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Quantification of Functional Aalterations after *In Vitro* Traumatic Brain Injury

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***Abstract*—Traumatic brain injury (TBI) is caused by mechanical forces, producing tissue deformation at the moment of injury. Complex cellular, neurochemical and metabolic alterations are initiated by the deformation and result in delayed cell death and dysfunction. Using an *in vitro* model of TBI based on organotypic brain slice cultures, we have quantitatively studied the relationship between tissue deformation and functional outcome. Specifically, we studied the effects of low levels of tissue deformation on the functional outcomes as measured by electrophysiology recordings. In response to 5% and 10% biaxial Lagrangian strain, the maximal evoked response and the excitability of neural networks were found to be decreased. Additionally, the different anatomic subregions of the hippocampus displayed different levels of impairment to the injuries. These results suggest that the network function was affected by low levels of applied strain which induced minimal cell death in previous studies.**

I. Introduction

Traumatic brain injury is a major public health problem, which is mainly due to motor vehicle accidents, falls and firearms. Over 1.4 million Americans experience a TBI every year. In this study, using our *in vitro* TBI model which allows for the precise control of tissue deformation[1-3], the relationship between the mechanical inputs that initiate TBI and the functional outcomes was tested.

The mechanically-induced injury model was developed using organotypic brain slice cultures to study TBI in a complex tissue culture preparation [4;5]. We previously developed cell death tolerance criteria for hippocampal slice cultures after mechanical stretch, and demonstrated that the posttraumatic cell death increased with applied strain [1;2;6]. Cell death in the hippocampus and cortex was dependent on strain [2;6], and the cortex was found to be much less vulnerable to mechanical deformation than the hippocampus.

In addition to cell death, neurons may become dysfunctional after injury, causing deficits in network function. Therefore, this study tested whether low levels of tissue deformation (5% and 10% biaxial Lagrangian strain) would affect the electrophysiological outcome of the hippocampus. The strains that we applied to the tissues were less than or equal to 10%, which has been demonstrated to induce only minimal cell death measured by propidium iodide staining in our previous studies [2;6]. Therefore, we were able to test whether functional electrophysiological changes could be induced in the absence of overt cell death.

We found that posttraumatic changes in function of the hippocampus were induced at both 5% and 10% strain and were correlated with strain. The maximal evoked response was decreased and the stimulus current required to evoke a half maximal response was increased in all three subregions of the hippocampus, including the CA1, CA3, and dentate gyrus (DG). These data suggest that mechanical deformation can disrupt the cellular/network function without concomitant cell death. These data have implications for the design of safety systems to prevent injuries, especially in the automotive environment, through incorporation with finite element models.

II. Methods

A. In vitro TBI Model

The *in vitro* biaxial-stretch model was developed previously in our laboratory [3]. In this model, brain tissue was cultured on a highly stretchable silicone membrane and was injured by precisely controlled, simultaneous biaxial stretch. The strain and strain rate of stretching can be controlled to generate various injury severities. Deformation was quantified by calculating Lagrangian strain from high speed video of the dynamic event:

$$E_{\text{Lagrangian}} = \frac{1}{2}\left(A_{max} / A_o - 1 \right) \qquad (1)$$

where the total area of the hippocampal tissue before stretch (A_0) and during stretch (A_{max}, at the maximum strain) was measured with ImageJ software.

B. Organotypic Hippocampal Slice Cultures

All animal procedures were approved by the Columbia University IACUC. The culture technique was developed previously in our laboratory [3]. Briefly, the hippocampus of a post-natal day 9 rat pup was removed aseptically and cut into sections 400 μm thick with a McIlwain tissue chopper (Brinkmann Instruments). Slices were transferred to a Millicell (Millipore Inc.) and silicone membrane complex pre-coated with laminin and poly-L-lysine. Cultures were

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maintained in a standard cell-culture incubator (37ºC, 5% CO_2).

C. Electrophysiological Recordings

At 6 days post injury, the neural activity was recorded and compared to age-matched, uninjured cultures. Cultures were transferred to pre-coated, 60 electrode microelectrode arrays (MEA, MultiChannel Systems) and perfused with artificial cerebrospinal fluid (aCSF in mM: 125 NaCl, 3.5 KCl, 26 $NaHCO_3$, 1.2 KH_2PO_4, 1.3 $MgCl_2$, 2.4 $CaCl_2$, 10 glucose; pH = 7.40) , which was bubbled with 95% O_2/5% CO_2 and pre-warmed to 37 °C. Neural signals were recorded at a sampling rate of 20 kHz and low passed with a 5 kHz analog, anti-aliasing filter. A programmable stimulator (MultiChannel Systems) generated constant current stimuli (100 μs per phase) to evoke responses.

Stimulus response (S/R) curves were generated for each culture[7]. Bipolar, biphasic stimuli of varying intensities (0μA-200μA in 10μA steps) were applied to the tissue through electrodes in the mossy fiber region. Mossy fibers are located in the hilus and are the cell processes of the cells in the dentate gyrus. Tissue response was recorded from all channels simultaneously, and the amplitude of the field potential response quantified as peak to peak. The response magnitude at each electrode was plotted versus stimulus intensity (Fig. 1) and then fit to a sigmoid function:

$$R(S) = \frac{R_{\max}}{1 + e^{m \bullet (I_{50} - S)}} \qquad (2)$$

R_{max} was the maximum response, I_{50} the current which produced a half maximal response, S the intensity of the stimulus, and m was proportional to the slope.

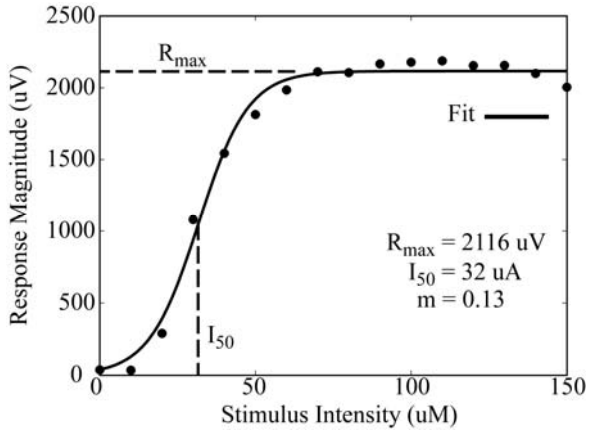

Fig. 1. The percentage change in I_{50} in the hippocampus (CA1, CA3 and DG regions) as a function of applied tissue strain (w/ standard error). The stimulus intensity to obtain 50% maximal response increased as injury severity increased, indicating that even 5% strain affects neuronal function of the hippocampus

III. Results

The evoked response in hippocampal slice cultures was affected at 6 days post injury as compared to uninjured, age matched controls. As shown in Fig.2, the R_{max} value was decreased after injury, indicating a decrease of the maximal evoked response even after 5% strain. In the CA1 region, R_{max} was decreased 40% and 51% after 5% and 10% strain, respectively. In the CA3 region, R_{max} was decreased less after 5% strain (7%), but was decreased after 10% strain by 54% similar to the CA1 region. In the DG, a 5% strain induced a 12% reduction of R_{max}, whereas 10% strain induced a 39% reduction.

The threshold intensity to obtain 50% maximal response (I_{50}) increased after injury compared to uninjured controls (Fig.3). In the CA1 region, I_{50} was increased 30% and 57% after 5% and 10% strain, respectively. I_{50} was increased in the CA3 region by 46% and 79% after 5% and 10% strain, respectively. I_{50} was increased in the DG by 23% and 49% after 5% and 10% strain, respectively.

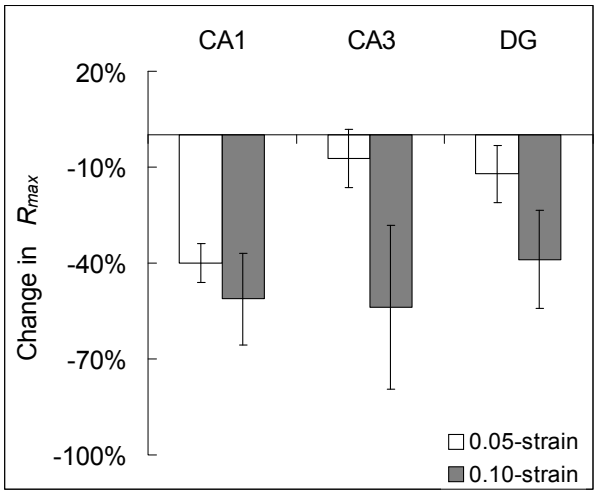

Fig. 2. The percentage change in R_{max} in the hippocampus (CA1, CA3 and DG regions) as a function of applied tissue strain (w/ standard error). The maximal evoked response decreased as injury severity increased, indicating that even 5% strain affects neuronal function of the hippocampus.

IV. Discussion

Our previous studies have determined the relationship between tissue deformation and cell death (measured by propidium iodide staining) for hippocampal and cortical slice cultures [2;6], and thereby the injury-induced cell death would lead to changes in function. However, it is still unclear whether cell death is a requirement for causing the disruption of neural circuits. Therefore, we applied biaxial strains to hippocampal slice cultures to generate mild injuries which induced minimal or no cell death, as determined from our earlier studies and quantified changes in neuronal activity after injury, to test whether the low level of tissue deformation could disrupt the normal function of neural

networks.

The maximum response (R_{max}) decreased in all regions, and the decrease was correlated with injury severity. This decrease in R_{max} indicated the attenuation of the capacity of the tissue to respond to electrical stimuli. This change is usually taken as evidence of nerve cell loss, however, after

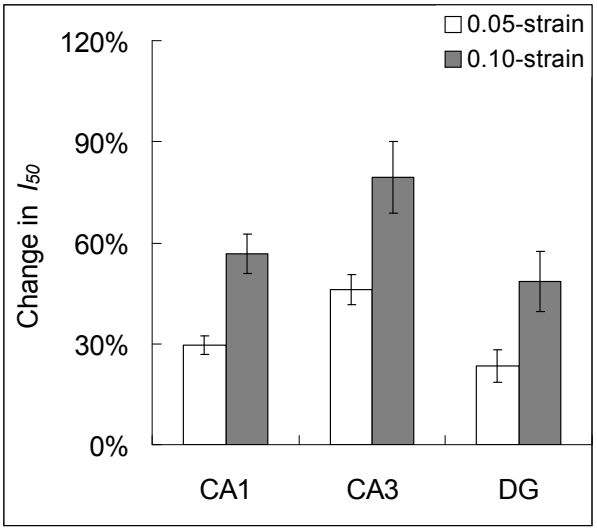

Fig. 3. The percentage change in I_{50} in the hippocampus (CA1, CA3 and DG regions) as a function of applied tissue strain (w/ standard error). The stimulus intensity to obtain 50% maximal response increased as injury severity increased, indicating that even 5% strain affects neuronal function of the hippocampus.

mechanical stimulation, cultures appeared healthy and without cytotoxic edema (i.e. absence of tissue swelling and darkening) which normally accompanies cell death. An alternative explanation is the attenuation of synaptic transmission caused by the mechanical stimulus, in the absence of cell death.

The threshold intensity to obtain 50% maximal response (I_{50}) was found to be increased in all regions, and the increase was also correlated with injury severity. This increase in I_{50} indicated the reduced excitability of the neuronal networks. This finding suggests that the machinery responsible for neuronal function was damaged. For example, there could be some post-synaptic mechanisms involved in this change such as proteolysis of post-synaptic neurotransmitter receptors or damage to voltage sensitive ion channels. An alternative explanation is that pre-synaptic mechanisms were involved with the net effect of reduced vesicular release of neurotransmitters after injury, thereby increasing the number of failed neurotransmission events.

MEAs were used for this study to quantify altered function after injury, taking the advantage of measuring activity throughout the slice to study long-range connectivity. Simultaneously quantifying neural transmission within the tri-synaptic circuit, which runs from the DG to the CA3 and finally to the CA1, was possible by employing MEA with 60 microelectrodes to interface within the entire circuit. In addition, recording separately from different subregions of the hippocampus is more appropriate since the CA1, CA3 and DG subregions have been shown to respond differently to mechanical inputs [2;6].

The effects of mechanical tissue deformation on electrophysiological function have been studied previously. In a study of CNS white matter injury due to elongation of the optic nerve, significant changes in visually evoked potentials were observed after a 25-30% elongation [11]. And the induction of morphological signs of damage was found after larger elongations of 35% strain. These strains, although substantially higher than those used in the current study, were uniaxial, whereas the strains in our study were biaxial, which could result in activation of different cell responses. Moreover, axons within the optic nerve are arranged in a sinusoidal pattern which could straighten before the axons are subjected to stretch, thus those fibers within the white matter could be less susceptible to damage with increasing tissue strain due to the micro-architecture of the tissue.

In most other studies of functional alterations after TBI *in vivo*, tissue deformation cannot be controlled as precisely, although injury severity can be graded. In these studies, considerable cell death was found after injury[15;16], and the evoked response magnitude and the excitability of the CA1 region was found to be decreased after injury[12-15]. These results agree with our current findings. However, we observed that these functional changes occur in the absence of cell death, suggesting that the neural network function can be affected by injury with no cell death involved. Previous studies have found that an approximately 40% cell death throughout the hippocampus after injury were accompanied with reductions in R_{max} [15;16].

The DG region has also been reported to be dysfunctional after injuries displaying hyperexcitability [17-20]. However, our results indicate a decreasing excitability in the DG region after injury. The differences could be due to the following two reasons. First, the previous studies quantified the DG function in response to perforant path stimulation, whereas we stimulated the tissues through the mossy fiber pathway due to the limitation of hippocampal slice cultures that the perforant path is usually not maintained in culture. Different neural transmission pathway may respond differently to mechanical injuries. Second, the hyperexcitability of the DG which was observed *in vivo* has been attributed to a selective loss of inhibitory interneurons [19;20], whereas our results did not show loss of any particular sub-type of neuronal cells. The low strains may be disrupting neural transmission throughout neural networks within the hippocampus, thereby resulting in the increased I_{50} and decreased R_{max} that we observed. Higher levels of strain greater than 10% may then be required to induce the loss of inhibitory interneurons to induce hyperexcitability of the DG, as reported elsewhere.

V. Conclusion

Our work found that low levels of mechanical deformation

affected neuronal function with no cell death, indicating that even mild events may lead to a quantifiable neuronal network dysfunction. One possible conclusion is that neuronal function is a more sensitive measure of injury than cell death. Our results will be significant for the development of safety systems designed to protect against TBI.

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