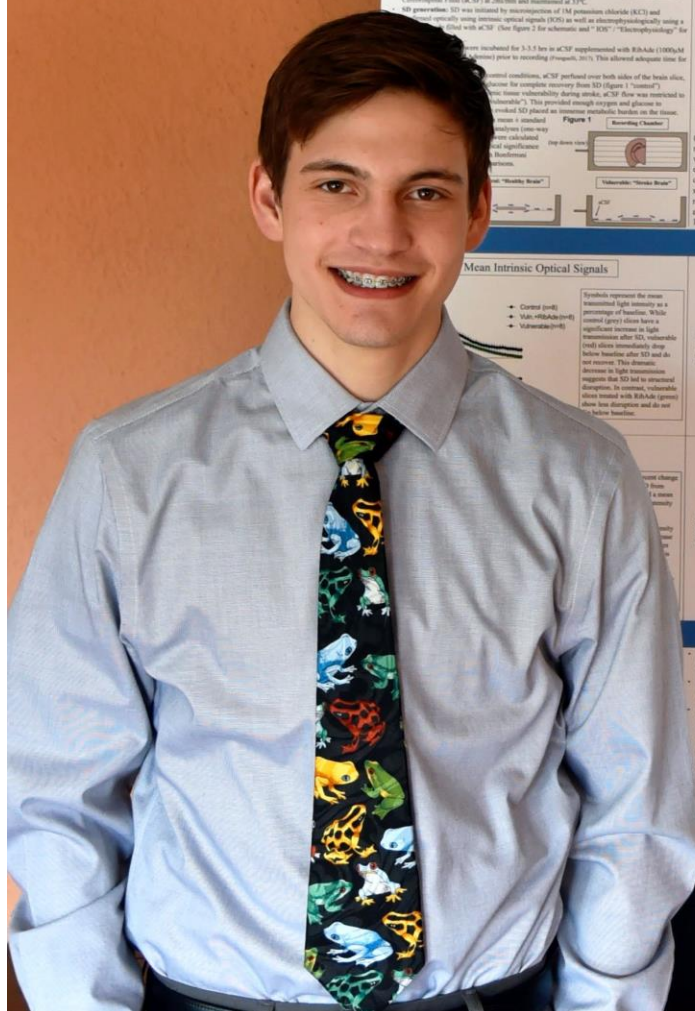


# 2019 State NMJAS Research Paper Competition Winner

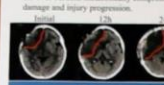
## Rusty Ludwigsen



### Increasing Metabolic Substrates Improves Spreading Depolarization Recovery in a Brain Slice Model of An Innovative Therapy for Reducing Brain Injury After Stroke


#### Strokes Continue to Get Worse

- Stroke is the second leading cause of death worldwide and a significant contributor to permanent disability (World Health Organization, 2016).
- After the initial stroke onset, brain injury can continue to progress for days or even weeks (see image series below) at a time where there is a striking lack of therapeutic intervention.
- Recent evidence shows that injury progression is caused by waves of neuronal excitation called **spreading depolarizations (SDs)**.
- SD is extremely metabolically demanding and thus depletes tissue of adenosine triphosphate (ATP), a source of cellular energy.
- SD over burdens metabolically compromised stroke tissue resulting in further damage and injury progression.



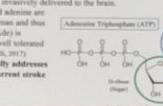
#### Spreading Depolarization (SD)

- SD is a slowly propagating wave of prolonged neuronal activation (several minutes) often referred to as a "brain tsunami."
- The duration of brain activation is massive during SD, significantly longer than normal brain activity and even longer than seizures.
- The core of stroke damage (infarct core) is tissue closest to the occluded blood vessel. This is the first brain region to succumb to irreversible injury.
- Penumbral tissue (surrounding the infarct core) experiences decreased blood flow after a stroke that leaves it in a vulnerable and metabolically compromised state.
- SD can overwhelm the metabolic capacity of vulnerable tissue resulting in tissue damage. Therefore, SD in penumbral tissue can lead to infarct core growth.
- While SD initiates in penumbral regions, it can also propagate into healthy brain regions with adequate metabolic capacity. In these regions, SD can stimulate beneficial recovery mechanisms.
- An ideal stroke treatment would protect **vulnerable penumbral tissue** while allowing the propagation of SD.



#### D-ribose + Adenine (RibAd)

- D-ribose and adenine (RibAd) are ATP precursors and exogenous supplementation has been demonstrated to increase ATP concentration (Frigault, 2017).
- ATP is the universal source of cellular energy and is critical for SD. By increasing ATP concentrations, RibAd may reduce the metabolic SD which may minimize damaging consequences.
- Both D-ribose and adenine can readily cross the blood brain barrier.
- RibAd to be most successfully delivered to the brain.
- Both D-ribose and adenine are currently used in mice and thus the mixture (RibAd) is expected to be a well tolerated treatment (Frigault, 2017).
- RibAd specifically addresses SD unlike any current stroke treatments.



#### Research Question

Can supplementation of ATP precursors reduce deleterious consequences of SD?

#### Procedure

Brain slices, 350 micron coronal hippocampal brain slices were prepared (by graduate students) from 6 female wild type mice and brain slices (C57BL/6). Individual brain slices were mounted in a recording chamber that was continuously perfused with oxygenated artificial CSF (aCSF) at 2ml/min and maintained at 37°C.

SD propagation was induced by microinjection of 50 μM potassium chloride (KCl) and SD propagated using intrinsic optical signals (IOS) as well as electrophysiologically using a 200 μm optical fiber (see figure 2 for schematic and "Electrophysiology" for details).

Brain slices were incubated for 3-5 hrs in aCSF supplemented with RibAd (1000 μM) (added prior to recording (Frigault, 2017)). This allowed adequate time for RibAd to penetrate the brain slice. This allowed adequate time for RibAd to penetrate the brain slice.

During control conditions, aCSF perfused over both sides of the brain slice. During the complete recovery from SD (Figure 1 "control"), brain slices were incubated with RibAd (1000 μM) for 3-5 hours (see figure 1 "control"). This provided enough oxygen and glucose to the brain slice to allow for recovery from SD. This provided enough oxygen and glucose to the brain slice to allow for recovery from SD.

Figure 1: Schematic of the experimental setup showing the brain slice, recording chamber, and optical fiber.

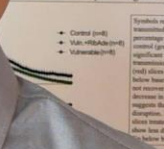
#### Electrophysiology

A small glass monopolar stimulating electrode was placed in the hippocampus (CA1). The electrode delivered a small electric current and results in a postsynaptic response referred to as an excitatory postsynaptic potential (EPSP). EPSP amplitudes were recorded from a recording electrode placed 2.25mm from the stimulating electrode and distant from the pipet containing KCl (figure 2). The temporary depolarization of EPSPs (resolving within milliseconds) is reminiscent of normal synaptic activity, and therefore EPSP amplitude was used as an indicator of neuronal recovery after SD. Pulses were delivered at 0.05Hz and an intensity necessary to achieve a response 40-60% of the maximum. In this study, baseline amplitude was set to 100% and graphs thus show the percent change in amplitude from baseline responses.

#### Results

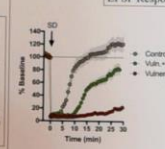
##### Mean Intrinsic Optical Signals

Hydrolyzed light intensity was measured using a photodiode. While control (grey) slices have a significant increase in light transmission after SD, vulnerable (red) slices immediately drop below baseline after SD and do not recover. The decrease in light transmission suggests that SD led to structural disruption. In contrast, vulnerable slices treated with RibAd (green) show less disruption and do not drop below baseline.



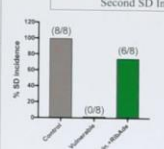
##### EPSP Responses After SD

Hydrolyzed light intensity is expressed as a percentage of baseline. Control (grey) slices return to baseline responses within 10 minutes post SD. After SD, vulnerable (red) slices show only partial recovery during the duration of the recording. RibAd (green) improves recovery in vulnerable slices.



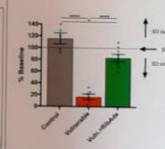
##### Second SD Incidences

SD cannot propagate the stroke because SD requires greater density to sustain of glutamate and K<sup>+</sup> ions. Under control conditions, some many repetitive SDs have multiple glutamate and K<sup>+</sup> ions. The first SD, however, is a single SD. The first SD, however, is a single SD. The first SD, however, is a single SD.



##### Average EPSP Amplitude 25-30min

The amplitude of EPSP responses between 25 and 30 minutes were averaged and expressed as a percentage of baseline. Control slices recovered to 113.8 ± 9.3% of baseline responses (n=6). Vulnerable slices recovered to 71.8 ± 4.7% of baseline responses (n=6). This is a significant decrease of 38.8 ± 9.3% from control (P < .0001). RibAd improved vulnerable slice recovery, with responses to 106.6 ± 8.7% of baseline responses (n=6). This is a significant increase of 34.8 ± 8.7% when compared to untreated vulnerable slices (P < .0001).



##### What These Graphs Imply

- IOS is valuable to evaluate overall tissue health.
- By preventing the decrease in light transmission seen in vulnerable brain slices (red), RibAd reduces the damage caused by SD.
- EPSP Recovery After SD
- EPSPs are valuable when evaluating functional recovery of neurons because EPSPs allow for significantly improved EPSP recovery which is an indicator of neuronal health.
- Control slices recover above baseline which is indicative of long term potential recovery through adequately perfused brain regions. RibAd is an ad-hoc because it prevents vulnerable tissue while allowing SD propagation.
- Second SD Incidences
- SD requires a critical density of functional tissue in order to propagate.
- Indicates that RibAd reduces widespread tissue damage in vulnerable tissue > from SD.

#### References

- World Health Organization. (2016). *World Health Statistics Quarterly*. Geneva: WHO.
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All images, videos, and graphs taken created by author unless stated otherwise.

# **Increasing Metabolic Substrates Improves Spreading Depolarization Recovery in a Brain Slice Model of Stroke: An Innovative Therapy for Reducing Brain Injury After Stroke**

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Worldwide, stroke is the second leading cause of death and the third leading cause of disability with approximately 15 million strokes reported each year. Recent work has demonstrated that an event in the brain referred to as a spreading depolarization (SD) is a significant contributor to injury progression in the days to weeks after stroke onset. SD, often referred to as a brain tsunami, is a slowly progressing wave of coordinated neuronal and glial depolarization. Even in the healthy brain, SD is extremely metabolically demanding and can deplete the building blocks of adenosine triphosphate (ATP), a source of cellular energy. In tissue compromised by a stroke or other acute brain injury, SD overburdens the metabolic capacity of tissue and results in irrecoverable injury. Clinically, there are no treatments available that target SD specifically. In this work, the exogenous supplementation of the mixture of the ATP precursors, D-Ribose and Adenine (RibAde), prior to SD was examined. RibAde supplementation has been shown to increase ATP production in brain tissue, and thus may reduce damaging consequences of SD in stroke conditions. Electrophysiology and transmitted light imaging were used to evaluate SD initiated by potassium chloride microinjection in metabolically compromised mouse brain slices. Exposure to RibAde improved recovery of brain slices likely by increasing ATP availability. These findings suggest that Adenine and D-ribose supplementation reduce the damaging consequences of SD in vulnerable tissue and may pose as an innovative approach in the treatment of ischemic stroke and brain injury worldwide.