# Influence of Cortical Spreading Depolarizations on Post-Traumatic Epilepsy in a Rodent Model of Neurotrauma

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## Background and Significance

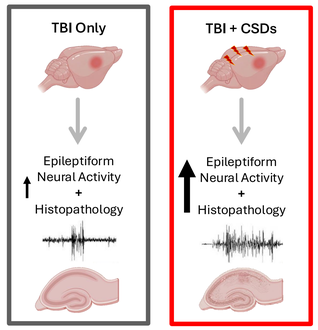
Post-traumatic epilepsy (PTE) is a frequent complication after traumatic brain injury (TBI) and significantly increases morbidity and mortality

The mechanisms underlying the pathogenesis of PTE are poorly understood.

Cortical Spreading Depolarizations (CSDs) are mass waves of neuronal depolarizations that spread throughout the cortex and facilitate abnormalities in brain structures, such as the hippocampus

**We hypothesize that CSDs after TBI increase the risk of developing epileptiform activity and related histopathological abnormalities.**

**Figure 1:** Visual representation of our hypothesis




## Methods

### Lateral Fluid Percussion Injury (LFPI) and Cortical Spreading Depolarizations

- After undergoing a craniectomy, eight male Sprague-Dawley rats received a TBI via LFPI, which delivers a fluid pulse onto the intact dura at the right parietal cortex **(Figure 2A)**.
- Approximately 30 minutes after LFPI, rats underwent eight CSDs over two hours (Figure 2B), with KCl applied directly to the right frontal cortex and saline for controls (n = 4/group).

### Electrocorticography (ECoG) and Pentylenetetrazol (PTZ)

- Three months post-injury, rats were implanted with bipolar ECoG electrodes (500Hz sampling rate) in both hemispheres.
- Six months post-injury, rats were assessed for seizure susceptibility via serial intraperitoneal injections of 5mg/kg PTZ, a potent $GABA_A$ antagonist and proconvulsant. Injections were terminated once the rat experienced a Racine 5 seizure or after a maximum of nine doses **(Figure 2C)**.

### Zinc Transporter 3 (ZnT3) Immunohistochemistry (IHC)

- ZnT3 is a marker of mossy fiber sprouting in the hippocampus, which is associated with epileptic activity
- Seven months post-injury, rats (n=3/group) were euthanized and processed for ZnT3 IHC. Stained tissue was assessed qualitatively.

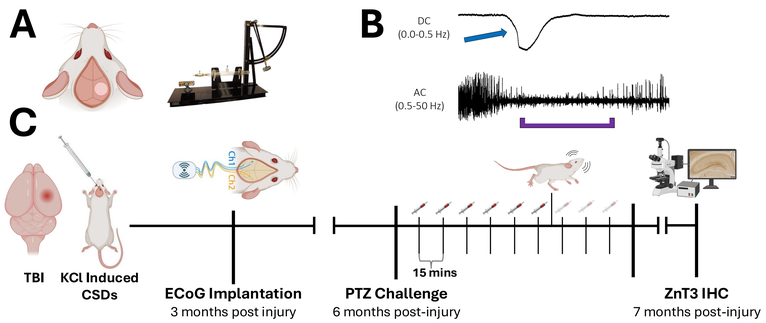



**Figure 2: (A)** Illustration of LFPI. A pendulum strikes a column of fluid, delivering a 2atm pulse onto the dura. **(B)** Canonical electroencephalographic trace of a CSD, showing a steep depression in the DC-current followed by temporary silencing in the AC-current. **(C)** Experimental timeline.

## Electrocorticography Results During PTZ Challenge

### CSDs after TBI do not increase seizure susceptibility

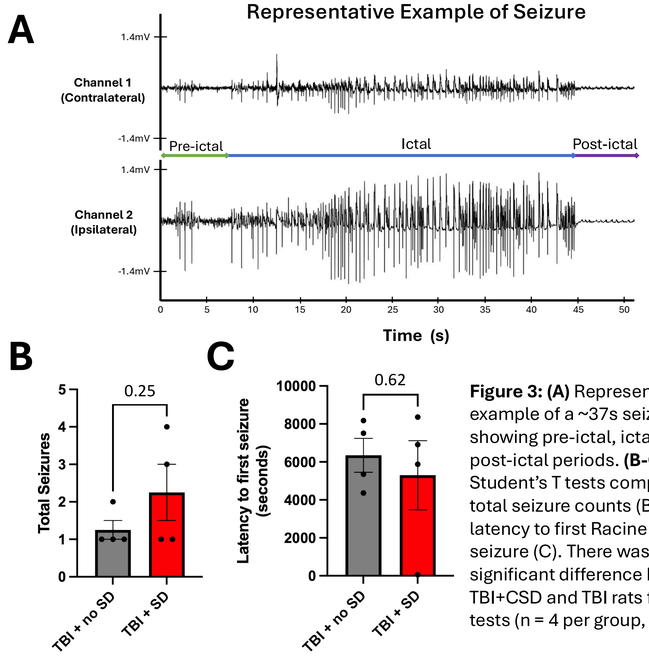



**Figure 3: (A)** Representative example of a ~37s seizure showing pre-ictal, ictal and post-ictal periods. **(B-C)** Student's T tests comparing total seizure counts (B) and latency to first Racine 4-5 seizure (C). There was no significant difference between TBI+CSD and TBI rats for both tests (n = 4 per group, p > 0.05).

### CSDs after TBI increase the amplitude (intensity) of epileptic spikes

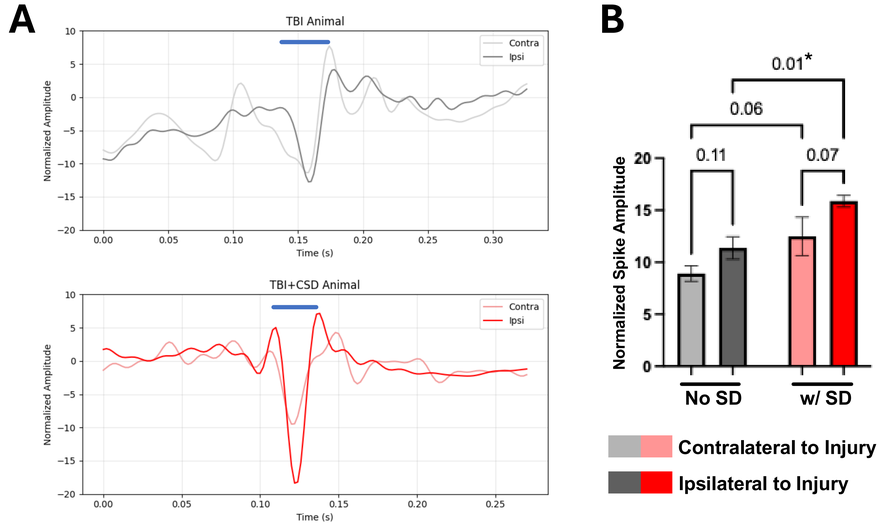



**Figure 4: (A)** Representative examples of epileptic spikes for TBI rats (upper panel) and TBI + CSD rats (lower panel). Blue line indicates the boundary of an epileptic spike. The maximum amplitude within this boundary was considered the spike amplitude **(B).** A within-subject linear mixed-effects model revealed that TBI+CSD rats had significantly larger spike amplitudes than TBI rats within the hemisphere ipsilateral to injury and CSD induction [p = 0.0115].

## ZnT3 Immunohistochemistry Results

### CSDs after TBI do not facilitate mossy fiber sprouting

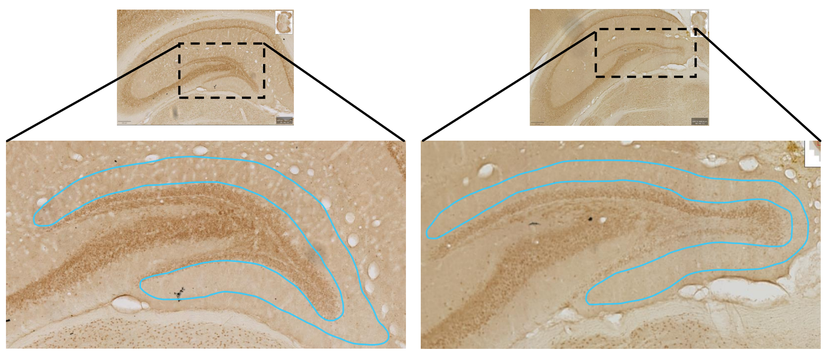



**Figure 5:** Representative examples of hippocampal ZnT3 immunohistochemistry in TBI and TBI+CSD rat. The cyan line approximately demarcates the molecular layer of the dentate gyrus. Greater ZnT3 staining in this region would suggest increased mossy fiber sprouting and susceptibility to seizures. Qualitative assessment of immunolabeling shows no appreciable difference between groups.

## Discussion and Future Directions

Our preliminary findings suggest CSDs may intensify cortical epileptic spikes. This effect was significant in the hemisphere ipsilateral to LFPI and CSD induction, indicating a localized pathophysiology.

**Overall, while CSDs may not increase seizure susceptibility or enhance our proposed histopathological marker of epilepsy, they appear to promote hyperexcitability of cortical neurons.**

**Next steps:**

1. Determine the prevalence of epileptiform activity before and after PTZ challenge.
2. Quantify ZnT3 immunolabeling and related histopathological markers using automated software such as Fiji or QuPath.

## Acknowledgments

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