Modeling the Effects of Astrocytic GLT-1 Downregulation and Therapeutic Agents on Tripartite Synapse in ALS

Binish Narang[1,6], Rishi Kanchi[2,6], Rohan Aluru[3,6], Theodore Kaczmarek[4,6], Daniel Stepanyan[5,6], Karla Montejo[6]

Ridge High School, Basking Ridge, NJ 07920[1]; William Fremd High School, Palatine, IL 60067[2]; Centennial High School, Frisco, TX 75035[3] ; New Trier Township High School, Winnetka, IL 60093[4]; The Roxbury Latin School, Boston, MA 02132[5]; Boston University, Boston, MA 02215[6]

Introduction

- **Amyotrophic Lateral Sclerosis (ALS)**
  - Neurodegenerative disease that impairs motor control
  - Affects approximately 30,000 individuals in the U.S.
- **Astrocytes**
  - Glial cells that regulate neuronal health and the uptake of glutamate, the primary excitatory neurotransmitter
  - Approximately 40% of sporadic ALS cases exhibit elevated levels of glutamate[1]
- **Glutamate Transporter 1 (GLT-1)**
  - Astrocytic transporter commonly downregulated in ALS responsible for 80% of total glutamate reuptake
  - Downregulation leaves excess synaptic glutamate, causing frequent action potentials and excitotoxicity, which damages motor neurons
- **Therapeutic Agents**
  - **Riluzole:** Most commonly prescribed medication for ALS. Reduces the release of glutamate into the synapse, decreasing excitotoxicity[2]
  - **FP802:** Experimental drug for ALS that inhibits the N-methyl-D-aspartate (NMDA) glutamate receptors that cause excitotoxicity by activating Ca^{2+} channels[3]
- **Goals**
  1. Develop a model of glutamate dynamics in healthy and ALS-affected synapses by integrating an astrocyte and a postsynaptic neuron
  2. Explore therapeutic effects of Riluzole and FP802 on ALS-affected glutamate pathways

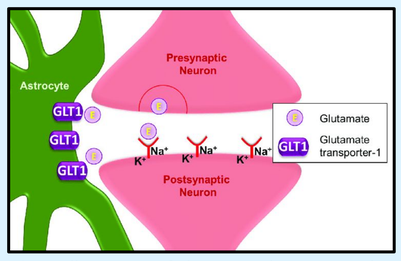

Fig. 1: Illustration of glutamate dynamics in the synapse, including astrocytic GLT-1 uptake

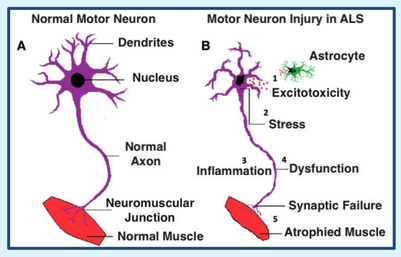

Fig. 2: Comparison between a healthy motor neuron and a neuron degenerated by ALS

Methods

- **Components of Python Ordinary Differential Equation Model**
  - **Synaptic Glutamate Model**
    - Created a model that reflects synaptic glutamate concentration over time

$$\frac{d[\mathrm{Glu_{syn}}]}{dt} = -k_{\mathrm{ast}}[\mathrm{Glu_{syn}}] - k_{\mathrm{post}}[\mathrm{Glu_{syn}}] + \mathrm{Glu_{stim}}$$

    - $[Glu_{syn}]$ represents the glutamate concentration in the synapse
    - k_{ast} is the rate constant for astrocytic glutamate uptake
    - k_{post} is the rate constant for postsynaptic glutamate uptake
    - Glu_{stim} is the glutamate stimulus from the presynaptic neuron (we manually input this)
    - We set k_{ast} = 0.08 and k_{post} = 0.02 based on empirical data to achieve the desired 4:1 ratio, as reported in previous studies[7]
  - **Postsynaptic Current Model[8]**
    - Outputs postsynaptic current based on glutamate concentration in the postsynaptic neuron
  - **Astrocyte Calcium Dynamics Model[4]**
    - Outputs calcium dynamics in the astrocyte based on glutamate uptake
  - **Hodgkin-Huxley Neuron Model[5]**
    - Simulates ion channel dynamics and synaptic activity in the form of action potentials
    - Modified the Hodgkin-Huxley model to include calcium ion channel dynamics[6]
  - **Computational Tools:** Model Created in Python 3.10.12
    - Matplotlib to create plots to analyze data
    - Scipy to solve ordinary differential equations
- **ALS Simulation:** Isolates the downregulation of astrocytic GLT-1 to model its impact on ALS
  - 81% decrease in k_{ast} from 0.08 to 0.0152[9]
- **Drug Simulation:** Simulates the effect of two different drugs on the ALS model
  - **Riluzole**
    - 50% decrease in glutamate stimulus representing reduction in presynaptic glutamate release[2]
    - 15% increase in k_{ast} from ALS-affected value[10]
  - **FP802**
    - 60% decrease in amount of postsynaptic glutamate uptake utilized in postsynaptic current[3]

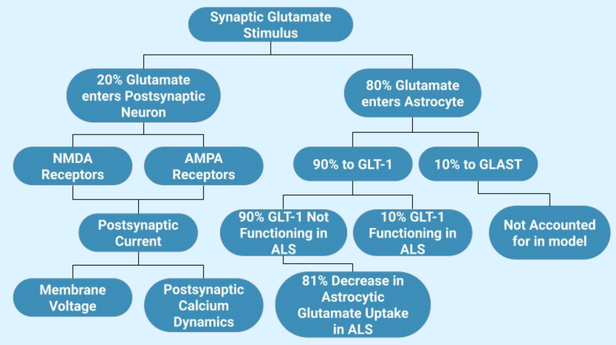

Fig. 3: Path of glutamate from the stimulus in the synaptic cleft to the postsynaptic neuron and astrocyte

Results

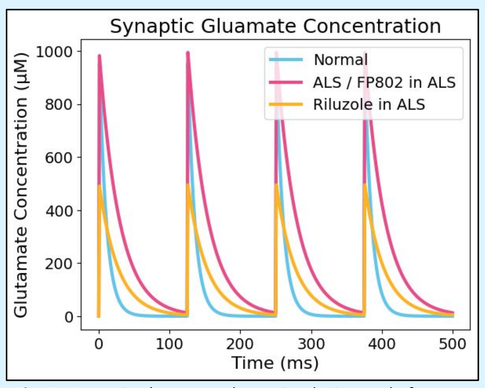

Fig. 4: Synaptic glutamate dynamics during and after stimuli. Different conditions result in different rates of glutamate exiting the synaptic cleft. FP802 shows the same pattern as ALS.

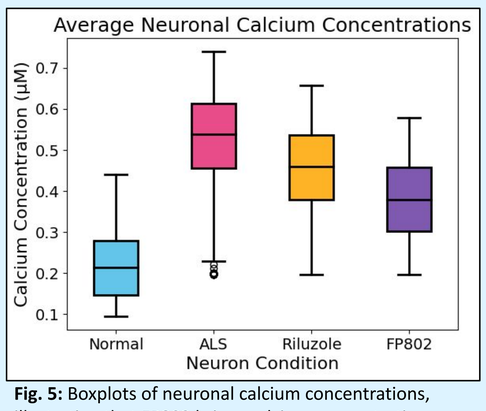

Fig. 5: Boxplots of neuronal calcium concentrations, illustrating that FP802 brings calcium concentrations closer to normal levels compared to riluzole

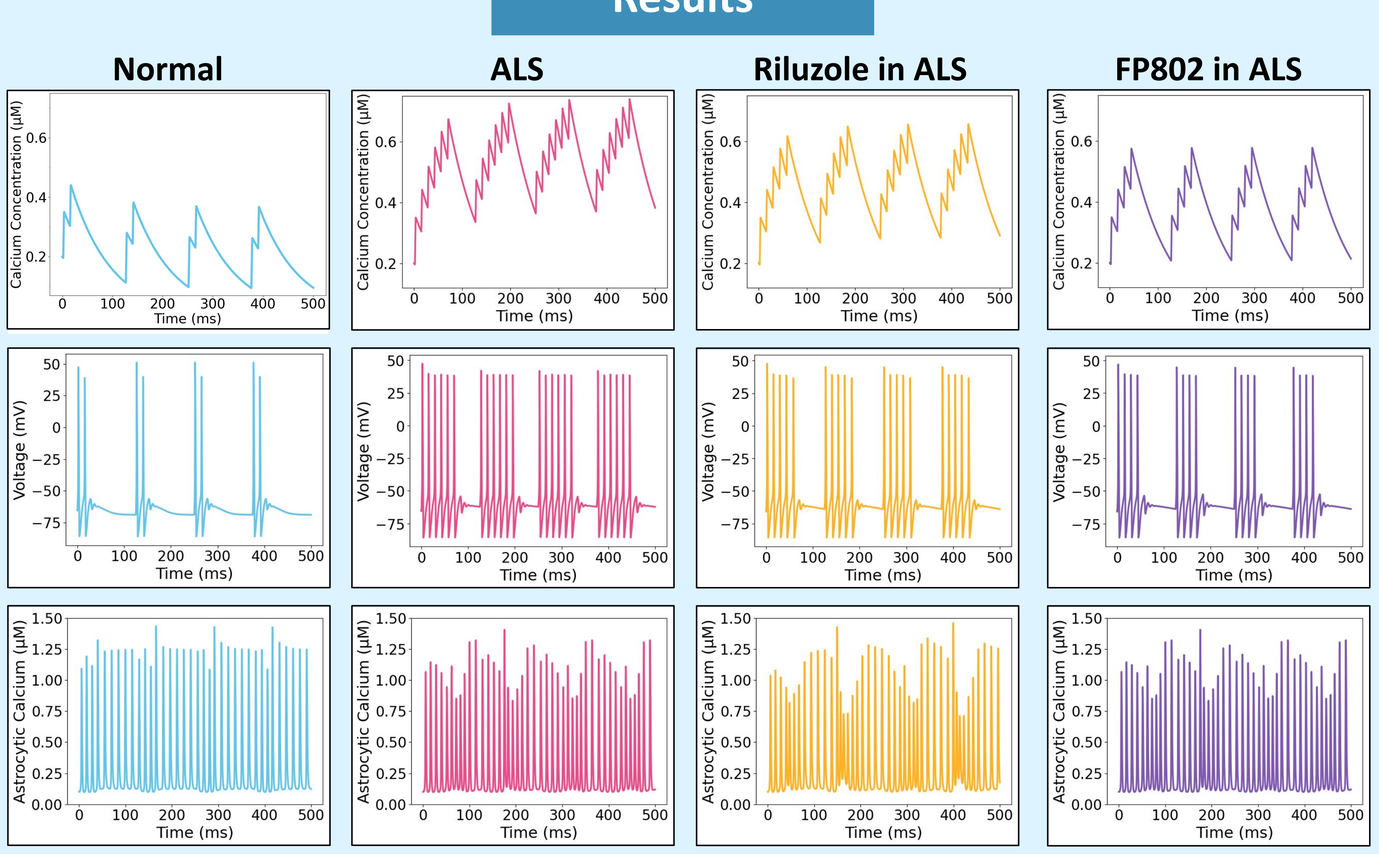

Fig. 6: Model results show neuronal calcium concentrations, postsynaptic membrane voltage, and astrocytic calcium concentrations in the first, middle, and last rows, respectively. The columns represent simulations under normal conditions, ALS, ALS with riluzole treatment, and ALS with FP802 treatment. In normal conditions, there are two action potential spikes per glucose stimulus, which increase to six under ALS conditions. Riluzole reduces this to five spikes, while FP802 further decreases it to four.

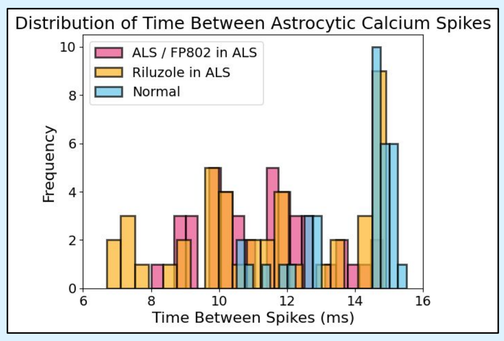

Fig. 7: Overlaid histograms of the time between astrocytic calcium spike peaks. FP802 shows the same pattern as ALS. Riluzole exhibits attributes of both the ALS and normal histograms, indicating it brings astrocytic calcium closer to normal levels.

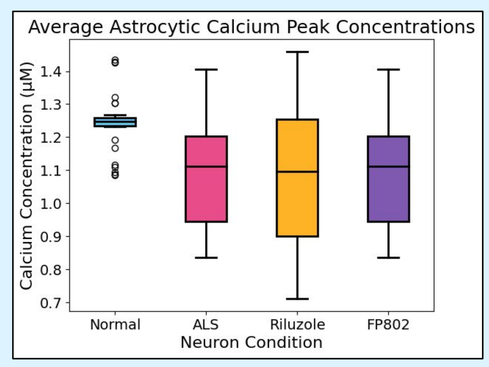

Fig. 8: Boxplots of average astrocytic peak calcium concentrations, illustrating that riluzole does not significantly alter astrocytic peak calcium concentrations

Discussion

Significance

- Our model provides a visualization of the complex interactions between an astrocyte, synapse, and neuron, mediated by glutamate
- **ALS Simulation**
  - Results show the multifaceted effects of GLT-1 downregulation on astrocytic and neuronal calcium dynamics, as well as neuronal action potentials
  - GLT-1 downregulation causes a significant increase in action potential spikes and calcium concentration, reflecting excitotoxicity in the postsynaptic neuron (Fig. 6)
  - GLT-1 downregulation also causes abnormal astrocytic calcium dynamics, as shown by the irregular shape of the calcium dynamics graph (Fig. 6) and a significantly shorter average interval between calcium spikes (Fig. 7)
- **Riluzole Simulation**
  - Riluzole causes a slight reduction in action potential spikes and neuronal calcium levels
    - Aligns with the limited effects of Riluzole on ALS, since studies show it prolongs patient tracheostomy-free survival by only 2-3 months[11]
- **FP802 Simulation**
  - FP802 causes significant reduction in neuronal action potentials and postsynaptic neuron calcium concentration
  - FP802 outperformed Riluzole in reducing action potential spikes and postsynaptic neuron calcium levels, highlighting its potential for future treatments
- Both drugs show minimal to no impact on peak astrocytic calcium levels, indicating that astrocytes would continue to exhibit abnormal calcium dynamics

Limitations

- **Assumptions in our Model:**
  - We assumed that all of the glutamate is taken up by only the astrocyte and postsynaptic neuron, although this may not accurately reflect actual conditions
  - Due to the limited research on FP802 as a relatively new drug, we had to make several assumptions to model its effects and address data gaps
  - Our model uses a generic Hodgkin-Huxley neuron to simulate ALS, assuming it resembles motor neurons despite inherent differences
- There is limited literature on the impact of ALS on the specific parameters used in both the Hodgkin-Huxley and astrocyte models
  - Limits the accuracy of our ALS-affected models, as some parameters may not accurately reflect the changes induced by the disease

Future Work

- FP802 effectively reduced excitotoxicity in ALS, indicating it could be a viable and potentially more effective alternative to Riluzole for treatment
- Results found that Riluzole and FP802 have limited effects on astrocytic glutamate dynamics, so astrocytes, which regulate homeostatic functions in neurons, will still function abnormally and contribute to excitotoxicity
- Further treatment targeting abnormal astrocytic calcium activity would be necessary to fully address the implications of GLT-1 downregulation
- Further research that accounts for motor neurons specifically in the model would also be more accurate in depicting ALS

References

Program Files

Acknowledgements

We would like to thank Karla Montejo, Ryan Senne, Steven Brandt, Patrick Bloniasz, Shahin Roozkhosh, and Krish Asija for their invaluable teaching, guidance, and assistance. We would also like to thank our families, the RISE program, and Boston University for their continued support and for this incredible opportunity.