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Orientation-Dependent Neuronal Degradation Resulting from Axonal Strain Experienced in Football-Realistic Acceleration

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ABSTRACT

Traumatic brain injury (TBI) is a common occurrence that results in neuronal death with hazardous long-term effects. Modeling TBI computationally is necessary in order to gain a better understanding of mechanical effects on neurobiological injury cascades and injury thresholds. A model of a single axon was submitted to accelerations observed in the sport of American Football to test for axonal membrane strains necessary to induce an apoptosis pathway. A neuronal membrane strain of 0.20 [1] has been found to cause a Ca^{2+} influx necessary to initiate a neuronal degradation pathway. The proposed model sought to identify if accelerations in American Football could cause such detrimental strains. To test this, forces

were applied in three directions: parallel to the axon, normal to the axon, and rotational about the axon to account for the multiple orientations forces can act upon to cause neuronal strain. Results from the different orientations with varying force magnitudes made it clear that stresses applied rotationally are the most detrimental and can cause a strain of 0.200 at an acceleration as low as 45g. Accelerations of 45g or greater are found in approximately 10% of the impacts observed in college football [2]. The resulting data from this model can be extrapolated to a larger scale to benefit the design of better head protection to include protection from shear forces.

INTRODUCTION

Traumatic brain injury (TBI) affects more than 2 million people in the United States each year and is associated with a high rate of morbidity [3]. These injuries can be caused by a variety of factors, including blunt trauma, penetrating injury, and concussive force. Many people place themselves in positions that increase the chance of such injuries, particularly those who play high-contact sports, such as American Football. Football is known to be a high-impact sport and, as a result, a large contributor to the 300,000 annual concussions reported for young adolescents in the United States [4]. However, the primary injury, such as cerebral contusions and hematomas, is not only the pathology associated with trauma; many patients suffer from a variety of secondary effects known as diffuse injuries that manifest hours, days, months, and years post-trauma. Secondary degradation arises from the inertial forces from rapid rotational and lateral motions of the head, which deform the white matter and lead to diffuse injury. The secondary effects can be as mild as a headache, dizziness, or nausea, or can be much more severe, such as the development of epilepsy or chronic traumatic encephalopathy (CTE). The vast majority of diffuse injuries evolve over time because of a series of deleterious cascades that include

the activation of proteases, second messengers, mitochondrial failures, and other apoptosis pathways [5]. This time delay makes secondary injury detection very difficult because initial brain scans are unable to show the full extent of the injury. Diffuse axonal injury (DAI) has been pinpointed as the primary focus for secondary brain injury. Disruption of the axon is an important pathology in mild, moderate, and severe traumatic brain injury [5]. The effects of high-impact collisions, especially head-to-head collisions, frequently witnessed in football games are mitigated by the use of a helmet as a form of head protection, yet the helmet does not fully protect the players from TBI. The secondary degradation observed in athletes in high-contact sports poses a major risk because these athletes can initiate DAI and neuronal degradation pathways without detection. Computational models are indispensable tools for gathering neurobiological data non-invasively in order to better guide neural protection strategies for these athletes.

Computational models for TBI have progressed from macroscale to microscale via simulations of the brain to neuronal initiation of DAI. When the brain experiences an impact, the force propagates through each individual cell of the brain, inducing both longitudinal and shear stresses upon each cell. This in

turn induces strain upon the neurons which begins an apoptosis pathway, resulting in DAI and loss of neuron functionality. The strain on the membrane creates a calcium influx into the cytoplasm, which leads to the phosphorylation of tau proteins and, ultimately, the aggregation of microtubules within the axon. Geddes and Cargill evaluated the dependence of intracellular calcium concentrations from applying various magnitudes of strain on neural cells. In order to obtain micromolar concentration fluctuations, a strain of 0.20 must be applied to the neuronal membrane [1]. These calcium fluctuations are significant enough to simulate an axonal degradation pathway as shown in Figure 1. Calcium fluctuations activate u-calpain, an isomer of a calcium-dependent cysteine protease. Calpain activation stimulates the proteolytic cleavage of p35, a neuron-specific activator for cyclin-dependent kinase 5 (CDK5), turning it into p25 [6]. This conversion of p35 to p25 creates a more stable protein with a longer half-life. Therefore, the generation of p25 causes prolonged activation of CDK5 through the p25-CDK5 complex. This complex actively phosphorylates tau proteins, unlike the p35-CDK5 complex, and disrupts the normal regulation of this tau phosphorylation pathway [7]. Tau proteins are structural microtubule-binding proteins that localize in the axon on a neuron [8]. Hyperphosphorylation of tau proteins leads to an abnormal amount of condensed microtubules into paired helical bundles. This high association of tau proteins with microtubules has been correlated to DAI and other axonal injury diseases, such as

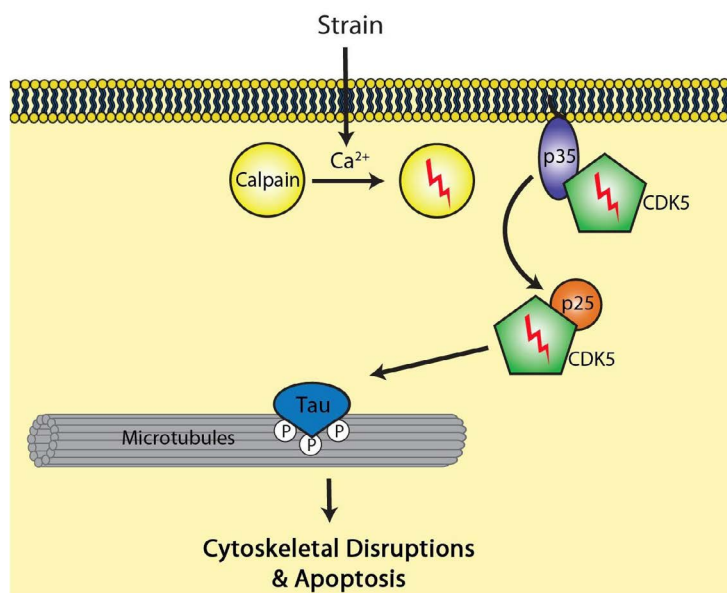


Figure 1: Biological pathway demonstrating the affect of a calcium influx on the phosphorylation of tau proteins resulting in cytoskeletal disruption and neuronal death

Alzheimer's Disease. It has been proposed that a change in the microtubule network via the association of phosphorylated tau proteins affects the transport of proteins and other intracellular components along the axon, which leads to a pathway that cleaves the axon [9]. Therefore, by applying accelerations to obtain a strain of 0.20 on the axonal membrane, a calcium dependent pathway leading to the hyperphosphorylation of tau proteins can be triggered, leading to loss of neuronal functionality.

The current standard to prevent TBI in activities with a high likelihood of sustaining an impact to the head, such as football, is the use of a protective helmet, typically composed of an outer plastic shell with cushioning foam on the inside. This current strategy functions by cushioning and slowing the brain during impact, decreasing the longitudinal stress on the neurons. However, this does not take into account the shear stresses applied to the neurons, a major cause of TBI. In order to further develop preventative measures for TBI and DAI that address the issue of shear stress, it first has to be known what stresses, particularly shear, induce the apoptosis pathways. This model attempts to find these forces necessary to induce these apoptosis pathways so that these results can then be extrapolated to help guide preventative measures directed at TBI.

METHODS

Model design

We decided to model the neuron in three dimensions as it is the most realistic way of approaching the design of the neuron. Our initial model was of both the axon and the myelin sheaths, modeled as viscoelastic isotropic materials with properties found in literature and recorded in Table 1. To make the model as true to reality as possible, we included the nodes of Ranvier, which we modeled as gaps between the cylindrical myelin sheaths coating the axon, and the ends of the myelin sheaths were tapered into the nodes. After demonstrating the strains are localized in the node, the model was reduced from the full axon model all the way down to a single segment of axon to alleviate the issues of meshing and computational time as seen in Figure 2. There were no intra- or extracellular structures or dendrites included because we were most focused on the nodes of Ranvier and how the stress is concentrated in that particular region. Our primary goal was to prove whether or not the nodes of Ranvier are under the most stress and are most affected by a rotational force versus a longitudinal or normal force, and so we only needed to model the basics - the axon, the myelin sheath, and

the nodes. After solving several models of this form, we decided to simplify our model to be a singular node, making solving in COMSOL more efficient.

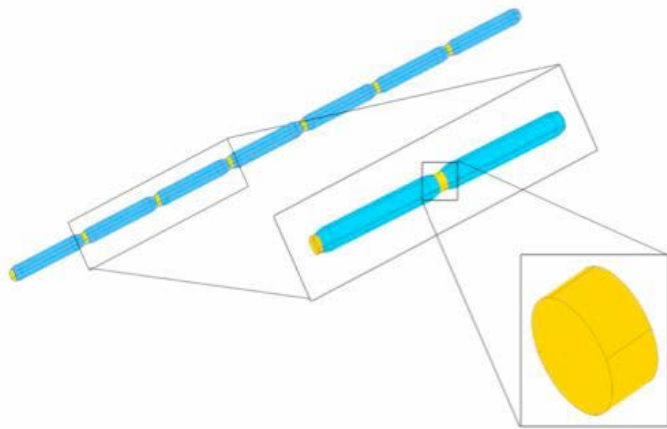


Figure 2: Schematic demonstrating how the model was simplified after gaining appropriate justification

Force Applications

We modeled three different neuron orientations with respect to load to model the primary ways in which the neuron may be affected in a head-on collision often seen in football. Our models depicted a force along the longitudinal axis of the axon, a force normal to the axon, and a rotational force on the axon. Figure 3 shows a schematic of the three different force applications. We chose these three orientations as they most accurately depicted the broadest generalizations of force application in head-to-head collisions.

The magnitudes of the forces applied were determined by taking three characteristic accelerations observed in football and converting them using Newton's second law. The mass of an axon was estimated using our model's volume and density taken from literature. These forces were converted

into stresses with units of N/m^2 by dividing the forces by the surface area of the side of interest.

Additionally, we applied a sinusoidal acceleration in a similar fashion to how a force was applied normally to the axon to test if oscillations would affect the viscoelastic axon differently than applying direct forces. An appropriate frequency was found by using the equation $F=Af^2m$, where F is a force equivalent to a known acceleration, A is the amplitude of the oscillation, f is the frequency, and m is the mass of the object. We used a force of $50 N/m^2$, which resulted in a 0.200 strain in the normal analysis, a given amplitude of 2 mm, and the mass of our proposed model to find an f of 138 Hz. This frequency was used to apply a sinusoidal acceleration, given by the equation $A\sin(f2(\pi)t)$, applied normal to the axon's membrane. Of course, there are other ways in which the axon may be affected that have not been explored here, including if the force is exerted on the neuron at an angle, but these three modes of force translation are the general ways in which a neuron may be affected.

RESULTS

When first starting with the axon and a single node of Ranvier, applying a rotational force and a normal force to this model showed that the myelin sheaths undergo the greatest displacement, yet there was a disproportionate strain at the node. Figure 4 shows a slice plot confirms that the node undergoes the greatest stress. These results justify refining the model to focus only on the node since it is the weakest portion of the neuron. Reducing the model to a single node allows for more a reasonable computational time. To identify which forces induced the greatest strain and DAI on the model, the reduced node model was subjected to three forces: a longitudinal force, a normal force, and a rotational force.

In the longitudinal model, the force was

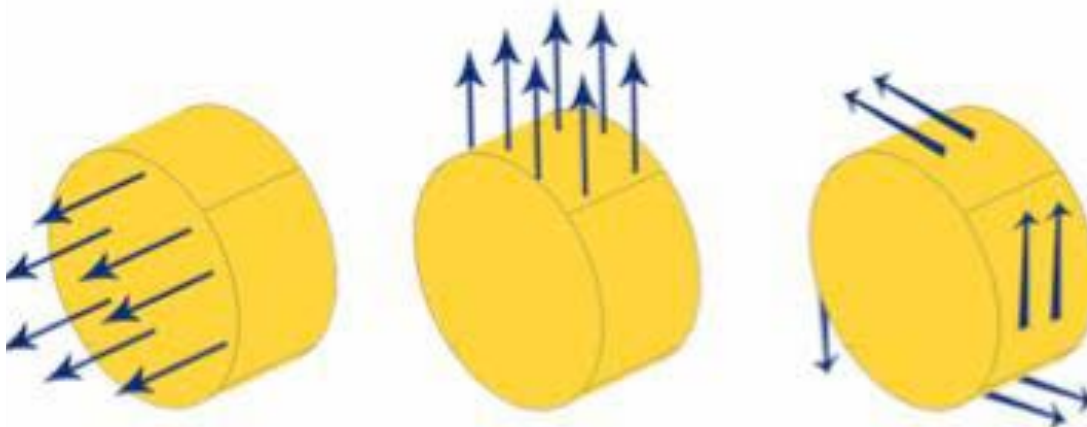


Figure 3: Node model under longitudinal (left), normal (middle), and shear (right) stresses

applied to the cross-sectional face of the node. When an acceleration of 120g, the maximum acceleration measured in intercollegiate football impacts, was applied to the model in a longitudinal fashion, it produced a strain of 0.129 as shown in Table 2, which is below the strain indicative of DAI. The strain distribution can be seen in Figure 5. Because this directional force could not produce a large enough calcium influx to cause secondary degradation, no further magnitudes of accelerations were tested.

When acceleration was applied to the model in the x-y plane, normal to its side edge, it was found that an acceleration of 101.38g causes a 0.200 strain, the approximate threshold for inducing the DAI pathway. These values can be seen in Table 3. The displacement and strain plot is shown in Figure 6. Accelerations of 101.3g corresponds to a 50% chance of getting a concussion. Therefore, the hundreds of thousands that have concussions each year are at risk of inducing this axonal degradation pathway for long-term damage.

For another model, acceleration was applied to the model in the x-y plane in a rotational manner about the center axis. Four different values of linear acceleration were applied – 120g, 80g, 45.16g, and 40g. The stress ranged between 20.6 and 61.79 N/m², with maximum stress occurring at the maximum acceleration and minimum stress occurring at the minimum acceleration, which is to be expected. The displacement ranged between 1.512*10⁻⁷ meters and 4.195*10⁻⁷ meters, with maximum displacement occurring at the maximum acceleration and minimum displacement occurring at the minimum acceleration, which is to be expected. The values of strain ranged between 0.185 and 0.553, with the target value of 0.200 occurring at a linear acceleration of 45.156g. More detailed values are described in Table 4. Figure 7 shows the stress distribution when a rotational linear acceleration is applied to the node of Ranvier.

The acceleration that was found to produce a strain of 0.200 in the normal direction was the acceleration used in the frequency analysis. This linear acceleration of 101.38g was applied to the nodular model as a sinusoidal frequency, resulting in 50 N/m² of stress applied to the node. The total displacement was 1.056*10⁻⁸ meters, resulting in a 0.166 strain. Figure 8 depicts the strain distribution on the nodular model resulting from normal stress applied in a sinusoidal manner.

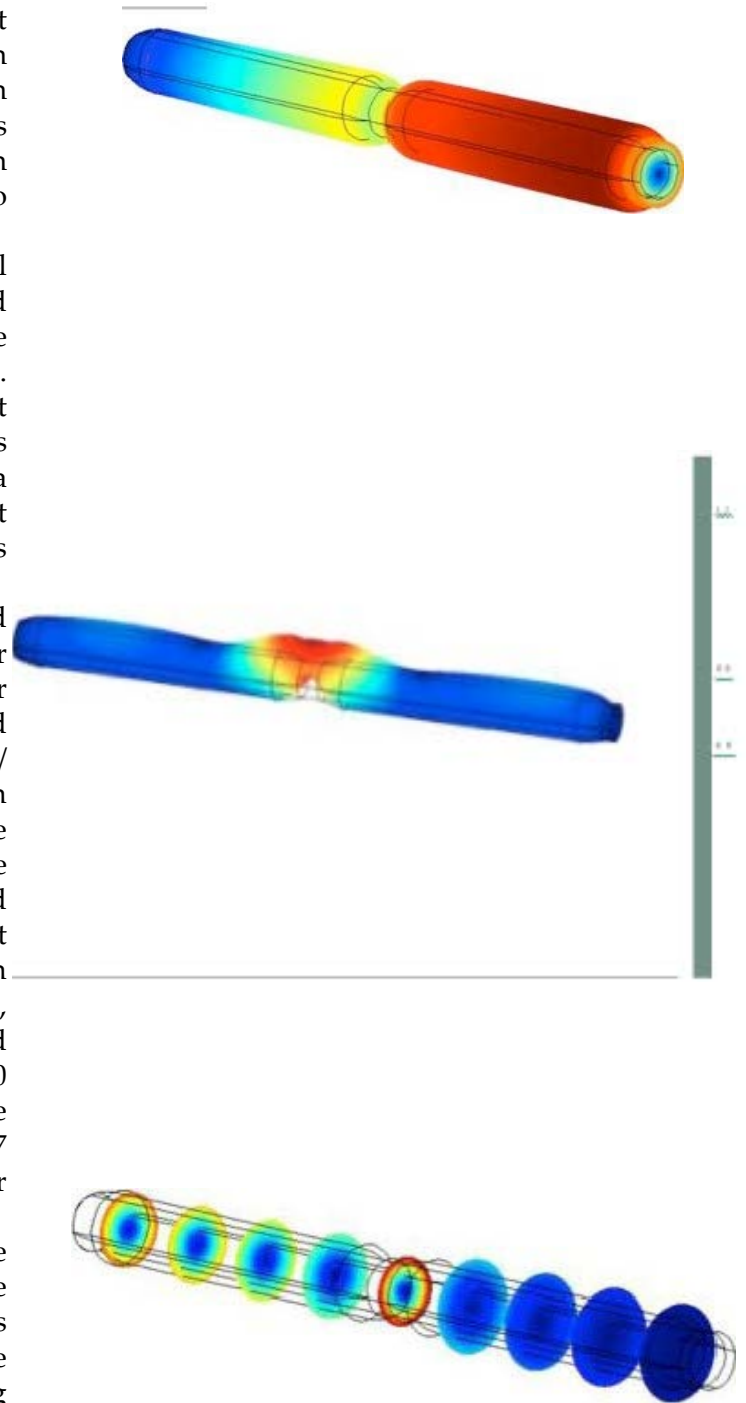


Figure 4: (top) Axon model under rotational stress showing greatest displacement concentrated at myelin sheaths and a disproportionate displacement at the node of Ranvier. (middle) Axon model under normal stress showing maximum displacement at node of Ranvier. (bottom) Axon model under rotational force showing stress through slices along the axon, proving the highest stress is concentrated at the node of Ranvier.

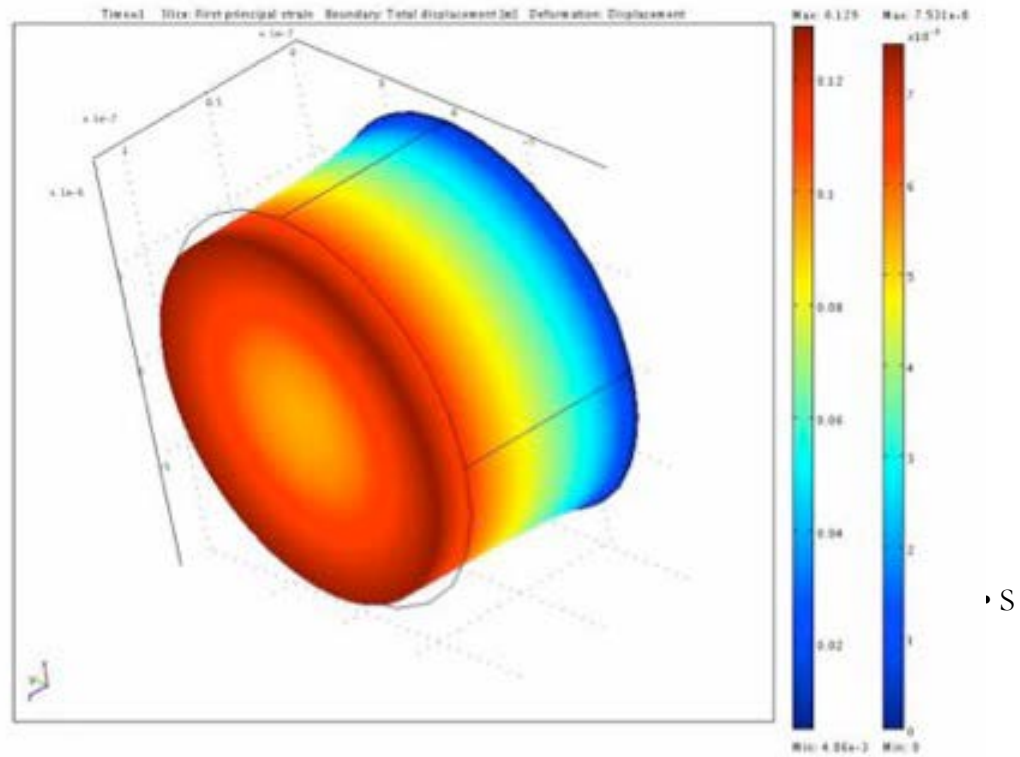


Figure 5: (Image of COMSOL node model showing strain distribution resulting from longitudinal stress application

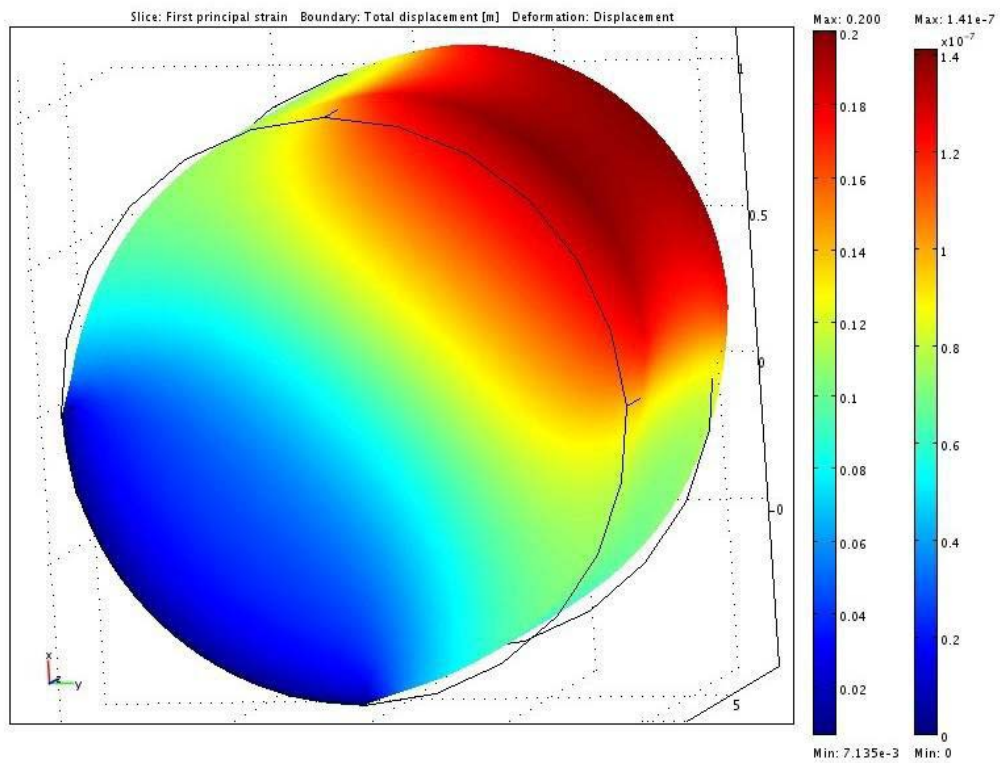


Figure 6: Image of COMSOL node model showing strain distribution resulting from normal stress application

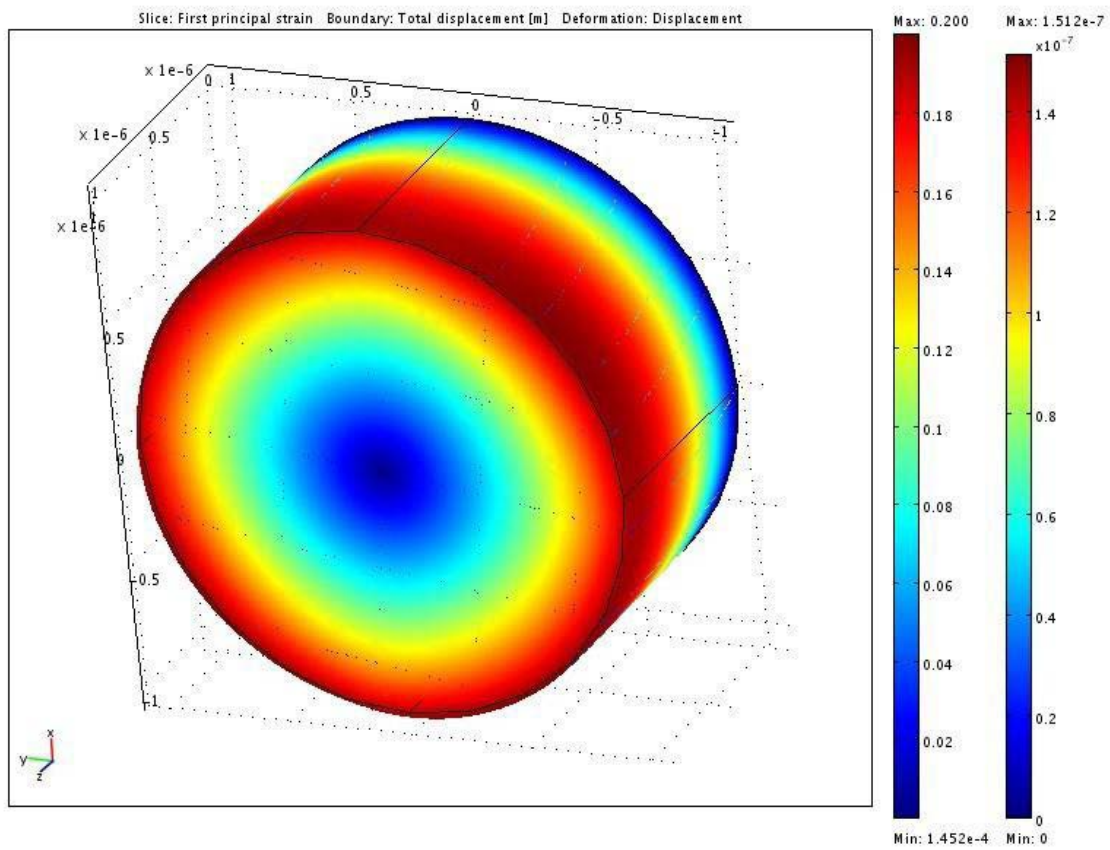


Figure 7: Image of COMSOL node model showing strain distribution resulting from rotational stress application

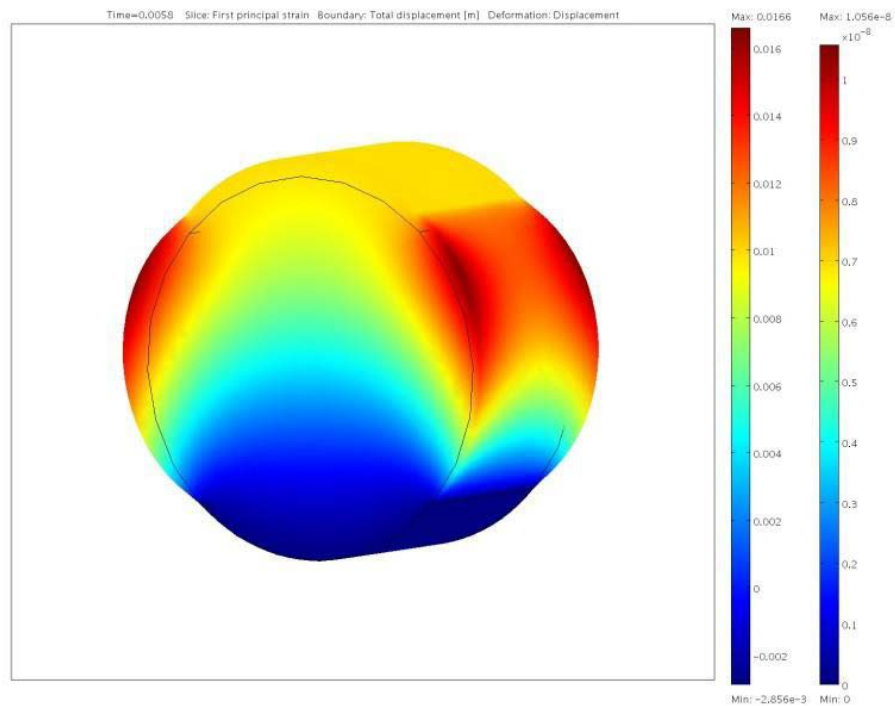


Figure 8: Image of COMSOL node model showing strain distribution resulting from a normal stress applied in a sinusoidal manner

DISCUSSION

In the original simulation modeling an axon with multiple nodes of Ranvier, the stress concentrates at the nodes rather than dissipating equally along the axon when the stress is applied normal to the axon. Furthermore, when the stress was applied rotationally to the axon, the myelin sheath was observed to displace the most, and the part of the axon covered by the myelin sheath was relatively unharmed. This finding agrees with the fact that one of the primary purposes of the myelin sheath is to act as a protective insulator surrounding the axon. The shear modulus of the myelin sheath is one-third of the shear modulus of the axon, which shows that myelin is a soft malleable coating to the axon that helps to dissipate the stress where the myelin is present. Additionally, the stress distribution was observed at several cross-sectional areas along the axon, and it was found that the node of Ranvier clearly felt the greatest magnitude of stress. This observation allowed us to simplify our model further and focus solely on the weakest part of the axon - the node of Ranvier.

The three simulations modeling a rotational force, a normal force, and a longitudinal force provided great insight as to what stresses are necessary to induce a 0.200 strain and, therefore, axonal degradation. The rotational model only required a stress of 22.27 N/m² in order to induce a 0.200 strain, whereas the normal model required a 50 N/m² stress, and the longitudinal model required a stress representative of an acceleration greater than those witnessed in collegiate football collisions. From these results, it is evident that rotational and shear forces on the axon cause damage at significantly lower stresses than forces applied linearly. Furthermore, the stresses we found necessary to induce the DAI pathway were translated into accelerations that the neuron would feel in a football-related collision. Translating the applied rotational stress gave a proposed linear acceleration of 45g necessary to initiate hyperphosphorylation of the tau proteins, while if the force is purely normal to the axon, the necessary acceleration was approximately 101g. Rowson et al. observed that 10% of the impacts in collegiate football were greater than 40 g in severity [2]. This means that roughly 10% of the hits in football could potentially initiate calcium influxes that result in secondary degradation of neurons even though the players might not experience concussions.

In order to demonstrate that these results could be tested in vitro, the force necessary to create 0.200 strain in the model utilizing a linear normal force application was applied in a sinusoidal manner, which represents the brain's oscillations during the course of an impact. Applying this force sinusoidally

resulted in a 0.166 strain, comparable to that generated from the direct force application. This shows that these computational models can be replicated in vitro utilizing a shaker plate model, with relative similarity in results.

Shear injuries, such as those commonly observed in football, cause more damage to the axon due to the rotational force component and thus are more likely to cause TBI. Compared to other directional forces, shear injuries have a significantly lower force threshold necessary to cause the same amount of strain. This makes hyperphosphorylation of tau protein induced by shear forces increasingly more common. Because these shear injuries are more likely to induce a wide range of long-lasting effects on football players, including TBI, it is even more important to find a way to improve the current preventative techniques against shear injuries.

CONCLUSION

Through applying a variety of stresses on a finite element model simulating an axon, important structural and mechanical properties were determined. Most importantly, the model demonstrated the ability of myelin sheaths to act as a protective border for the axon. This observation led to the discovery that the nodes of Ranvier were thus subjected to the greatest stress, as they were the only part of the axon not protected by the myelin. The model was then further simplified in accordance to this finding so that only the weakest part of the axon would be studied. This simplified model, representing a single node of Ranvier, established forces applied rotationally as the most detrimental compared to longitudinal and normal. This finding suggests that football players can be subjected to diffuse axonal injury at relatively low accelerations not even indicative of concussions. The forces experienced in 10% of collegiate hits can induce a calcium influx into neurons large enough to induce hyperphosphorylation of tau proteins, which leads to secondary axonal degradation.

Because of the danger of secondary degradation that such shear stresses can cause, it is recommended that more effective helmets at preventing shear forces be developed and implemented for use in football. Additionally, this strain-induced pathway allows for secondary protection through a chemical regulation of the tau pathway. By blocking the phosphorylation pathway for a limited amount of time, these players would reduce the long-term risks of secondary degradation. By combining both a chemical and mechanical approach, axonal degradation could greatly be reduced in high impact sports including football.

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