



US DEPARTMENT OF DEFENSE

BLAST INJURY RESEARCH PROGRAM COORDINATING OFFICE

Preclinical Models of Blast Injury Mechanical/Electrical/Chemical Response of Biological Cells to Blast and Impact

Traumatic brain injury (TBI) occurs when a primary insult causes brain deformation resulting in secondary injury and ultimately neuronal degeneration and death. Data suggest that injury to the brain is susceptible to both the strain and strain rate of the insult (*Margulies and Thibault 1992, Margulies, Thibault, and Gennarelli 1990, Meaney et al. 1995*). To understand how altering the rate of injury affects secondary injury, researchers at the Army Research Laboratory (ARL; Aberdeen Proving Ground, Maryland) cultured primary hippocampal neurons on silicone membranes and stretched them using the cell injury controller II (Figure 1).

First, two-dimensional strain tensor of the membrane, at three different pressures (23, 47 and 63 pounds per square inch), and three different times to peak (25, 50 and 75 milliseconds), was measured using digital image correlation (Figure 2). Strains increased as either pressure or time to peak increased. Maximum principle strain rates were also obtained during the rising part of the strain waveform. Using these strain rates and interpolating between these measurements, maximum principle strain rates were obtained for each pressure and time to peak used in the experiments (Figure 3). Researchers identified the region of the membrane with equal-biaxial tensile strain with negligible shear strain through calculated principal strains. Only the middle region (8-10 millimeter diameter) of the membrane experiences equal-biaxial tensile strain and will be utilized for analysis in cell culture studies (Figure 4).

Second, hippocampi were harvested from embryonic day 18 (E18) rat embryos, cultured and subjected to a biaxial stretch of 10 millimeter peak deformation at three different rates, 10-12 days in vitro (Table 1). Structural damage to neurons was assessed using immunocytochemistry of microtubule associated protein 2 (MAP2). Qualitative analysis of preliminary data showed neurons stretched at three different rates (albeit all relatively low) exhibit evidence of dendritic beading suggesting that at low strain rates, 0.2 or above, strains are sufficient to induce beading (*DiLeonardi, Gunnarsson, and Weerasooriya 2017*). Experiments to visualize functional deficits are in progress using live cell imaging to visualize changes to cellular transport (Figure 5).

The objective of this work was to determine thresholds of cellular injury following controlled mechanical loading. These thresholds will be used to create injury criteria which will better inform numerical models, evaluate future protective equipment, and provide further insight into cellular mechanisms of traumatic brain injury, thus providing a better understanding of the link between how the load resulting from a blast or impact affects the brain.

This study was funded by ARL.





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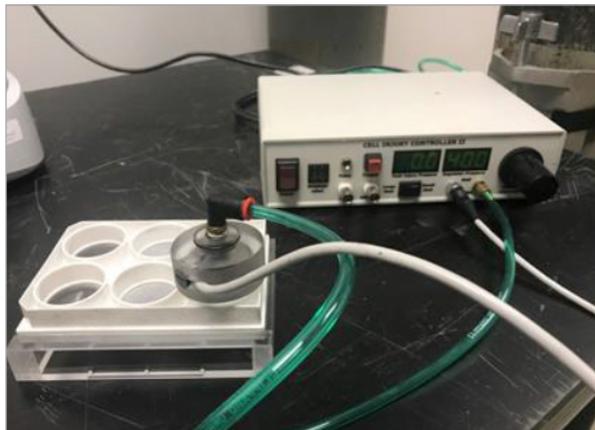


FIGURE 1: Cell Injury Controller II device delivers a pressure pulse to stretch cells adhered to a silastic membrane. (Figure used with permission from the authors)

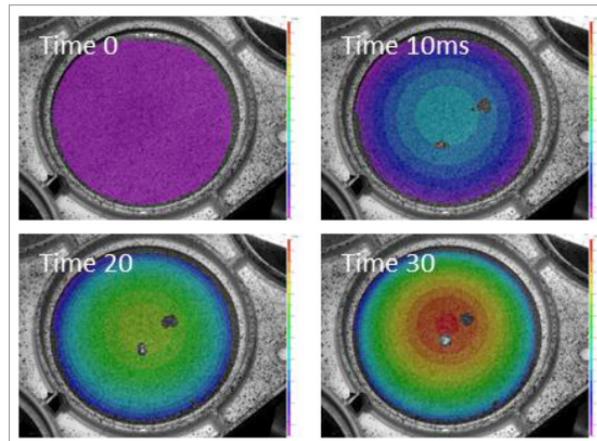


FIGURE 2: Example images captured from using digital image correlation every 10 millisecond shows the z-direction displacement contours. (Figure used with permission from the authors)

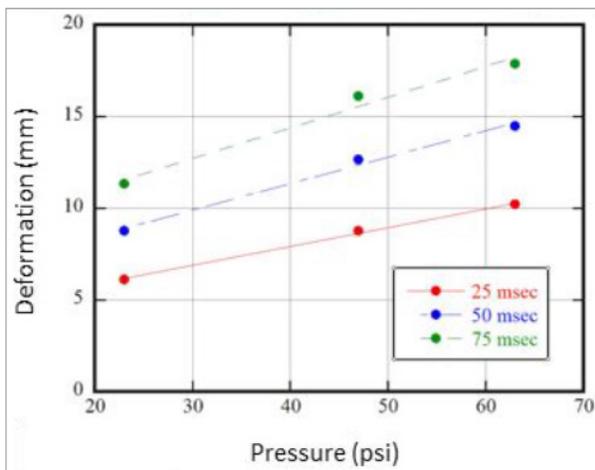


FIGURE 3: Membrane deformation as a function of pressure. (Figure used with permission from the authors)

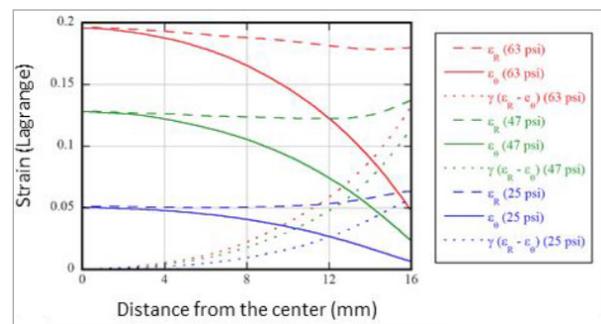


FIGURE 4: Identify the region of the membrane with equal-biaxial and negligible shear strain through calculated principal strain rates. Only the middle region (8-10 millimeter diameter) of the membrane experiences equal-biaxial tensile strain and will be utilized for analysis in cell culture studies. (Figure used with permission from the authors)





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TABLE 1: Table denotes the parameters used to stretch cells using the cell injury controller II. All three levels stretch the membrane to 10-millimeter deformation in the z direction but do so at different rates. (Table used with permission from the authors)

	Pressure (psi)	Time to Peak (ms)	Strain Rate (s ⁻¹)
Level 1	60	25	~7.8
Level 2	30	50	~5
Level 3	13.7	75	~3

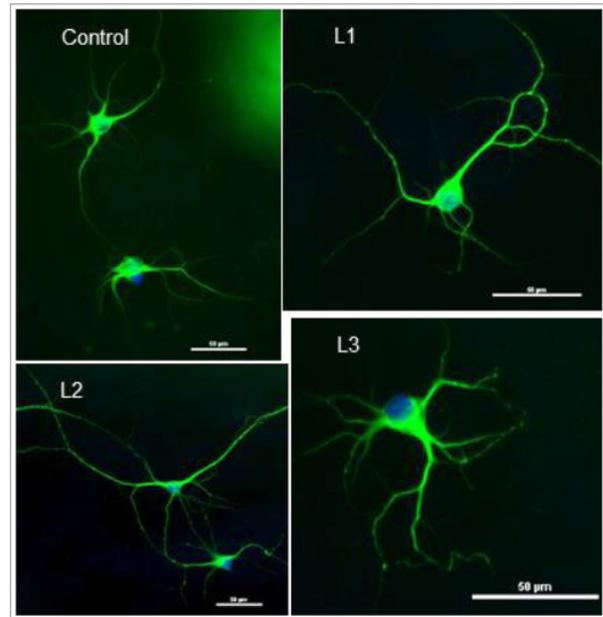


FIGURE 5: Immunocytochemistry for MAP2 to visualize structural alterations of cells revealed injured neurons at all levels exhibited dendritic beading. (Figure used with permission from the authors)

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