The Effects of Cortical and Hippocampal Spreading Depolarizations on Glutamatergic Signaling Proteins

Kinsey Barhorst BS\textsuperscript{1}, Yara Alfawares\textsuperscript{2}, Jennifer L McGuire PhD\textsuperscript{3}, Jed Hartings PhD\textsuperscript{3}, Laura B Ngwenya MD PhD\textsuperscript{3,4, 5}

\textsuperscript{1} University of Cincinnati, College of Medicine, Cincinnati, USA; \textsuperscript{2} University of Cincinnati, Undergraduate Neuroscience Program, Cincinnati, USA; \textsuperscript{3} University of Cincinnati, Department of Neurosurgery, Cincinnati, USA; \textsuperscript{4} University of Cincinnati, Department of Neurology and Rehabilitation Medicine; \textsuperscript{5} University of Cincinnati, Neurotrauma Center UC Gardner Neuroscience Institute, Cincinnati, USA.
Introduction

• Traumatic brain injuries (TBI) are one of the United States’ biggest health epidemics, accounting for 2.8 million ED visits, hospitalizations, and deaths in 2013.

• TBIs are the leading cause of death and disability in persons under 45 years of age and can severely impair cognition and other tasks that affect daily function.

• Following TBI, ion homeostasis can be interrupted causing waves of neuronal and glial activation followed by a reduction of spontaneous electrical activity known as a spreading depolarization (SD).

• Cortical SD are found in 50-60% of patients with TBI and are associated with unfavorable outcomes.

• SD are found to increase extracellular glutamate release, leading to neurotoxicity and cell death.

• AMPA receptor subunits (GluR1 and GluR2) and NMDA receptor subunits (NR2A and NR2B) are all implicated in glutamatergic signaling that underlies neuronal plasticity.

• Our study investigates the secondary cellular changes within the hippocampus that arise as a consequence of hippocampal and cortical SDs.
Disclosures

I have no financial or organizational disclosures to report.
Methods

Subjects
1. 21 male Sprague-Dawley Rats were assigned to one of three experimental groups:
   1. 6 were sham procedures that received 0.9% NaCl injections in frontal cortex (.1 sec at a PSI=7-20)
   2. 6 received cortical SD induction with 1 M KCl injections in frontal cortex (.1 sec at a PSI=7-20)
   3. 9 received hippocampal SD induction with 1 M KCl injections in hippocampus AP:-2.2, ML: -1.8, DV: -3.4 (.1 sec at a PSI=7-20)

Procedure
- 2 large craniectomies were made over the parietal and frontal cortex.
- Small holes were made in the dura immediately superior to the electrode placements.
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Electrophysiology
- SDs were induced using a PicoSpritzer every 15 minutes for 2 hours.
- AC and DC current were recorded from both the ipsilateral parietal cortex and ipsilateral hippocampus.
- Recording from hippocampus at AP:-5.0, ML: -3.4, DV: -3.2.

Western Blot
- Tissue was collected 3 days post-SD induction following live rapid decapitation. The brain was dissected according to the image below.
- Ipsilateral hippocampus and parietal cortex samples were homogenized and quantified using BCA assay prior to western blot.
- Tissue samples were probed using primary antibodies then visualized using secondary antibodies.
- Tissue was analyzed for NMDA receptor subunits NR2A and NR2B along with AMPA receptor subunits GluR1 and GluR2.

Figure 1 a) Diagram of craniectomies made in the frontal bone (blue box) and parietal bone (black box). b) Diagram of hippocampal stimulating electrode placement. c) Diagram of hippocampal recording electrode placement.
Results

Electrophysiology

Figure 2. R477 Hippocampal SD induction. a) Hippocampal DC current. b) Hippocampal AC current. c) Cortical DC current. d) Cortical AC current.

Figure 3. R469 Cortical SD induction. 1) Hippocampal DC current. 2) Hippocampal AC current. 3) Cortical DC current. 4) Cortical AC current.
Results

Western Blot - Hippocampal Tissue

Figure 4. The difference in AMPA receptor subunits in ipsilateral hippocampal tissue between groups. *p=0.0233 **p=0.0016

Figure 5. The difference in NMDA receptor subunits in ipsilateral hippocampal tissue between groups. GluR1 *p=0.0233 **p=0.0016. GluR2 *p=0.0394
Results

Western Blot - Cortical Tissue

*Figure 6.* The difference in glutamate receptor subunits in ipsilateral parietal cortex. *p=0.0005**

*NR2A* & *NR2B* with significant differences noted at *p=0.0309*.
Discussion

- Cortical SDs did not spread to the hippocampus nor did hippocampal SDs spread to cortex.
- NR2A is increased in the hippocampus during HSD while NR2B is unaffected.
- GluR1 is increased in the hippocampus during HSD compared to both sham and CSD while GluR2 is decreased in hippocampus during HSD compared to CSD.
- NR2B is increased in the cortex during HSD while all other receptor subunits show no significant difference in the cortical tissue.
- SDs had a greater impact on glutamatergic subunit composition within the hippocampus, implicating a change in the excitatory neuronal mechanisms that underly cognitive processes.
- SDs have both local and distant effects on glutamatergic signaling proteins that may impact neuronal plasticity.
- Future directions include further investigation of proteins that interact with AMPA receptor subunits as well as how neurogenesis within the hippocampus is impacted by SDs.
Summary

• Spreading depolarizations play an intricate role in the pathogenesis of TBI.

• SDs have been shown to have both local and remote effects on the glutamatergic subunits that underly neuronal plasticity, especially within the hippocampus.

• Further investigation is needed in order to fully elucidate the effects that SDs have on remote and local glutamatergic subunit composition.

• Implications could be important for determining the effects that ketamine, an NMDA antagonist and potential TBI treatment option, might have on plasticity post-TBI.
References


