



Background

- Amyotrophic Lateral Sclerosis is a neurodegenerative disease that specifically targets the motor neurons
- In ALS patients, excitotoxicity occurs
- Calcium dysregulation is a major part of ALS
- Glutamate can increase the influx of calcium through NMDA receptors

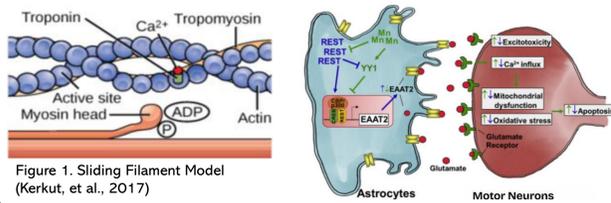


Figure 1. Sliding Filament Model (Kerkut, et al., 2017)

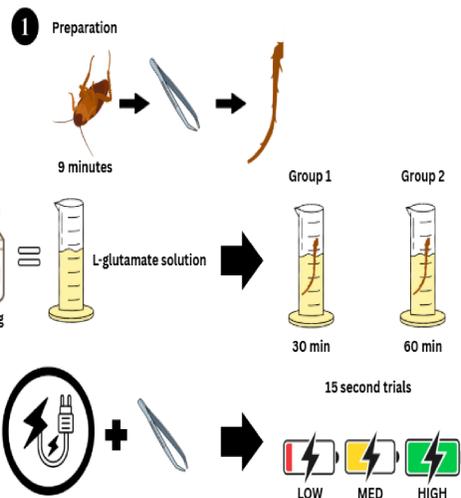
Figure 2. Diagram of EAAT2 on Astrocytes. (Pajarillo, et al., 2021)

Purpose

- Discover alternate methods to treat ALS:
 - **Determine:** Whether lower or higher frequencies of electrical stimulation benefit motor neurons the best.
 - **Understand:** How these frequencies affect motor neurons over exposure time periods.

Methodology

The experiment examines cockroach leg motor neurons in an increased glutamate environment to model ALS and investigate the effect of different electrical frequencies.



Experiments were conducted using a SpikerBox from BackyardBrains and a Faraday Cage to retrieve accurate measurements limiting background noise.

Research Question

How do different frequencies of electrical current affect the action potential of cockroach motor neurons under different exposure levels to L-glutamate?

Hypothesis

If a lower frequency of electrical current is applied to the neuromodulated cockroach, this will result in the largest decrease in intercellular calcium levels, because in a normal system, lower frequencies of electrical current lead to less neural activity and less glutamate release so calcium levels do not rise.

Graphical Abstract



Main Takeaways

These results indicate an optimal stimulation window that reduces excitotoxic activity in glutamate treated motor nerves:

- Action potential amplitude (mV) reflects neuronal activity and calcium stress
- In healthy (control) nerves, stimulation intensity has little effect
- In glutamate-treated (hyperexcitable) nerves, high stimulation worsens overactivation
- Low-moderate stimulation hits a "sweet spot", reducing excessive activity without silencing signaling
- The effect is conditional: low stimulation helps only in hyperexcitable systems

Analysis

- Two sample T-tests were conducted, all p-values lower than 0.01
- All p-values lower than 0.05, except for 60-minute hair stimulation data as "sweet spot" has shifted, and neuron becomes unstable after that point
- Standard deviation is low, low variance in data

Conclusions

- Neuronal response exhibits a frequency "sweet spot" where activity is strong but remains stable, avoiding calcium overload and excitotoxic stress
- In control nerves, sweet spot is broad, indicating a robust system tolerant to stimulation changes
- After short glutamate exposure (30 min), low-frequency stimulation produces the largest responses by allowing recovery between spikes
- After prolonged glutamate exposure (60 min), the optimal window shifts, reflecting increased fragility and excitotoxic vulnerability
- Low-frequency stimulation transitions from optimal performance early to protective stabilization later, rather than being universally "best."
- Lower electrical frequencies can help lower excitotoxic stress in ALS-like conditions

Future Work

- Observe internal processes using calcium imaging with Fluo 4AM
- Conduct experiments for global cockroach environment to understand the larger implications
- Mathematically model equations for each level of electrical stimulation to provide a quantifiable relationship between calcium dynamics, electrical stimulation, and ALS
- Experiment on different stages of ALS progression

Results

Baseline control (frequency only)

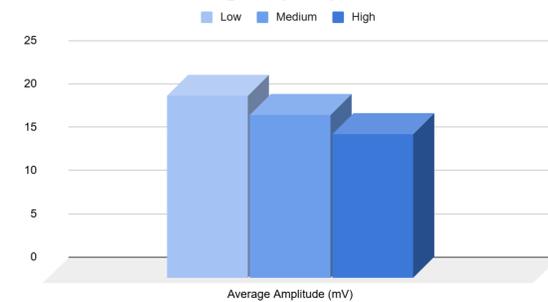


Figure 3: Baseline Control Bar Graph, electrical frequency only. Cockroach leg motor neurons have constant action potential wavelength of 15-16mV.

30 minute glutamate with hair stimulation - enhancement

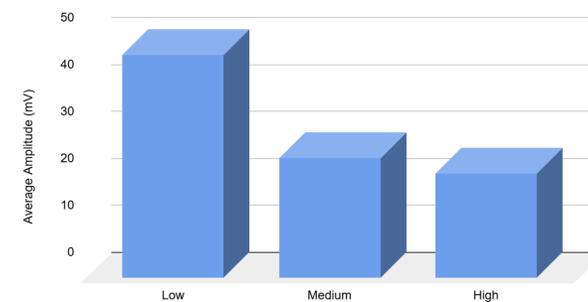


Figure 4: 30 Minute Exposure to L-glutamate Bar Graph, low, medium, high frequencies. In an ALS environment, higher frequency led to higher action potential output. **p<0.01.

60 minute glutamate with hair stimulation - system instability

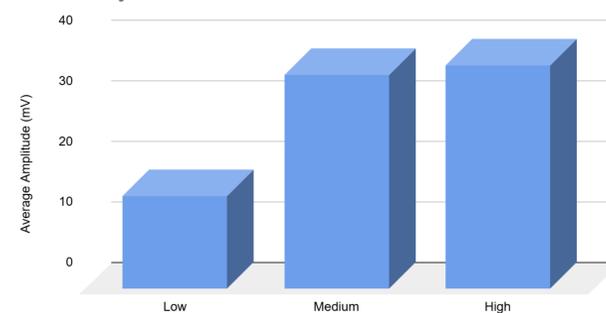


Figure 5: 60 Minute Exposure to L-glutamate Bar Graph, shows the instability of the system and how low frequency is now protecting the motor neurons. * p<0.05

Hair stimulation + frequency (sweet spot graph)

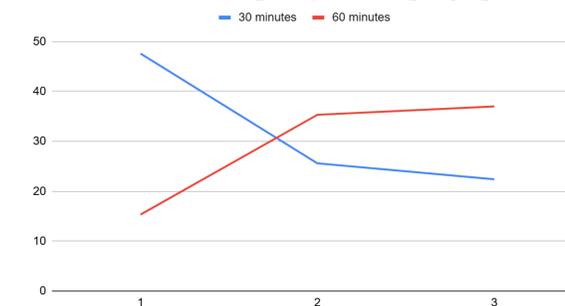


Figure 6: Line Graph representing the sweet spot shift over time With exposure to L-Glutamate.