needed to produce tissue deformation. These behaviors are defined by a mechanical property termed *viscoelasticity*.

Types of traumatic CNS injury

Focal injuries result from direct loading and can often occur without widespread, or diffuse, damage. Focal injuries are typically induced when an object penetrates the skull or vertebral column as a result of a motor vehicle accident, gunshot wound, or a blow. As a result, macroscopically visible damage is typically visible at the site of impact, and the clinical symptoms are often very specific to the area that is directly injured. Focal injuries to the brain include epidural hematomas and skull fracture (with or without brain damage). When

there is osteal or dural compromise, this is often termed *open head injury* in the clinical setting. Contact loading can also result in coup (at the site of impact) and contra-coup (away from the site of impact) contusions to the brain, involving both cellular and vascular components. Focal injuries account for one-half of all severe head injuries, but two-third of all deaths in this group (Thurman and Guerrero, 1999; Adekoya et al., 2002).

SCI is most commonly caused by fracture and dislocation of the spinal column, resulting in a focal injury. The mechanical impact causes displacement of bone fragments, intervertebral discs, or ligaments, resulting in transient compression or contusion of spinal cord tissue. Spinal cord is compressed at the site of impact that causes the surrounding tissue to lengthen in the longitudinal direction. Tissue near the center of the spinal cord is most vulnerable, suggesting that the mechanical loads are highest in this anatomical region. Large myelinated axons in the surrounding white matter are also highly susceptible to mechanical damage, due to stress concentrations at the nodes of Ranvier (Maxwell, 1996). As in TBI, the rate, magnitude, and duration of the biomechanical insult can dictate the injury response and may affect functional outcome. Slow stretching of the spinal cord results in very little tissue damage. In fact, increasing the length of the spinal cord up to twice the original length results in very little damage if the elongation is applied slowly (Shi and Whitebone, 2006). However, biomechanical inputs applied rapidly or for an extended duration (longer than 20–30 min) may surpass tissue thresholds and result in irreversible damage.

Diffuse injuries are most often caused by inertial loading, which describes the motion of objects. The acceleration (velocity change divided by change in time) is an important parameter in determining tissue response. Higher accelerations correspond to higher forces (force equals mass times acceleration, Newton's second law). This must be taken into account when establishing thresholds for tissue damage. Because of the complex head-neck dynamics, the brain can undergo high acceleration when subjected to an external load and therefore TBI often manifests as a diffuse injury. When the acceleration is translational, injuries tend to be localized to a smaller area. Rotational acceleration, on the other hand, can lead to large strains deep within the brain, resulting in diffuse axonal injury (DAI) (Gennarelli et al., 1982). Most injuries seen clinically are a combination of translational and rotational accelerations (referred to as *angular acceleration*). Diffuse injuries are thought to occur as a result of not only the acceleration portion of loading, but also from the deceleration portion of the insult, creating very fast moving, uneven load distributions (Margulies et al., 1990). Diffuse strains can lead to differential movement of the skull relative to the brain, causing parasagittal bridging vein injury, as well as intracerebral hemorrhage. Diffuse injury to the brain tends to lead to widespread dysfunction, making these injuries the most prevalent cause of persistent neurological disability. Clinically, diffuse injury is often seen in closed head injury and arises most often from motor vehicle accidents.

Experimental modeling of traumatic CNS injury

Experimental models of CNS injury have been invaluable in the investigation of pathological mechanisms and treatment strategies. However, due to the variable nature of clinical traumatic CNS injury (e.g., inconsistencies in the anatomical location of impact and the magnitude and duration of loading), experimental models must simplify the human condition in order to create a reproducible injury that can be utilized for controlled experimental testing. Although relevance to the clinic may be sacrificed, these simplifications allow the assessment of various outcome measures at the cellular, tissue, and organism level in response to defined bulk loading parameters.

In vivo animal models preserve much of the complexity associated with human traumatic CNS injury while allowing the investigator to experimentally manipulate certain parameters (e.g., treatment variables, time of sacrifice) that are not possible in humans. In the study of injury biomechanics, in vivo models provide a more complete representation of the human brain and spinal cord

because they more closely mimic the material properties and anatomical architecture. Therefore, the load distribution and structural failure in animal models are expected to be similar to human injury when clinically relevant biomechanical loading parameters are applied in a scale-appropriate manner.

In vivo models commonly used in TBI and SCI research have been used to experimentally represent aspects of the biomechanics of CNS trauma. Direct loading has been mimicked using contusion, weight drop, fluid percussion, or compression injuries. Contusion or weight drop involves brief, rapid loading of CNS tissue using a piston or a weight dropped from various heights (Dixon et al., 1991; Anderson and Stokes, 1992; Marmarou et al., 1994; Young, 2002; Scheff et al., 2003). These models are designed to deliver a rapid bulk insult that has both impact and inertial components. Compression injury is also used to experimentally replicate mechanical loads applied to spinal cords over long durations (e.g., due to abnormal, prolonged twisting of the spine during an automobile accident) (Rivlin and Tator, 1978; Dolan and Tator, 1979). Inertial loading experienced during TBI is modeled with fluid percussion injury (Dixon et al., 1987; McIntosh et al., 1989; Thibault et al., 1992) (which has components of impact loads as well) and angular acceleration of the head (Gennarelli et al., 1982; Smith et al., 1997), which results in characteristic pathophysiological changes such as DAI.

In vitro TBI models offer several advantages over whole animal models, including control over cellular components and real-time measurement of acute responses. Neural cultures and tissue explants have been subjected to compression, tension, or shear to experimentally mimic aspects of CNS trauma (see Morrison et al., 1998b). Models include deformable membranes that are stretched biaxially (Ellis et al., 1995; Cargill and Thibault, 1996; Geddes and Cargill, 2001; Morrison et al., 1998a) or uniaxially (Pfister et al., 2003; Lusardi et al., 2004) to transfer strain to attached cells, some with the capability of deforming neurites aligned longitudinally to the strain field (Galbraith and Thibault, 1993; Smith et al., 1999). These in vitro models allow for isolation of specific biomechanical parameters (e.g., deformation mode, rate, and magnitude), allowing for systematic assessment of cellular responses to defined inputs. The recent development of a three-dimensional (3-D) model in which neural cells are cultured in a hydrogel offers an intermediate degree of complexity, as bulk deformation of the culture results in heterogeneous strain fields at the cellular level depending on the orientation of the cell within the matrix (LaPlaca et al., 2005; Cullen and LaPlaca, 2006).

Tolerance criteria for CNS injury

To date, several cellular tolerance criteria have been established to describe the contribution of both acceleration and pulse duration for a specific head injury (e.g., skull fracture, concussion), including the Wayne State Tolerance Criteria (Lissner et al., 1960), the Gadd Severity Index (Gadd, 1966), and the Head Injury Criterion (Versace, 1971). The basic overlying principle is that short pulses of high acceleration can produce injury, while lower accelerations require longer pulses to produce injury. These criteria have contributed to the development of a fundamental foundation; however, the tolerance stipulations have been based on cadaver or primate data in which the measure of injury did not consider damage at the cellular level. The efforts at the National Highway Traffic & Safety Administration (NHTSA) have produced models of the head in the SIMon project. The predictive capability of SIMon and other computational models hinge on adoption of rational and experimentally verified thresholds for damage. Because different regions of CNS tissue have different cellular orientations and tissue composition, resulting in nonuniform (or heterogeneous) mechanical properties, structural and functional tolerances of the brain and spinal cord differ depending on the region affected. More complex and realistic computer models have been developed to provide more accurate information relevant to the biomechanics of injury (e.g., Zhang et al., 2004). Iterative verification of these models is imperative to their successful application. Measurement of brain tissue strain

during a dynamic mechanical event is exceedingly difficult in the intact animal or postmortem human subject (Hardy et al., 2003). Consequently, it has proven challenging to determine quantitative tolerances to be used for the damage measures embedded in computer models. Current efforts have utilized existing experimental data and scaling relationships to empirically derive thresholds to predict physiological outcome in animal experiments (Takhounts et al., 2003). Therefore, experimental models that enable the correlation of strain and acute injury could potentially determine detailed cellular tolerances.

Response phases of traumatic CNS injury

Acute cellular response

The initial damage that is a direct result of loading to the brain is defined as the *primary phase* of injury. Biomechanicians study this phase in order to determine tissue tolerances to mechanical loading because the effects of the mechanical insult can be more easily isolated from biochemical events occurring in the secondary or more chronic phase. Our understanding of tolerances at the cellular level is vital to developing better safety equipment and understanding mechanotransduction in the pathological range. At the time of the insult there may be a varying amount of primary damage that results from the physical force itself. This includes compromised skin, bony fractures, tissue tearing, cellular rupture, and reorientation of the tissue components. If a deformation threshold is surpassed, these structural failures result and can severely compromise brain function.

Due to the heterogeneity of CNS tissue, it is likely that loads and deformations experienced by cells in various anatomical regions are not consistent and cannot be accurately estimated by simplistic models assuming homogeneity. Certain anatomical regions may be subjected to more severe loading during impact because of differences in the material properties in that particular location (due to variations in cellular orientation, myelination, etc.). Anatomical regions experiencing larger strains would therefore be expected to be more susceptible to primary damage caused by the mechanical insult itself. Although identification of these regions would allow more accurate correlations between the mechanical input and pathophysiological responses, very little is currently known about local cellular strains in animal models of CNS trauma, mainly due to limitations in detection techniques.

One approach for addressing these technical limitations is the development of more sensitive methods for the detection of mechanically induced damage. Although detection of structural failures can be relatively obvious in some instances (such as the presence of large focal lesions), more subtle damage may also be present and can provide a unique opportunity for assessment of local cellular strains after trauma. Visualization of the anatomical localization of this mechanical damage can provide a more sensitive measure of the load distribution throughout the tissue. We and others have investigated nonspecific plasma membrane damage as an indicator of mechanical damage in various models of TBI and SCI (Pettus et al., 1994; LaPlaca et al., 1997; Shi and Borgens, 2000; Geddes et al., 2003; Farkas et al., 2006). This type of cellular damage occurs as a direct result of mechanical loading, creating rips or tears in the plasma membrane at regions of high local strain.

We have utilized Lucifer yellow as an indicator of acute biophysical membrane failure after TBI and SCI. Lucifer yellow is normally membrane-impermeable; therefore, cellular presence of this molecule can be used to detect plasma membrane compromise. In these experiments, Lucifer yellow was injected intrathecally 3 h prior to brain or spinal cord contusion, and animals were sacrificed 10 min after injury (a schematic of the injury devices are illustrated in Fig. 3). Histological evidence demonstrated heterogeneous uptake of the permeability marker in various anatomical locations (as shown in Fig. 4), indicating that the distribution of mechanical loading in CNS tissue is complex and not well understood. Although we have focused on acute membrane damage as an indicator of the load distribution throughout the brain and spinal cord, others have explored membrane compromise as an initiator of downstream pathological events. Cell membrane damage can

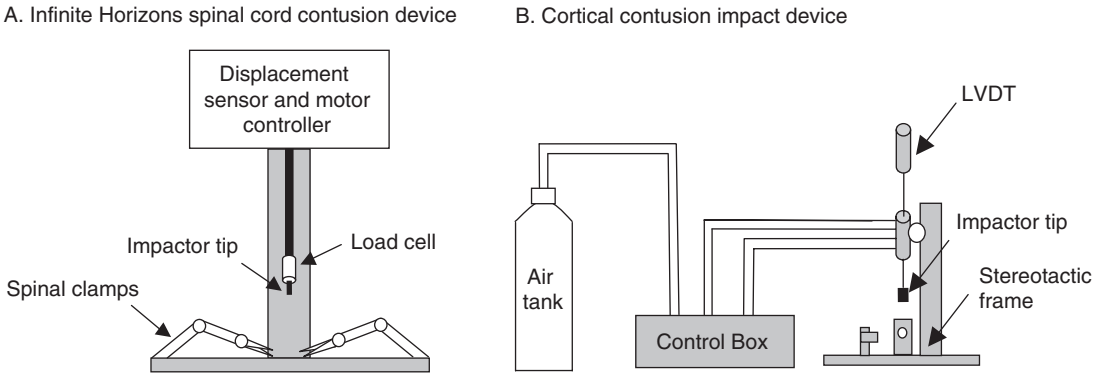


Fig. 3. In vivo contusion injury devices. Injury devices are used to experimentally deliver prescribed injury parameters to the exposed brain or spinal cord. For example, the Infinite Horizons spinal cord contusion device (A) allows the user to select an impact force for injury, while the controlled cortical impact device (B) utilizes a pneumatic system to injure the brain at a defined tissue displacement.

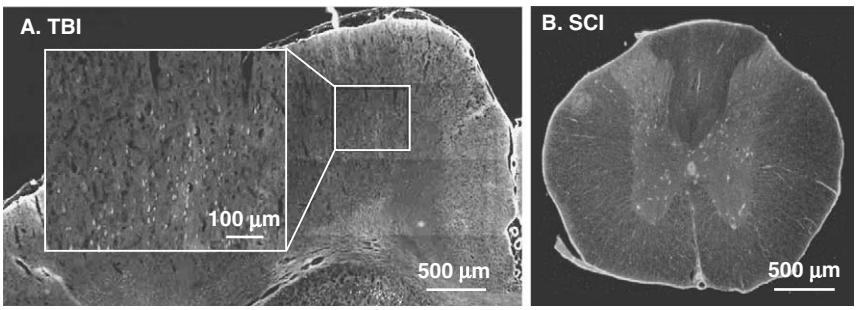


Fig. 4. Acute cellular permeability following TBI and SCI. In the acute phase of traumatic injury, the plasma membrane becomes damaged due to local cellular strains that exceed structural thresholds. Lucifer yellow uptake in the injured brain (A) and spinal cord (B) demonstrates a heterogeneous distribution of membrane failure, suggesting that loading is not evenly distributed throughout the CNS parenchyma.

lead to abnormal ion movement across the membrane, resulting in pathophysiological changes such as conduction block, neurofilament compaction, and impaired axonal transport (Pettus et al., 1994; Shi and Pryor, 2002). Thus, mechanical loading may directly result in pathophysiological changes.

Experimental evidence has demonstrated that the extent of membrane compromise is dependent on the magnitude and rate of strain (LaPlaca et al., 1997; Geddes et al., 2003; Shi and Whitebone, 2006). In addition, others have suggested that the mode of injury may play a critical role in dictating the extent of mechanically induced cell membrane damage (Geddes-Klein et al., 2006). After TBI, membrane disruption has been shown to occur after focal injury in a contusion model (Fig. 4) as well as diffuse loading after impact acceleration injury (Farkas et al., 2006), with patterns of marker uptake specific to the mode of impact. Because there is a correlation between injury severity and membrane compromise, permeability markers can therefore be used as an indicator of the extent of local cellular loading parameters. For example, experiments conducted in our laboratory have demonstrated more extensive permeability marker uptake in specific hippocampal regions after contusion injury, suggesting that local cellular loading is more severe in certain anatomical locations. These data may explain the preferential cell death seen in these regions in the subacute and chronic phases, as mechanical damage during the initial

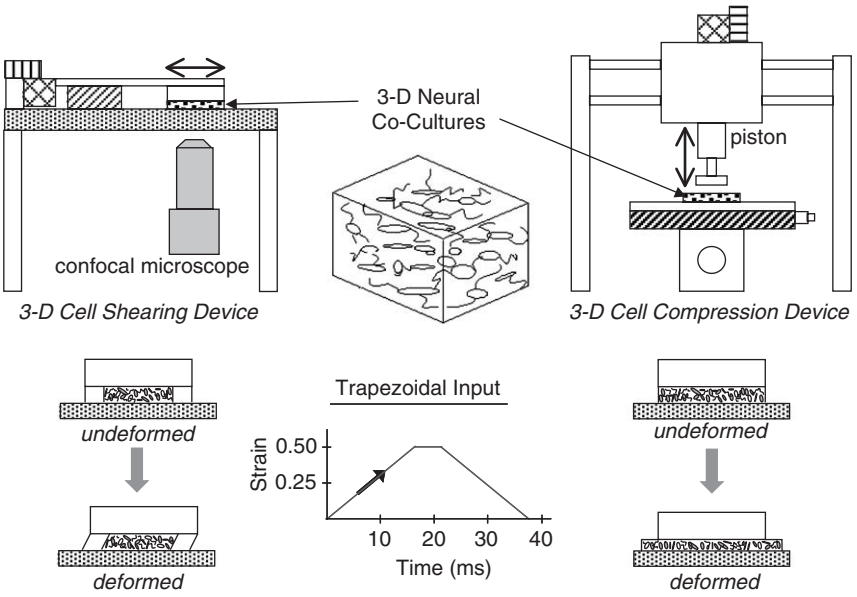
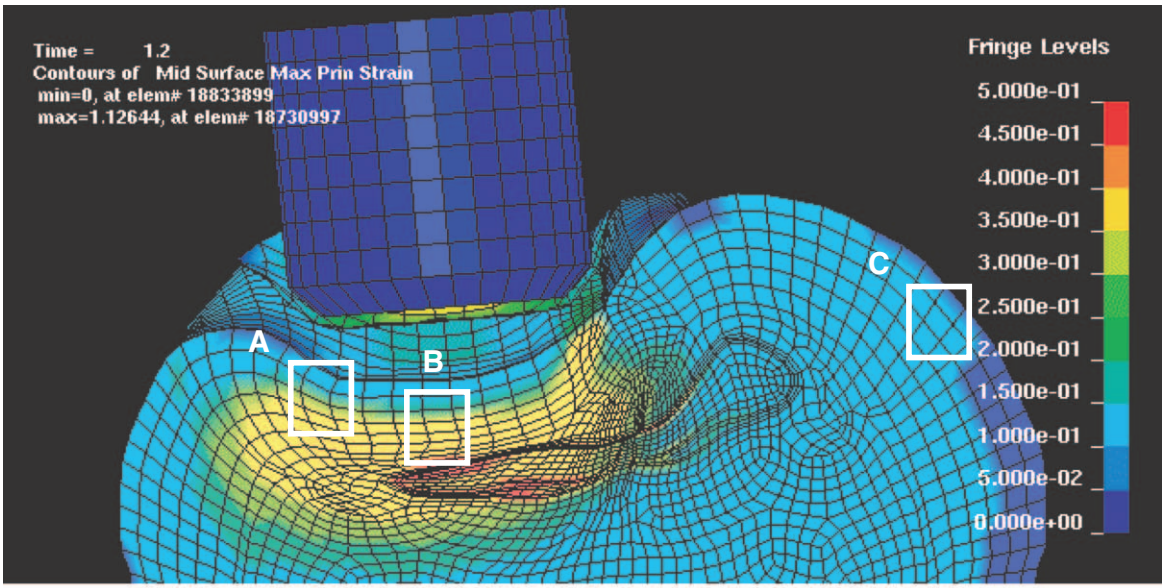


Fig. 5. In vitro injury devices for shear and compression injuries. Neural cells cultured in a 3-D configuration were subjected to either shear or compression injury with a prescribed strain magnitude and rate. This experimental model provides control over bulk material deformation, while local strains may vary due to cellular orientation within the matrix.

impact in these regions may make the cells more susceptible to death and/or dysfunction during the secondary phase of injury.

Although in vivo models can provide a more anatomically accurate representation of the structural and functional damage associated with human CNS injury, in vitro models allow for more thorough investigation of tissue tolerances because biomechanical insult parameters can be more precisely controlled and manipulated. In a recent study, the effects of both shear and compression modes of impact were investigated (Fig. 5). This is an example of how strain estimations derived from finite element analysis (FEA) can be applied to simplified culture environments to isolate components of the heterogeneous mechanical response (Fig. 6). Briefly, mixed cultures consisting of neurons and astrocytes were plated in a 3-D matrix and subjected to either shear or compressive loading (0.50 strain at strain rates of 1, 10, or 30 s^{-1}). Both types of loading resulted in significant increases in membrane permeability in a strain rate dependent manner, with no differences in the density or percentage of permeabilized cells based on mode of deformation. However, the degree of permeability marker uptake per permeabilized cell, potentially a gauge of local cellular strain/stress concentrations, was greater following shear deformation (Fig. 7). Interestingly, the density of dead cells was also significantly greater following shear deformation (5–7 fold increase) compared to compression (2-fold increase), suggesting that there is a correlation between the degree of membrane permeability and the extent of cell death. This study agrees with previous work demonstrating that shear deformation is the primary mode of tissue failure (Holbourn, 1943; Sahay et al., 1992).

Although this study evaluated cellular responses based on different modes of *bulk* deformation, local cellular strains are heterogeneous, and may be a function of cell orientation with respect to the bulk strain field (amongst other factors) (LaPlaca et al., 2005; Cullen and LaPlaca, 2006). We have demonstrated that neuronal response to loading depends on cell orientation, and hence local cellular strain, where maximal neurite loss occurred at shear-dominated strain regimes (LaPlaca et al., 2005). Ongoing in vitro studies are aimed at defining the biomechanical parameters (deformation mode, rate, and magnitude) that lead to structural



Finite element modeling of strain propagation following a focal insult (controlled cortical impact) in a rat.

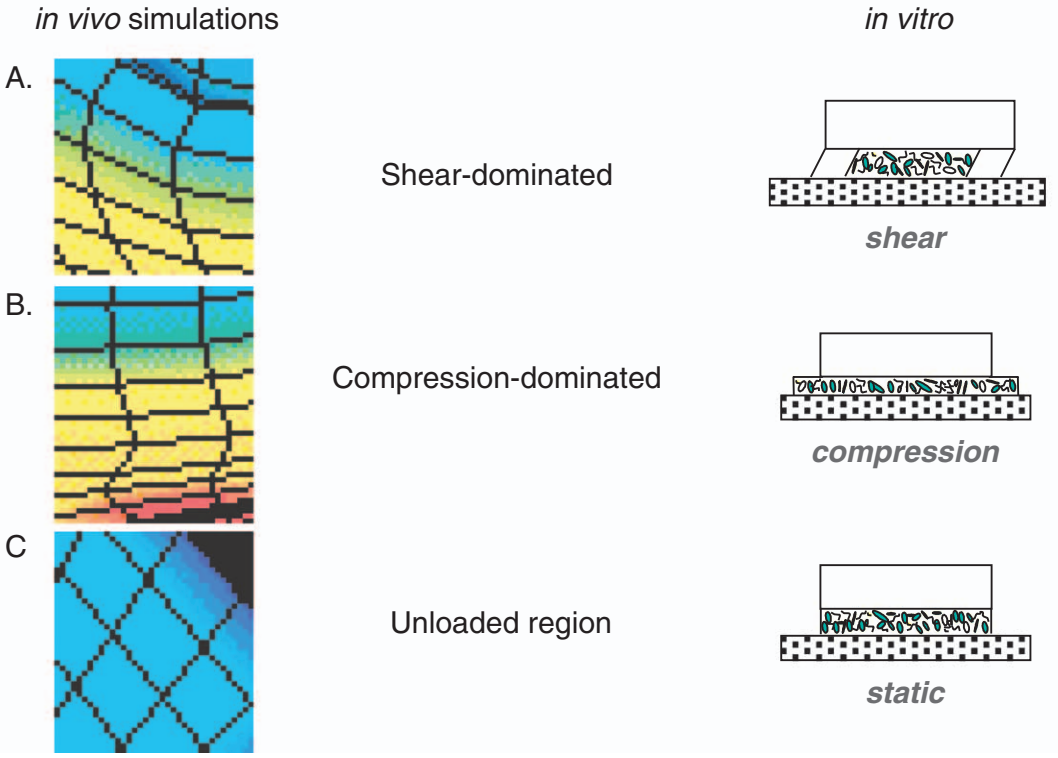


Fig. 6. Finite element model (FEM) simulations and corresponding isolation of loading components in vitro. Traumatic loading to the brain results in the generation of complex, heterogeneous strain patterns at tissue and cell levels. Heterogeneity in the cellular response to traumatic loading may be due to several factors, including mode of deformation, cell population, and cell orientation. Neural cell tolerances to traumatic loading may therefore be elucidated based on these parameters.

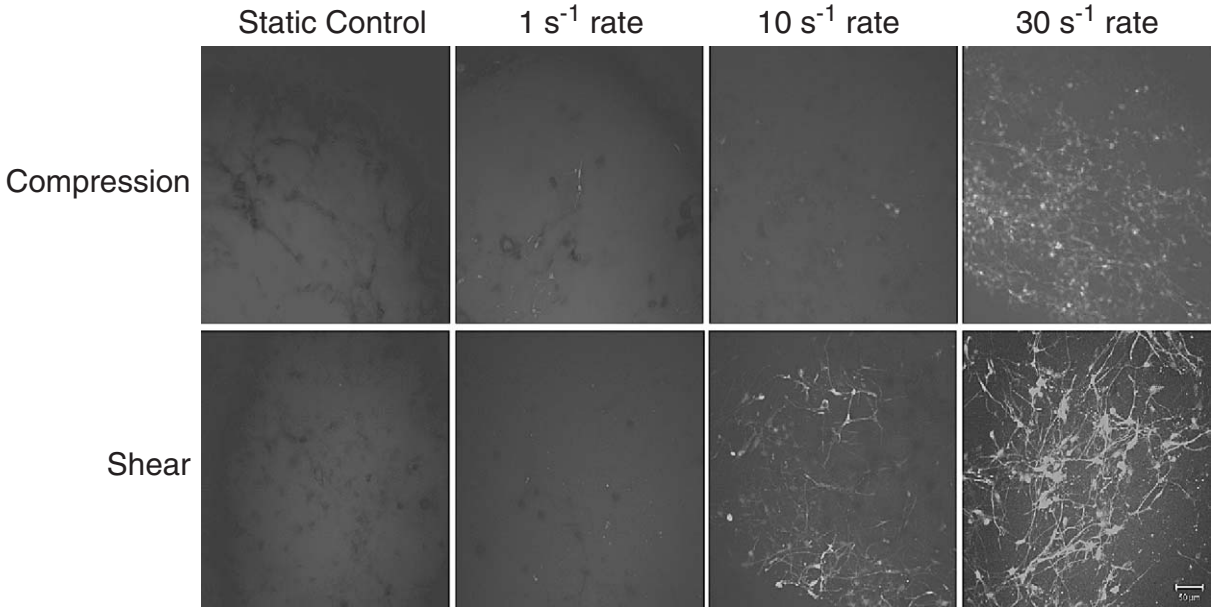


Fig. 7. Acute cellular permeability increases in vitro depend on mode of bulk loading. Representative confocal reconstructions of calcein^{+} cells following static control conditions or mechanical loading (0.50 strain at 1, 10, or 30 s^{-1} strain rate). Calcein, a normally cell-impermeant molecule, was added to the extracellular space prior to loading but becomes intracellularly sequestered following loading. Reconstructions from 50 μm thick *z*-stacks are shown here.

failure at the cellular level. Models of neural trauma that represent the related biomechanics and pathophysiology are important for the elucidation of cellular tolerances and the development of mechanistically driven intervention strategies.

Secondary response

Primary damage initiates a cascade of secondary responses, leading to cell death, network dysfunction, and system-level changes (Fig. 8). While there is no absolute time when primary damage evolves into delayed effects, the *secondary phase* of injury can be defined as any consequence of the primary insult. This may be in the acute (minutes to hours) period or in a more delayed fashion (days to months) and is dependent on the severity of the initial insult, as well as the health and age of the individual. There is a role for biomechanics in determining injury mechanisms in both the primary and secondary phases of the injury response by utilizing laboratory models that best mimic the forces/stresses and deformations/strains that occur during a traumatic insult. The response (whether cellular or whole organism) can better represent the clinical setting and therefore potential treatments can be evaluated in a more relevant setting.

Future directions

Determination of tolerance criteria for traumatic CNS injury will likely require a multilevel approach that incorporates both existing data and new knowledge from animal and cellular studies with more refined computer modeling. Computer modeling in the form of FEA can provide estimates of the mechanical response of tissue to a large range of traumatic insult parameters, allowing parametric analysis. These models need to contain anatomical detail (for both human and animals) and corresponding mechanical property data to maintain the highest possible fidelity. In addition, they should be able to simulate large, high rate deformations for both impact and inertial insult conditions. These estimated strain and stress patterns should be verified with in situ measurements when possible. This

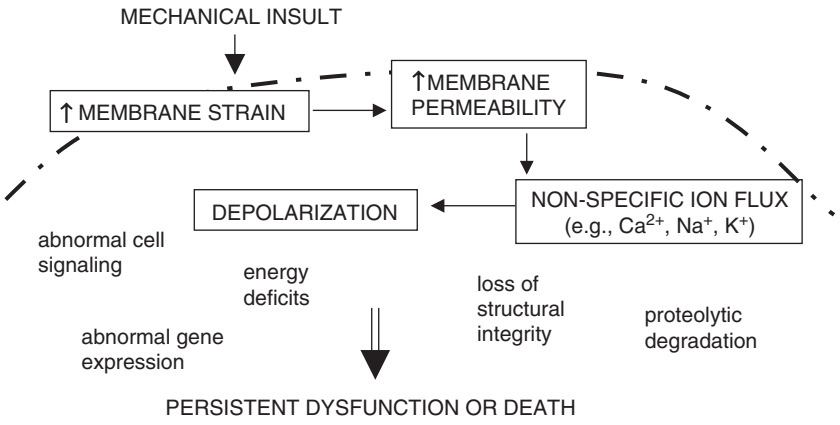


Fig. 8. Simplified schematic of injury cascades initiated by mechanical loading. Mechanical injury may directly initiate downstream pathophysiological events, but the cause-and-effect relationship has not been thoroughly explored. Plasma membrane damage is hypothesized to trigger cell death or dysfunction through the inability to regulate ion flux.

represents an experimental challenge and is worthy of consideration with new advances in nano- and micro-fabrication techniques, which permit electromechanical sensors to be instrumented. Animal models provide an opportunity to study the acute phase of injury and therefore can be correlated with estimated strain patterns in order to improve our current understanding of mechanotransduction. In addition, parallel long-term studies of delayed cell death and functional outcome can provide correlative data to acute responses. Furthermore, the cell response can be studied under very controlled conditions, and in vitro models of traumatic injury can be used to isolate elements of the mechanical response and refine our understanding of cellular tolerances. Altogether, these data (with known temporal responses) can be applied to human models of traumatic injury (with unknown temporal responses) and tolerance criteria for humans extracted and predicted for specific scenarios.

Conclusion

Given the tremendous consequences that TBI and SCI have on society, it is important to better understand the biomechanical circumstances as they relate to the physiological and clinical implications. Biomechanics can play a role in improving preventative measures such as safety design in automobiles and sports equipment, as well as highway and road safety by determining loading thresholds to the soft tissue of the brain and spinal cord. In addition to preventative strategies, biomechanics plays an important role in experimental modeling which, in turn, is vital to the development and application of mechanistically inspired pharmaceutical agents. By applying consistent and clinically relevant mechanical parameters (e.g., shear strain applied at high rates) to isolated neural cells or animal tissue, the response to mechanical disturbances can be assessed. The strain response is dependent on the tissue heterogeneity, namely the region-specific material properties and tissue orientation, therefore making elucidation of the cellular-level response to mechanical-trauma complex. The correlation of the injury response with strain enables detailed cellular tolerances that can be used to predict human injury criteria using FEA. In addition to cellular-level investigations, biomechanical models can be utilized at the animal level to achieve preclinical testing settings. Taken together, multilevel investigations can be used to eventually decrease the incidence of traumatic CNS injury and improve clinical outcomes.

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