

REPORT DOCUMENTATION PAGE				Form Approved OMB NO. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 30-08-2012		2. REPORT TYPE Abstract		3. DATES COVERED (From - To) -	
4. TITLE AND SUBTITLE NR2B-N-METHYL-D-ASPARTATE RECEPTORS CONTRIBUTE TO NETWORK ASYNCHRONY AND LOSS OF LONG-TERM POTENTIATION FOLLOWING MILD MECHANICAL INJURY IN VITRO				5a. CONTRACT NUMBER W911NF-10-1-0526	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER 611103	
				5d. PROJECT NUMBER	
6. AUTHORS Tapen P. Patel, Scott C. Ventre, Pallab K. Singh, David F. Meaney				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES University of Pennsylvania Office of Research Services University of Pennsylvania Philadelphia, PA 19104 -				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211				10. SPONSOR/MONITOR'S ACRONYM(S) ARO	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) 58155-LS-MUR.40	
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
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15. SUBJECT TERMS synchrony, NR2B-NMDA receptor, network activity					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON David Meaney
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 215-573-2726

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ABSTRACT

To understand how mechanical injury affects neural circuits, we 1) built a multi-scale stochastic integrate-and-fire model of network activity, 2) simulated an injured network, 3) predicted an important role for the NR2B-NMDA receptor in mediating network asynchrony, and 4) tested our predictions using in vitro stretch injury

NR2B-N-METHYL-D-ASPARTATE RECEPTORS CONTRIBUTE TO NETWORK ASYNCHRONY AND LOSS OF LONG-TERM POTENTIATION FOLLOWING MILD MECHANICAL INJURY *IN VITRO*

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Introduction: To understand how mechanical injury affects neural circuits, we 1) built a multi-scale stochastic integrate-and-fire model of network activity, 2) simulated an injured network, 3) predicted an important role for the NR2B-NMDA receptor in mediating network asynchrony, and 4) tested our predictions using *in vitro* stretch injury.

Methods:

Computational model: Population of pyramidal-like neurons were assembled into a network following the topology found *in vitro* cultures. Dendritic spines were populated with AMPARs and NR2A- and NR2B-NMDA receptors. Synaptic transmission was simulated by vesicular release of glutamate onto the spine head, and the activation of these receptors was modeled using a stochastic diffusion-reaction scheme. The resulting current, summed at the soma, was used to update the cell membrane potential. Injury was simulated by larger glutamate release and partial loss of NMDAR Mg²⁺ block, consistent with *in vitro* and *in vivo* observations.

In vitro experiments: Primary cortical neurons were plated on silastic membrane. Mature cultures were bulk loaded with fluo4-AM, and the calcium activity of the network was imaged pre- and post-stretch. The relative contribution of NR2A and NR2B receptors in altering network activity, functional connectivity and LTP potential of the post-injured network were assessed using subtype specific antagonists.

Results:

Since calcium is a crucial secondary messenger in neurons and serves as a proxy for underlying electrical activity, we first profiled the source of calcium and the temporal pattern of calcium dynamics of a spontaneously oscillating network. We found that, in mature cultures, the main sources of calcium during spontaneous activity are NR2A-NMDARs and VGCCs. In contrast, acutely (1-hr) following dynamic stretch injury, NR2B containing NMDARs become a significant source of calcium, likely due to partial loss of its native Mg²⁺ block.

To investigate the effects of enhanced NR2B activity on network dynamics, we built a multi-scale model of network activity (spines -> dendrites -> soma -> network) and simulated activity patterns of a population of neurons and matched to those of an *in vitro* culture (DIV 18-20). Next, we simulated the activity of an injured network and found that relief of NR2B Mg²⁺ block at the spine-level results in asynchrony at the network-level. Our simulations also predicted that the asynchronous activity in the simulated-injured region can propagate into adjacent regions in a distance dependent manner, influenced by the topology and connectivity of the network.

We conducted *in vitro* stretch injury experiments to validate our *in silico* predictions and found that following injury, 1) there is greater variability in the amplitude of calcium transients for a given neuron, 2) there is loss in synchrony and decline in functional connectivity in the injured

region, 3) synchrony is rescued by antagonizing NR2B-containing NMDARs, and 4) disruptions of network activity in the mechanical penumbra are negligible beyond 700um.

Functionally, synaptic calcium asynchrony limits LTP potential in the injured region. However, NR2B antagonism during LTP induction allows a fraction of injured neurons to undergo chemical LTP as measured by persistent increase in the amplitude of Ca²⁺ transients.

Conclusion: Despite its enormous incidence, mild TBI is not well understood. One aspect that needs more definition is how mechanical energy disrupts neural circuitry, in the absence of overt cell death. In this study, we merged information from different perspectives (*in silico* and *in vitro*) and across different length scales (spines to networks) to uncover an important role of the NR2B-NMDAR in mediating network asynchrony. Traditionally, NR2B antagonism has been used to blunt pro-apoptotic pathways, but we show that in mild injuries, NR2B receptors may be an important target for reverting the injured network to its pre-injury state and rescue plasticity in an injured network.

Acknowledgments: We thank the contribution of Patricia Slobodnick (cell culture), and YungChia Chen and David Gullotti for helpful discussions. This work was funded by National Institutes of Health and the Department of Defense (NIH RO1 NS35712, HD41699, and PO1 NS056202, W911NF-10-1-0526).

Keywords: synchrony, NR2B-NMDA receptor, network activity