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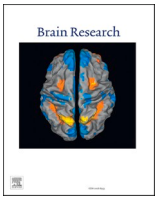

Physical memory of astrocytes

Tasnim Shireen [a], Frederick Sachs [b], Susan Z. Hua [a,b,*]

[a] Department of Mechanical and Aerospace Engineering, University at Buffalo, Buffalo, NY 14260, USA
[b] Department of Physiology and Biophysics, University at Buffalo, Buffalo, NY 14260, USA

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ABSTRACT

Traumatic brain injury (TBI) is a major risk factor for development of neurodegenerative disorders later in life. Short, repetitive, mechanical impacts can lead to pathology that appears days or months later. The cells have a physical "memory" of mechanical events. The origin of this memory is not known. To examine the properties of this memory, we used a microfluidic chip to apply programmed fluid shear pulses to adherent adult rat astrocytes. These caused a transient rise in intracellular Ca^{2+}. In response to repeated stimuli, 6 to 24 hrs apart, the Ca^{2+} response increased. This effect lasted longer than 24 hrs. The Ca^{2+} responses were more sensitive to the number of repetitions than to the rest time between stimuli. We found that inhibiting the Ca^{2+} influx during conditioning stimulus did not eliminate the stress potentiation, suggesting that mechanical deformation during the primary injury is accountable for the later response. The mechanical mechanism that triggers this long term "memory" may act by plastic deformation of the cytoskeleton.

1. Introduction

Traumatic brain injury (TBI) is a significant public health concern and one of the leading risk factors for development of chronic neurodegenerative disorders including Alzheimer's disease (AD) (Cruz-Haces et al., 2017; Sivanandam and Thakur, 2012). In sports, repetitive mild and sub-concussive head impacts increase the risk of chronic traumatic encephalopathy (CTE) later in life (DeKosky and Asken, 2017; Harmon et al., 2013; Zetterberg et al., 2019). Despite statistical evidence, the risk of developing chronic diseases after TBI is unpredictable. The severity of chronic diseases does not correlate with the magnitude of TBI but does with recurrence (Ikonomovic et al., 2019; Smith et al., 2013). The insults during mild TBI are typically short, but produce a prolonged effect we label "memory" (Bell et al., 2009; Maneshi et al., 2015). This memory could be the result of plastic deformation of the cytoskeleton system or from cascades of downstream processes involving biochemical and metabolic dysfunctions (Chen et al., 2009; Maneshi et al., 2015; Smith et al., 2013).

During TBI, brain cells experience transient stresses that deform the cytoskeletal structures with elastic and plastic deformation of cross-linking proteins (Bursac et al., 2005; Gardel et al., 2004). Using fluorescent force probes, we previously reported that astrocytes subjected to short (millisecond) shear pulses showed an immediate and long lasting increase of tension in the cross-linker α-actinin (Maneshi et al., 2018). The increases in tension were visible throughout the cell at times > 10 s, suggesting plastic deformation (Maneshi et al., 2018). This plastic deformation alters the force landscape of the cytoskeleton.

External force produces a Ca^{2+} influx and associated neurotransmitter release via mechanosensitive ion channels and receptors during TBI (Fineman et al., 1993; Mills et al., 2004; Ravin et al., 2012; Shapira et al., 1989). This overload of cytosolic Ca^{2+} may initiates biochemical cascades leading to secondary injury and cell death. Disruption of Ca^{2+} homeostasis can modify numerous biochemical reactions including ion transport and ER storage (Hilge, 2012; Weber, 2012), neuronal functions (Danoff et al., 1991; Ruiz et al., 2009), ATP-dependent membrane pumps (Davalos et al., 2005; Unterberg et al., 2004), mitochondria and apoptosis (Orrenius et al., 2003; Pivovarova and Andrews, 2010; Singh et al., 2006). It has been reported that stretching Purkinje neurons induces a GluR2 endocytosis that causes an increase in Ca^{2+} response to AMPA in long-term (Bell et al., 2007). This stimulus promoted the cells' permeability to AMPA incited Ca^{2+} uptake (Bell et al., 2007). The disruption of Ca^{2+} homoeostasis and Ca^{2+} pathways could account for the complex downstream reactions.

We previously reported that in astrocytes, fluid shear stress incites a Ca^{2+} influx through mechanosensitive NMDA receptors and Piezo1-type channels in the absence of agonists (Maneshi et al., 2017). Rapid shear pulse generates non-uniform forces in cells that can increase the open probability of mechanosensitive ion channels (MSCs) in force focusing

* Corresponding author at: 340 Jarvis Hall, University at Buffalo, Buffalo, NY 14260, USA.
E-mail address: zhua@buffalo.edu (S.Z. Hua).

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regions (Maneshi et al., 2018). This leads to our hypothesis that the early TBI-like mechanical events could cause plastic deformations in the cytoskeleton, increasing the susceptibility of MSC activations in the cells for a long time. This mechanical property of cells can provide the mechanical 'memory'.

Astrocytes are the most abundant cell type in the brain, and it plays a critical role in brain injury. During TBI, astrocytes propagate damage signals via Ca^{2+} waves, signal to neurons through neurotransmitters, and alter ion concentrations in the extracellular space (Chen and Swanson, 2003; Niggel et al., 2000). They also shown progressive changes of gene expression, morphology and functions, involving secondary cellular injury (Burda et al., 2016).

In this study, we use high-speed programmable microfluidic chips to apply repeatable fluid shear stimuli and measure Ca^{2+} responses in primary rat astrocytes. We show that the Ca^{2+} response to a second stimulus was significantly higher than the first, and the effect further enhanced with multiple additional stimuli. The effect lasted longer than our detailed observation period of 24 hrs. To discriminate Ca^{2+} effects from mechanical deformation, during the first stimulation, we inhibited Ca^{2+} influx using reversible channel inhibitors. This did block Ca^{2+} uptake during the conditioning pulse but did not remove cell memory suggesting that the cells are not using Ca^{2+} to store the early information.

2. Results

2.1. Stimulus history affects astrocyte response to fluid shear stimuli

Using the chips, we measured the Ca^{2+} response to fluid shear pulses (23 dyn/cm^2, 10 ms), 6 hrs apart, that served as conditioning and test stimuli (Fig. 1a). The first pulse produced a sharp transient in Ca^{2+}. The response to the second stimulus was ~ 40 % greater than the first (Fig. 1c,d, red curves), showing the cells have a memory of the early mechanical event. Control cells lacking a conditioning pulse but otherwise following the same measuring protocol showed a typical level of response (Fig. 1c,d, gray curves). The results were consistent across

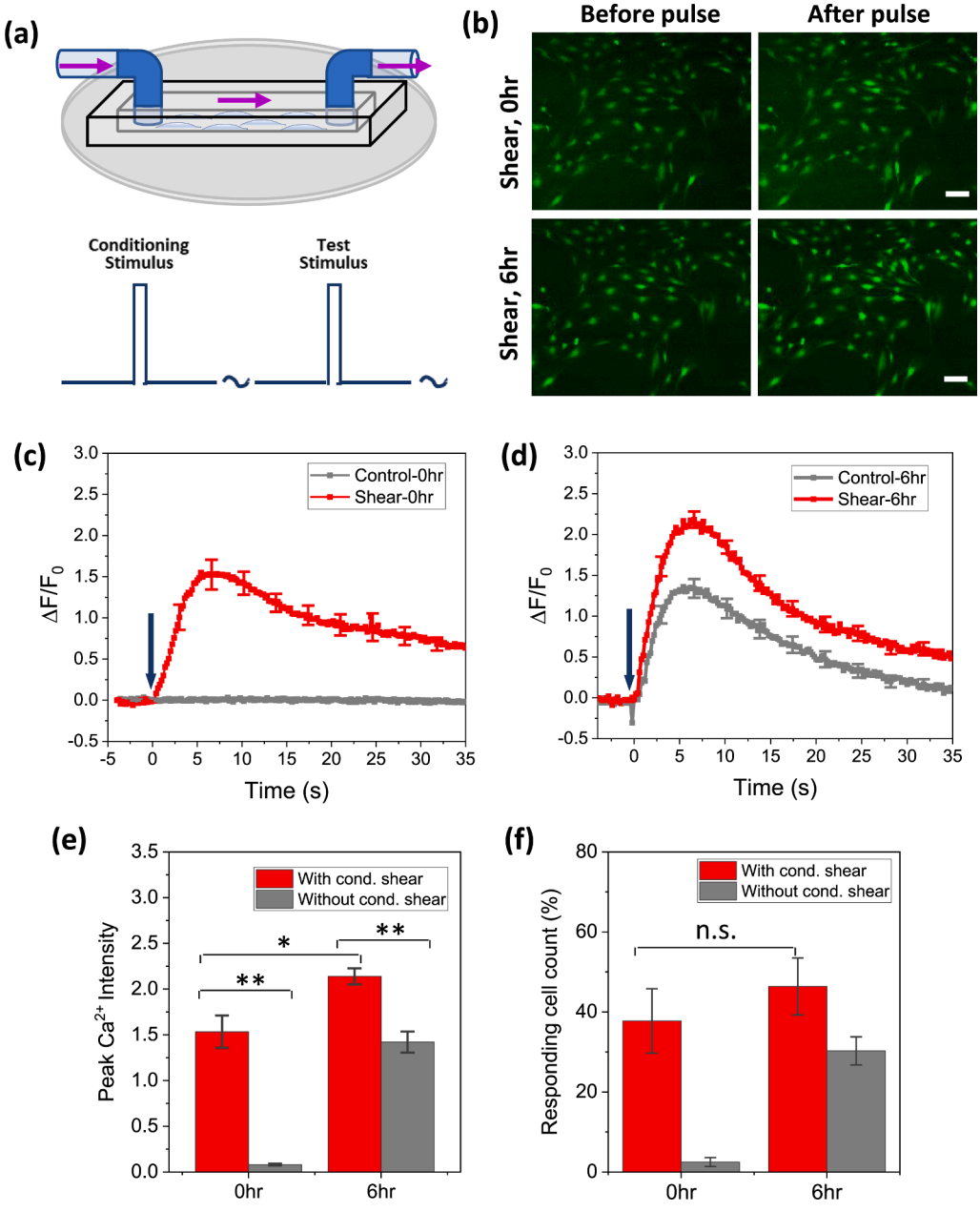

Fig. 1. Effect of stimulus history on subsequent Ca^{2+} response in astrocytes. (a) Schematics of the microfluidic chip and the stimulus waveform that was applied repeatedly. **(b)** Typical fluorescence images showing Ca^{2+} response to conditioning (0 hr) and testing (6 hr) shear pulse of 23 dyn/cm^2 and 10 ms wide from cells on the same chip. Scale bars indicate 20 μm. **(c, d)** Ca^{2+} response to conditioning (0 hr, **c**) and testing shear pulses (6 hr, **d**). The cells showed a stronger response at 6 hr with conditioning pulse (red curves) compared to control (without conditioning pulse, gray curves) (n = 60, averaged over 6 experiments for each condition). The arrow indicates the time when shear pulse was applied. **(e)** Mean Ca^{2+} peak values of repeated stimuli for shear and control experiments (n = 60, *p < 0.005, **p < 0.001). **(f)** Percentage of responding cells for each experiment. The number of responding cells increased slightly with second stimulus (n = 6 for each condition).

multiple preparations (Fig. 1c,d, n = 60 with six experiments for each condition). The effect of a conditioning stimulus on the later Ca^{2+} response is statistically significant (Fig. 1e, n = 60 for all conditions, *$p < 0.005$, **$p < 0.001$). The fraction of responding cells increased slightly by the second stimulus (Fig. 1f, 6 experiments for each condition). Some cells didn't respond to shear stress possibly because those cells were better reinforced by the cytoskeleton raising the threshold for activation (Maneshi et al., 2015). The shear pulses did not cause notable changes in cell adhesion or viability within the experiment periods (Fig. 1b). A rapid shear pulse generates heterogeneous forces in cells that may cause plastic deformation of the cytoskeleton (Maneshi et al., 2018).

To evaluate the lifetime of the cells' memory, we varied the interval between the conditioning and the test pulses to 6, 12, 18, and 24 hrs. The cells consistently showed a stronger response to the later stimuli. The test Ca^{2+} peaks were ~ 40 % higher than the conditioning response for all durations (Fig. 2a-d). Statistical analysis for multiple experiments showed that the effect was significant (Fig. 2e, *$p < 0.005$, **$p < 0.001$). The cells' memory clearly is > 24 hrs. To test if the memory requires time to form, we tested a short interval of 1 hr. The cells showed similar increased response to second stimuli (SM Fig. 1). We have previously shown that astrocytes can respond to low amplitude shear pulse train with 1 s separation, that pulse alone was not able to produce Ca^{2+} peaks (Maneshi et al., 2015).

2.2. Repeated stimuli increased cell susceptibility

To assess whether the cell memory is cumulative, we applied the shear pulses sequentially at 0, 6, and 24 hrs to the same group of cells, and found that the responses increased consistently with repetition (Fig. 3a). The second Ca^{2+} peak (6 hr) was ~ 40 % higher than the first one (0 hr), and the third peak (24 hr) was ~ 30 % higher than the second one (Fig. 3b). The results are consistent with multiple experiments (Fig. 3d, *$p < 0.005$, **$p < 0.001$). In contrast, without conditioning pulse cells showed lower responses at 6 and 24 hrs compared with pre-sheared cells, but the responses ramped up with repeated stimuli (Fig. 3c). Cells not only have long-term memory, but the effect is cumulative. As expected for a potentiating stimulus, the number of responding cells increased with repeated stimuli (Fig. 3e). Two types of changes may contribute to the incremental rise of Ca^{2+}, the additional plastic deformations of cell cytoskeleton that changes the local settings of MSCs, or an accumulation of Ca^{2+} in ER stores due to Ca^{2+} uptakes. We could rule out standard biochemistry since the concentration of reactants appears to be increasing and not decreasing as one might expect for repeated stimuli. Possibly increased mechanosensitive storage/release of Ca^{2+} as some enters with each stimulus. The following

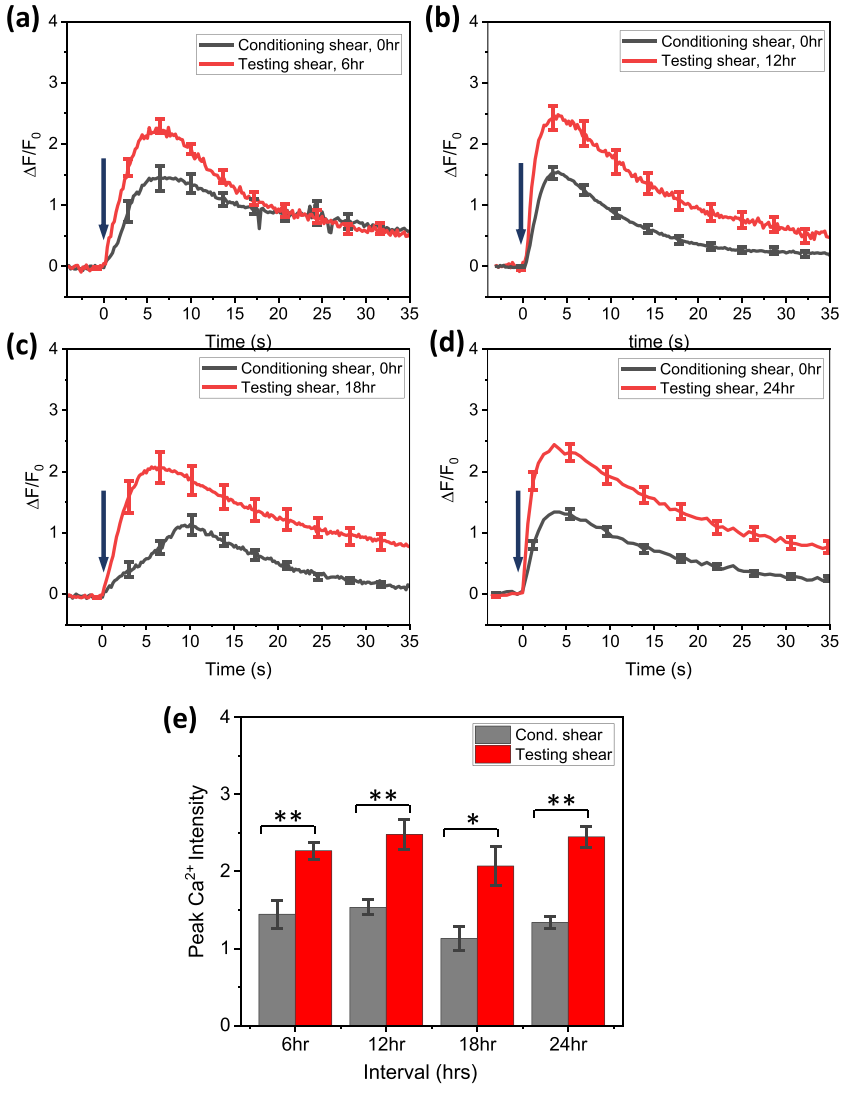

Fig. 2. Effect of stimulus interval on cells' memory. Two shear pulses (23 dyn/cm^2) with various intervals were applied to each preparation. **(a-d)** The Ca^{2+} responses to conditioning pulse (0 hr, gray curves) and testing pulse (6 hr, red curves) separated by 6 hr **(a)**, 12 hr **(b)**, 18 hr **(c)**, and 24 hr **(d)**, showing the responses to the second stimuli are consistently higher. **(e)** Mean Ca^{2+} peaks from two shear stimuli of different intervals (n = 50, 50, 30, 100 for 6, 12, 18, 24 hrs intervals, respectively, *$p < 0.005$, **$p < 0.001$).

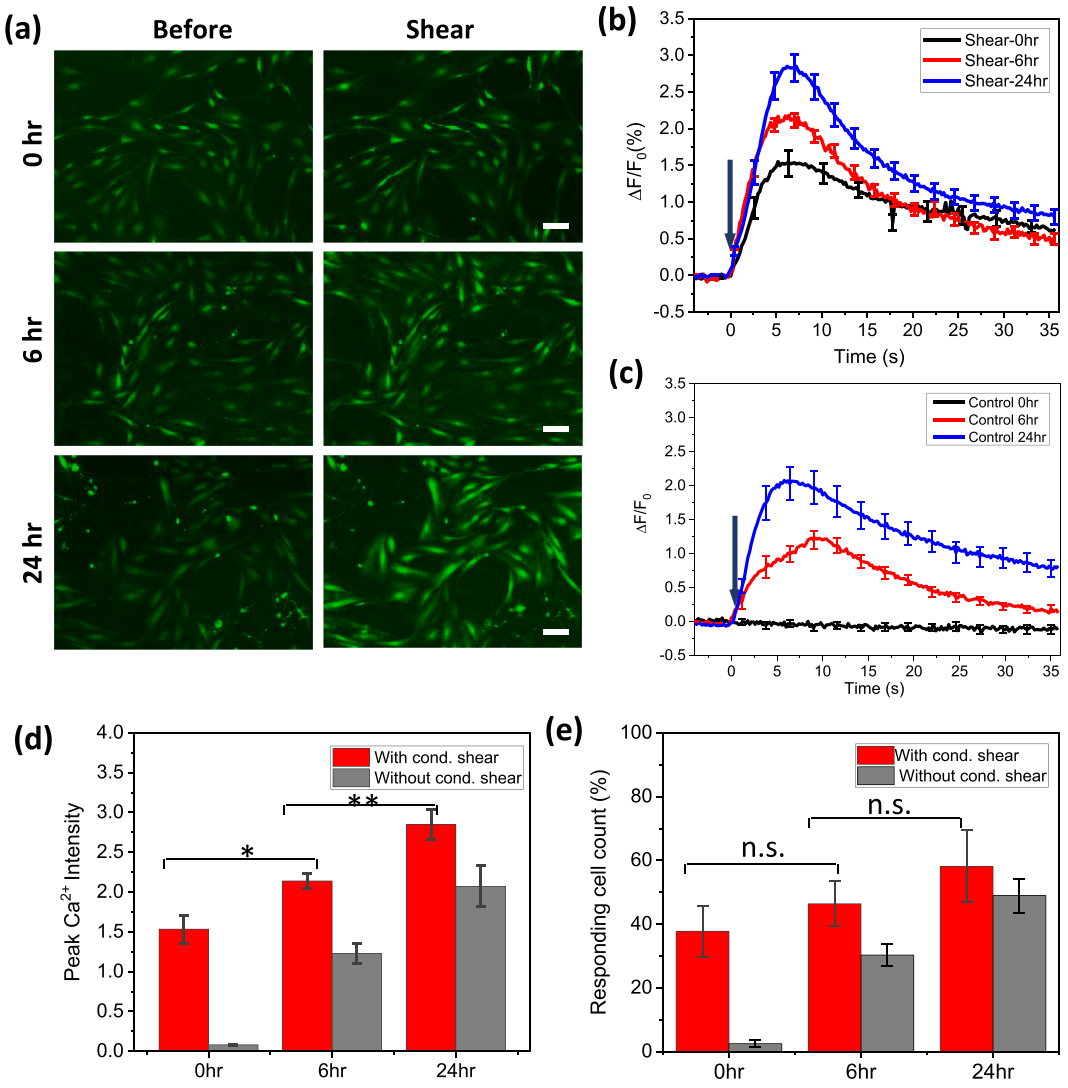

Fig. 3. The Ca^{2+} response to repetitive fluid shear stimuli. (a) Ca^{2+} images before and after a shear pulse of 23 dyn/cm^2 applied to the same chip at 0, 6, and 24 hrs. **(b)** Ca^{2+} responses to repeated shear pulses, each curve was averaged over 6 experiments (n = 60). It shows the response increased consistently with repeated stimuli. The Ca^{2+} dye (Flou-4-AM) was freshly loaded for each measurement. **(c)** Same experiments as **(b)** but without the conditioning stimulus (n = 40). Without conditioning stimulus, the cells showed lower responses at 6 and 24 hrs. **(d)** Statistical analysis of mean Ca^{2+} peak values in response to repeated stimuli (n = 60 for shear test, n = 40 for controls, $*p < 0.005$, $**p < 0.001$). **(e)** Percentage of responsive cells to each stimulus, which increased slightly with the number of repetitions. Scale bars indicate 20 μm.

experiments will show that to be unlikely.

2.3. *Repeated sub-threshold forces can be registered by cells*

To understand whether low magnitude stimuli can be registered and stored by cells, we applied repeated sub-threshold shear pulses (15 Dyn/cm^2, 10 ms) (Maneshi et al., 2015) at 0, 6, and 24 hrs, and measured the Ca^{2+} response. Only < 15 % of the cell population responded to a single stimulus of this magnitude. Nine experiments fell into three characteristic groups based on cell behavior. In Group-1, cells did not respond to any of the three repeated stimuli, a typical response is shown in Fig. 4a. In Group-2, cells did not respond to the first stimulus, but responded to the second and the third stimuli (Fig. 4b). In Group-3, the cells responded to all three stimuli with increasing magnitude (Fig. 4c). Peak Ca^{2+} for each group is summarized in Fig. 4d. In all experiments ~ 50 % cells were not able to respond to at least three repeated stimuli at this level. Among the responsive cells, most of them responded to the third pulse. This data shows that the cells can register and memorize near-threshold forces when they are subjected to repetitive challenges. These data provide the evidence that repetition plays a crucial role in long-term injury, consistent with clinical observations.

2.4. *Cell memory has a mechanical origin*

Testing the role of a Ca^{2+} influx seems unlikely since repeated stimuli would have to increase influx and accumulation will decrease the driving force. To discriminate Ca^{2+} influx as a mechanism of cell memory, we blocked Ca^{2+} influx during the conditioning (first) stimulus and applied a test pulse six hours later. There are at least two types of MSCs, NMDAR and Piezo, and a combination of MK801 and Ruthenium Red efficiently inhibits both types (Maneshi et al., 2017). Using the inhibitors during the first (conditioning) stimulus, did not affect subsequent responses (Fig. 5a). Control experiments without blockers conducted in parallel (Fig. 5b), showed comparable Ca^{2+} responses to test stimulus (Fig. 5a,b, black curves). As second control, we used a Ca^{2+}-free solution during the initial stimulation, and that too eliminated

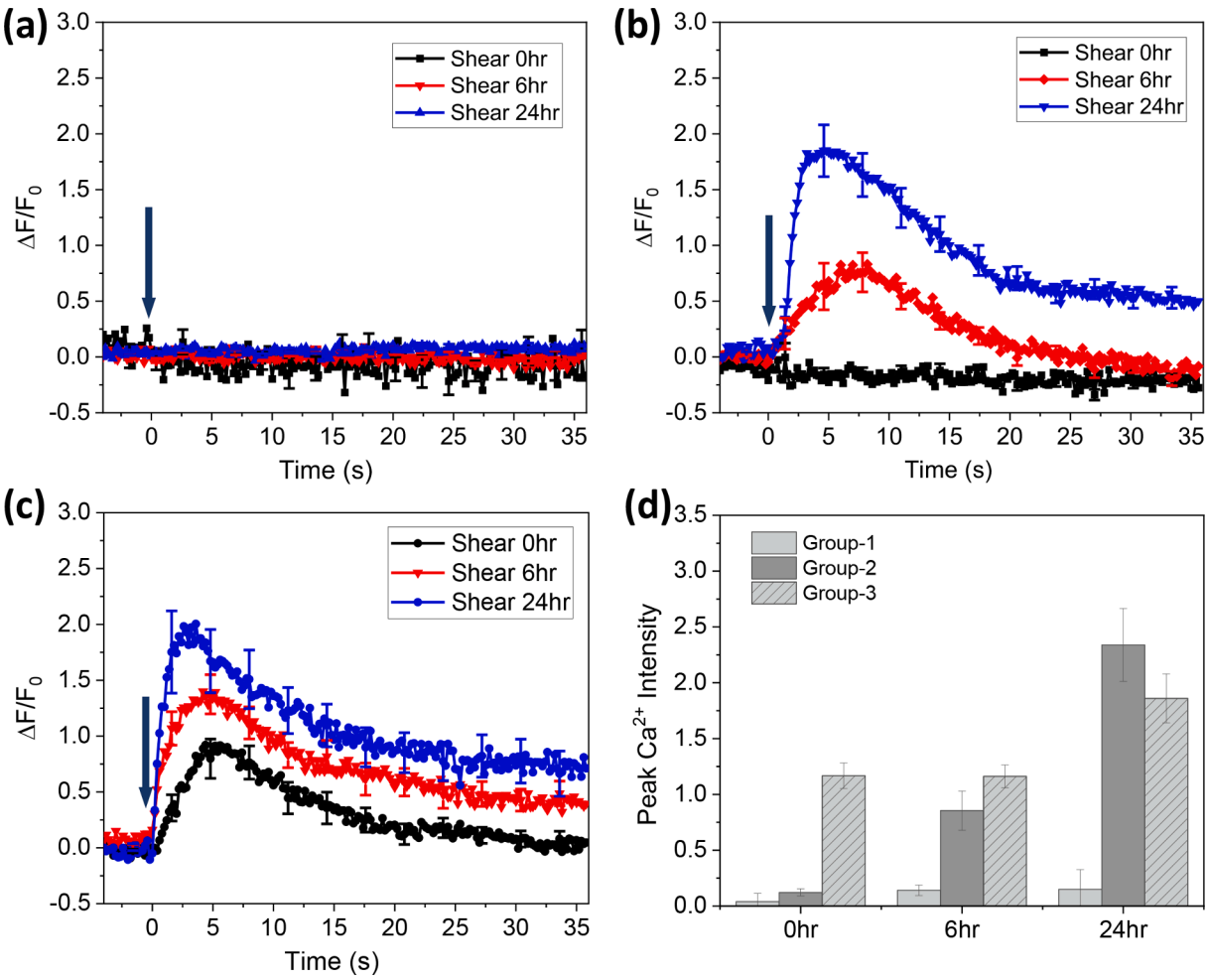

Fig. 4. The Ca^{2+} response to repeated sub-threshold shear stimuli. (a-c) Typical Ca^{2+} responses to shear pulse of 15 dyn/cm^2 and 10 ms duration that fall into three characteristic groups, **(a)** Group1 (three experiments), cells did not respond to the stimuli; **(b)** Group-2 (two experiments), cells responded from the second stimulus at 6 hr; **(c)** Group-3 (four experiments), cells responded from the first stimulus at 0 hr. **(d)** Statistical analysis of Ca^{2+} peak values for repeated stimuli showing a diverse pattern (n = 30, 20, 40 for three groups respectively).

the initial Ca^{2+} rise (Maneshi et al., 2015). And this too did not affect the Ca^{2+} response to repeated stimuli (Fig. 5d, $*p < 0.001$). The memory of early shear events persisted without a Ca^{2+} overload, suggesting that the enhanced response to repeated stimuli is not via Ca^{2+}-dependent biochemical process but occurs through a modulation of mechanical properties involving plastic deformation.

To assess the role of intact cytoskeleton, we modified F-actin networks with cytochalasin-D during the conditioning shear, followed by washout, and applied the test stimulus after 6 hrs. Cells responded to repeated pulses with similar Ca^{2+} peaks. The memory effect was diminished (Fig. 5e,f, red curves). In controls, the cells were subjected to the same drug treatment but without conditioning shear pulse, they also showed similar Ca^{2+} response to test stimulus (Fig. 5e,f, gray curves). The history of conditioning shear did not affect the later response in cytochalasin-D treated cells. This result indicates that an intact cytoskeleton is essential to cell's memory.

3. Discussion

A Ca^{2+} rise in brain cells caused by mechanical perturbations is likely during TBI (LaPlaca and Thibault, 1998; Ravin et al., 2012; Weber et al., 2001). Ca^{2+} overload can trigger biochemical cascades leading to cell death (Farkas and Povlishock, 2007; Fineman et al., 1993; Mills et al., 2004; Shapira et al., 1989). Astrocytes use both influx and store-operated Ca^{2+} pathways in response to stress. Influx is required for a global rise in intracellular Ca^{2+} (Maneshi et al., 2015). The Ca^{2+} overload could be an effector that further alters the function of intracellular Ca^{2+} stores and other Ca^{2+}-dependent channel activities. We have shown that the shear stress induced Ca^{2+} influx in astrocytes is mainly mediated by mechanically sensitive NMDA receptors (Maneshi et al., 2017). Other MSCs, such as Piezo-type channels, also participate but to a lesser extent (~20 %) (Maneshi et al., 2017). The inhibition of Ca^{2+} influx by blocking both channel types or using Ca^{2+}-free solution during the conditioning stimulus did not eliminate the effect of early stimuli (Fig. 5), demonstrating that Ca^{2+} overload and Ca^{2+}-dependent processes are not responsible for cell memory.

The forces exerted during TBI, are commonly short duration but lead to long-term disruptions, that we term memory. To examine this memory, we applied mechanical shear pulses to astrocytes to emulate TBI. Our results show that exposure to a shear stimulus enhanced the Ca^{2+} response hours later (Fig. 1). The effect lasts longer than 24 h and multiple stimuli enhanced the response (Figs. 2 and 3). Further limiting our choice of mechanism, inhibiting the Ca^{2+} influx during conditioning stimulus eliminated the initial Ca^{2+} rise, but enhanced the response to later stimuli (Fig. 5). What is the mechanism of this memory?

We can dismiss most biochemical equilibria since inhibiting the influx of calcium and the long lifetime argues against soluble mediators that would be depleted with repeated stimulation. We are driven to think of cytoskeletal involvement since that is directly responsive to mechanical inputs. Our data shows that disruption of intact cytoskeleton

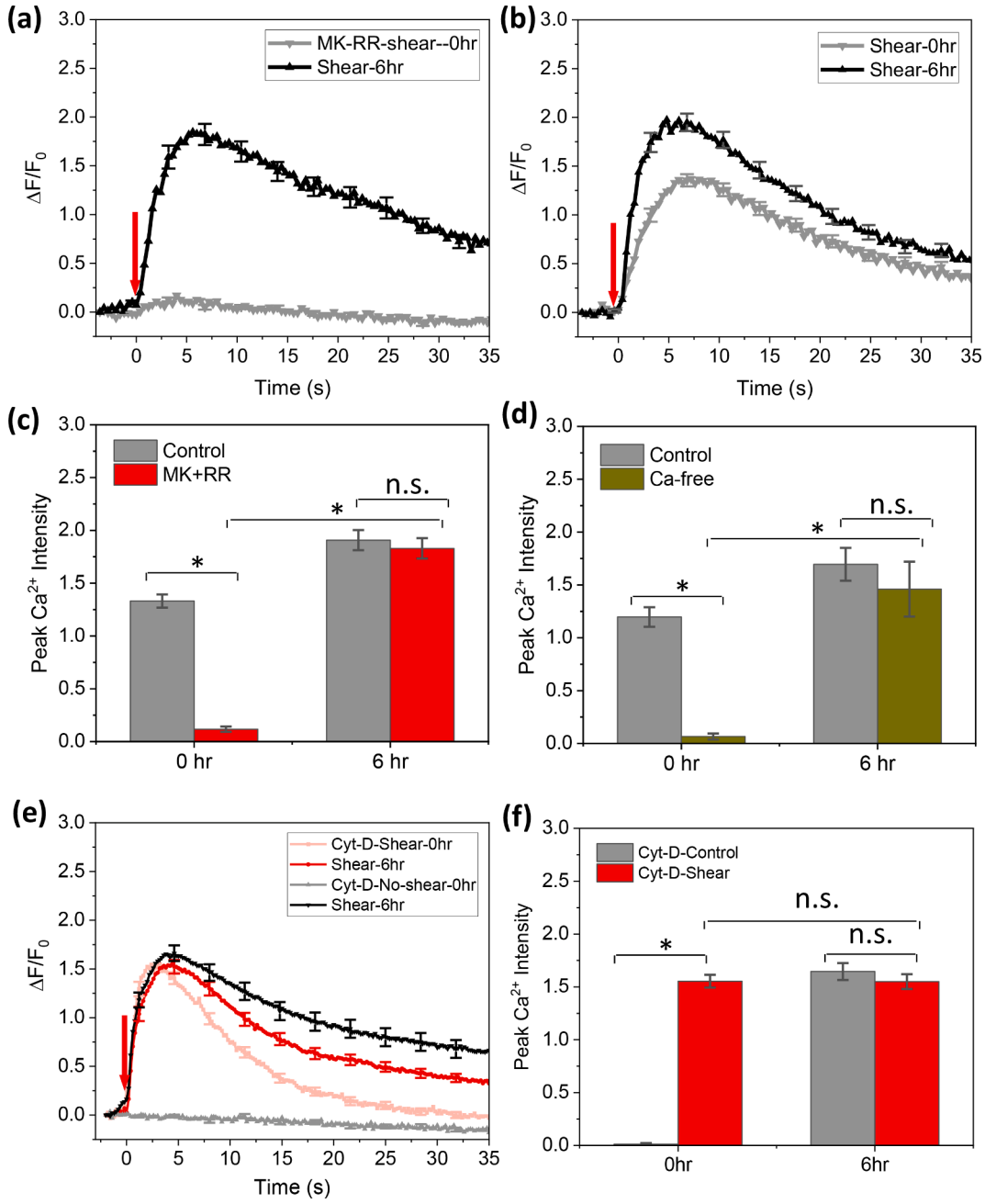

Fig. 5. Inhibiting Ca^{2+} uptake during conditioning stimulus did not affect the enhanced responses. (a,b) The Ca^{2+} response to repeated shear stimuli at 0 and 6 hr with inhibitor (MK801 and RR) **(a)** and without inhibitor **(b)**, showing the enhanced Ca^{2+} response persisted regardless of the presence of inhibitors. **(c)** Statistical analysis of peak Ca^{2+} with and without inhibitors from 9 experiments under each condition (n = 90 for all, $*p < 0.001$). **(d)** The Ca^{2+}-free solution was used during the conditioning stimulus (0 hr) and normal saline was replenished after the measurement (n = 20, $*p < 0.001$). **(e)** Cells were treated with cytochalasin-D (10 μM) during conditioning shear followed by washout. The figure shows the Ca^{2+} response to shear pulse at 6 hrs with (red curves) and without (gray curves) conditioning shear at 0 hr, showing disruption of cytoskeleton diminished cells' memory effect. **(f)** Statistical analysis of peak Ca^{2+} in cytochalasin-D treated cells with and without conditioning shear from 8 experiments under each condition (n = 80 for all, $*p < 0.001$).

with cytochalasin-D eliminated enhanced Ca^{2+} response to later stimulus, indicating cells may store the early mechanical input via cytoskeleton deformation (Fig. 5). To obtain long lasting mechanical effects, we reject models with diffusible messengers (Krieger et al., 2011) for the same reason. The fact that the response increases with repeated stimuli shows that a reactive pool is not being depleted. Sub-threshold stimuli that have a low probability of generating a Ca^{2+} rise when given in isolation, do respond with the number of repetitions (Fig. 4). Thus, the reactive system is not being depleted but potentiated by repeated stimuli and argues again against the role of diffusible reactants. However, imagine that stress breaks actin (or other linker) bonds to large cytoskeletal structures like spectrin, dystrophin or tubulin. While being nominally reversible, they might not rebind to the previous site, but with other equivalent sites that have a long lifetime. Using sub-threshold stimuli, we showed that the effect of stimuli is cumulative (Fig. 4). This is consistent with the idea that each stimulus breaks occasional bonds. These broken bonds change force landscape in cells and potentially alters later local stimulus seen by the MSCs, leading to a higher susceptibility to stimuli. Using mechanically sensitive stress probes, we found that short shear pulses applied to astrocytes cause a prolonged

increase in actinin tension throughout the cell for 10–100 s (Maneshi et al., 2018). This would cause decrease in tension in parallel components. This sustained change of the force landscape could be the major source of a cell's physical memory.

In conclusion, our study shows that cells respond to repeated fluid shear stimuli with a successive increase in Ca^{2+} susceptibility, showing the cells have a memory to previous mechanical events. The memory is longer than 24 hrs. This long-term effect is mediated by mechanical deformation but not by Ca^{2+}-dependent biochemical changes. These results provide an important mechanical pathway that links the initial and repetitive mild mechanical stimuli to the long-term risk of chronic disease development.

4. Materials and methods

4.1. Microfluidic flow chamber for repeated shear stimuli

The microfluidic chips were used to apply fluid shear stress to an astrocyte monolayer. Fluid shear stress was used as mechanical input to the cells because it is a well-defined system with well-established input/output control over the mechanical input. Although a stretchable membrane provides an environment closer to *in vivo*, the viscoelastic property of the membrane will modify the forces so the stimulus to the cells cannot be precise. The chips were made of PDMS flow chamber bonded to a glass cover slip. The chamber dimensions were 1000 μm wide, 100 μm high, and 15 mm long, with inlet and outlet holes at the end of the chamber. The flow chamber was connected to a high-speed pressure servo (ALA Scientific Instruments, NY), that is capable of generating rapid pressure pulses with ~ 1 ms resolution (Besch et al., 2002), generating transient shear forces. The flow velocity in the chamber was calibrated using fluorescence microbeads as described previously (Maneshi et al., 2015), and the wall shear stress was calculated using the empirical equation, $\tau = 6\mu Q/wh^2$, where μ is the dynamic viscosity of the fluid, Q is the volume flow rate, w and h are the width and the height of the chamber, respectively. The surfaces inside the chamber were coated with human fibronectin (Thermofisher Scientific, MA) and washed with culture media before seeding the cells. A new flow chamber was used for each experiment.

4.2. Cell culture

Adult rat astrocytes (ScienCell, CA, R1860) were cultured in DMEM containing 10 % fetal bovine serum and 1 % Penicillin/Streptomycin. Cells were resuspended in fresh media and seeded into the flow chambers when the flasks reached 90 % confluence. The cells were then cultured on the chip in an incubator for 3–4 days to reach ~ 90 % confluency. Media in the chamber was changed every 24 hrs to maintain the cell growth. The cells between passages 3 and 8 were used in the experiments that reduce the possibility of phenotypic changes (Liao and Chen, 2001).

4.3. Ca^{2+} measurements

Intracellular Ca^{2+} was measured using a cell permeable Ca^{2+} indicator, Fluo-4 AM (Invitrogen). The dye was dissolved in DMSO and diluted to a final concentration of 5 μM in saline. For repeated shear stimuli, the cells were washed with saline followed by culture media at the end of each run and cultured in an incubator for additional periods. A fresh dye was loaded for each subsequent experiment.

Fluorescence images were acquired using an inverted microscope (Axiovert 200M, Zeiss) with a 10x objective lens and a CCD camera (AxioCam, Zeiss). The filter set (Ex: 470/40 nm; Em: 525/50 nm) was used for Ca^{2+} imaging. Time-lapse images were obtained using Zeiss software (AxioVision, Ziess).

4.4. Solutions and reagents

During the shear experiments, saline containing 150 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM HEPES were used to perfuse the flow chamber. In Ca^{2+}-free solutions, $CaCl_2$ was replaced by $MgCl_2$. Two inhibitors, (+)-MK-801 hydrogen maleate and Ruthenium Red (both from Sigma, St. Louis, MO), were diluted to 10 μM and 30 μM, respectively. A mixture of the two solutions were used in the experiments. Cytochalasin-D (Sigma, St. Louis, MO) was dissolved in DMSO to a 10 mM stock solution, then diluted to a final concentration of 10 μM.

4.5. Data analysis

The relative change in Ca^{2+} intensity was calculated using.

$$\frac{\Delta F}{F_0} = \frac{F - F_0}{F_0} \quad (1)$$

where F and F_0 are the mean fluorescence intensities of selected cells at time t and $t = 0$, respectively. The background fluorescence was subtracted before the calculation. The normalized Ca^{2+} intensity was averaged over 10 cells from the middle section of each chamber and from multiple experiments. Each experiment and corresponding controls were conducted and compared using the same batch of cells. A minimum of 4 experiments were performed for all conditions. Statistical analysis used the standard error of the mean (s.e.m.). The statistical significance was analyzed using two-sample t-test, with $p < 0.005$ considered significant.

5. Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Tasnim Shireen: Data curation, Formal analysis, Investigation. **Frederick Sachs:** Conceptualization, Writing – review & editing. **Susan Z. Hua:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brainres.2022.148076.

References

Bell, J.D., Ai, J., Chen, Y., Baker, A.J., 2007. Mild in vitro trauma induces rapid Glur2 endocytosis, robustly augments calcium permeability and enhances susceptibility to secondary excitotoxic insult in cultured Purkinje cells. Brain. 130, 2528–2542.

Bell, J.D., Park, E., Ai, J., Baker, A.J., 2009. PICK1-mediated GluR2 endocytosis contributes to cellular injury after neuronal trauma. Cell Death Differ. 16 (12), 1665–1680.
Besch, S., Suchyna, T., Sachs, F., 2002. High-speed pressure clamp. Pflugers Archiv-European Journal of Physiology. 445 (1), 161–166.
Burda, J.E., Bernstein, A.M., Sofroniew, M.V., 2016. Astrocyte roles in traumatic brain injury. Exp. Neurol. 275 (Pt 3), 305–315.
Bursac, P., Lenormand, G., Fabry, B., Oliver, M., Weitz, D.A., Viasnoff, V., Butler, J.P., Fredberg, J.J., 2005. Cytoskeletal remodelling and slow dynamics in the living cell. Nat. Mater. 4 (7), 557–561.
Chen, Y.C., Smith, D.H., Meaney, D.F., 2009. In-vitro approaches for studying blast-induced traumatic brain injury. J. Neurotrauma 26 (6), 861–876.
Chen, Y., Swanson, R.A., 2003. Astrocytes and brain injury. J. Cereb. Blood Flow Metab. 23 (2), 137–149.
Cruz-Haces, M., Tang, J., Acosta, G., Fernandez, J., Shi, R., 2017. Pathological correlations between traumatic brain injury and chronic neurodegenerative diseases. Transl Neurodegener. 6, 20.
Danoff, S.K., Ferris, C.D., Donath, C., Fischer, G.A., Munemitsu, S., Ullrich, A., Snyder, S. H., Ross, C.A., 1991. Inositol 1,4,5-trisphosphate receptors: distinct neuronal and nonneuronal forms derived by alternative splicing differ in phosphorylation. Proc Natl Acad Sci U S A. 88 (7), 2951–2955.
Davalos, D., Grutzendler, J., Yang, G., Kim, J.V., Zuo, Y.i., Jung, S., Littman, D.R., Dustin, M.L., Gan, W.-B., 2005. ATP mediates rapid microglial response to local brain injury in vivo. Nat. Neurosci. 8 (6), 752–758.
DeKosky, S.T., Asken, B.M., 2017. Injury cascades in TBI-related neurodegeneration. Brain Inj. 31 (9), 1177–1182.
Farkas, O., 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.
Fineman, I., Hovda, D.A., Smith, M., Yoshino, A., Becker, D.P., 1993. Concussive brain injury is associated with a prolonged accumulation of calcium: a 45Ca autoradiographic study. Brain Res. 624 (1-2), 94–102.
Gardel, M.L., Shin, J.H., MacKintosh, F.C., Mahadevan, L., Matsudaira, P., Weitz, D.A., 2004. Elastic behavior of cross-linked and bundled actin networks. Science 304 (5675), 1301–1305.
Harmon, K.G., Drezner, J.A., Gammons, M., Guskiewicz, K.M., Halstead, M., Herring, S. A., Kutcher, J.S., Pana, A., Putukian, M., Roberts, W.O., 2013. American Medical Society for Sports Medicine position statement: concussion in sport. Br. J. Sports Med. 47 (1), 15–26.
Hilge, M., 2012. Ca2+ regulation of ion transport in the Na+/Ca2+ exchanger. J. Biol. Chem. 287 (38), 31641–31649.
Ikonomovic, M.D., Abrahamson, E.E., Carlson, S.W., Graham, S.H., Dixon, C.E., 2019. Novel therapies for combating chronic neuropathological sequelae of TBI. Neuropharmacology 145, 160–176.
Krieger, C.C., An, X., Tang, H.-Y., Mohandas, N., Speicher, D.W., Discher, D.E., 2011. Cysteine shotgun-mass spectrometry (CS-MS) reveals dynamic sequence of protein structure changes within mutant and stressed cells. Proc Natl Acad Sci U S A. 108 (20), 8269–8274.
LaPlaca, M.C., Thibault, L.E., 1998. Dynamic mechanical deformation of neurons triggers an acute calcium response and cell injury involving the N-methyl-D-aspartate glutamate receptor. J. Neurosci. Res. 52 (2), 220–229.
Liao, S.-L., Chen, C.-J., 2001. Tyrosine kinase signaling involves in glutamate-induced astrocyte proliferation. NeuroReport 12 (16), 3519–3522.
Maneshi, M.M., Sachs, F., Hua, S.Z., 2015. A Threshold Shear Force for Calcium Influx in an Astrocyte Model of Traumatic Brain Injury. J. Neurotrauma 32 (13), 1020–1029.
Maneshi, M.M., Maki, B., Gnanasambandam, R., Belin, S., Popescu, G.K., Sachs, F., Hua, S.Z., 2017. Mechanical stress activates NMDA receptors in the absence of agonists. Sci. Rep. 7, 39610.
Maneshi, M.M., Sachs, F., Hua, S.Z., 2018. Heterogeneous Cytoskeletal Force Distribution Delineates the Onset Ca(2+) Influx Under Fluid Shear Stress in Astrocytes. Front. Cell. Neurosci. 12, 69.
Mills, L.R., Velumian, A.A., Agrawal, S.K., Theriault, E., Fehlings, M.G., 2004. Confocal imaging of changes in glial calcium dynamics and homeostasis after mechanical injury in rat spinal cord white matter. Neuroimage. 21 (3), 1069–1082.
Niggel, J., Sigurdson, W., Sachs, F., 2000. Mechanically induced calcium movements in astrocytes, bovine aortic endothelial cells and C6 glioma cells. J. Membr. Biol. 174 (2), 121–134.
Orrenius, S., Zhivotovsky, B., Nicotera, P., 2003. Regulation of cell death: the calcium-apoptosis link. Nat. Rev. Mol. Cell Biol. 4 (7), 552–565.
Pivovarova, N.B., Andrews, S.B., 2010. Calcium-dependent mitochondrial function and dysfunction in neurons. FEBS J. 277, 3622–3636.
Ravin, R., Blank, P.S., Steinkamp, A., Rappaport, S.M., Ravin, N., Bezrukov, L., Guerrero-Cazares, H., Quinones-Hinojosa, A., Bezrukov, S.M., Zimmerberg, J., 2012. Shear forces during blast, not abrupt changes in pressure alone, generate calcium activity in human brain cells. PLoS One. 7, e39421.
Ruiz, A., Matute, C., Alberdi, E., 2009. Endoplasmic reticulum Ca(2+) release through ryanodine and IP(3) receptors contributes to neuronal excitotoxicity. Cell Calcium 46 (4), 273–281.
Shapira, Y., Yadid, G., Cotev, S., Shohami, E., 1989. Accumulation of calcium in the brain following head trauma. Neurol. Res. 11 (3), 169–172.
Singh, I.N., Sullivan, P.G., Deng, Y., Mbye, L.H., Hall, E.D., 2006. Time course of post-traumatic mitochondrial oxidative damage and dysfunction in a mouse model of focal traumatic brain injury: implications for neuroprotective therapy. J. Cereb. Blood Flow Metab. 26 (11), 1407–1418.
Sivanandam, T.M., Thakur, M.K., 2012. Traumatic brain injury: a risk factor for Alzheimer's disease. Neurosci. Biobehav. Rev. 36 (5), 1376–1381.
Smith, D.H., Johnson, V.E., Stewart, W., 2013. Chronic neuropathologies of single and repetitive TBI: substrates of dementia? Nat Rev Neurol. 9 (4), 211–221.
Unterberg, A.W., Stover, J., Kress, B., Kiening, K.L., 2004. Edema and brain trauma. Neuroscience 129 (4), 1019–1027.
Weber, J.T., 2012. Altered calcium signaling following traumatic brain injury. Front. Pharmacol. 3, 60.
Weber, J.T., Rzigalinski, B.A., Ellis, E.F., 2001. Traumatic injury of cortical neurons causes changes in intracellular calcium stores and capacitative calcium influx. J. Biol. Chem. 276 (3), 1800–1807.
Zetterberg, H., Winblad, B., Bernick, C., Yaffe, K., Majdan, M., Johansson, G., Newcombe, V., Nyberg, L., Sharp, D., Tenovuo, O., Blennow, K., 2019. Head trauma in sports - clinical characteristics, epidemiology and biomarkers. J. Intern. Med. 285 (6), 624–634.