Inhibiting Molecular Dynamics of Apoptosis and Oxidative Stress Pathways in Parkinson's Neurodegeneration

Connor Jin[1,5], Adrian Tomaszewski[2,5], Ashley Yan[3,5], Isha Harbaugh[4,5]

Syosset High School, Syosset, NY[1]; Leland High School, San Jose, CA[2]; Amador Valley High School, Pleasanton, CA[3]; Bloomington High School South, Bloomington, IN[4]; Boston University, Boston, MA[5]

Introduction

- Parkinson's Disease (**PD**) is a neurological disorder that causes degraded motor function, resulting in symptoms that massively debilitate patients
- PD is characterized by the loss of dopaminergic Substantia Nigra pars compacta (**SNc**) cells in the brain
  - Death of SNc cells is caused largely by mitochondrial dysfunction, resulting in elevated reactive oxidative species **(ROS)** and eventual apoptosis

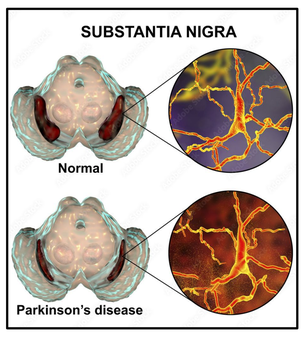

Figure 1: 3D illustration of the Substantia Nigra decreasing in volume and individual neurons during neurodegeneration

- Mechanisms for potentially inhibiting activation of ROS and apoptosis have been explored
  - Overload of mitochondrial calcium can cause oxidative stress
    - Prevented using calcium channel blockers (**CCBs**), notably the drug nimodipine in the nervous system
  - Stimulation of apoptosis inhibitor (**IAP**) release inhibits the caspase pathway in apoptosis
  - Inhibition of **Calpain protease** in the apoptotic pathway
- **Goal**: Using computational methods, we aim to simulate various potential mechanisms of inhibiting apoptosis and oxidative stress for PD treatment on the single-cellular level

Methods

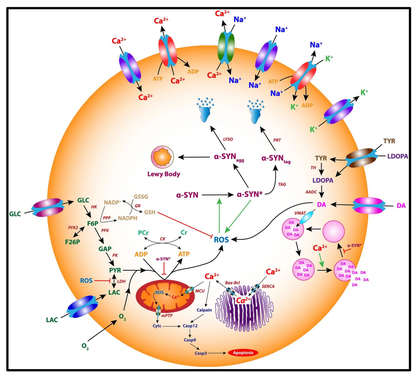

Figure 2: Diagram of the many cellular processes involved in SNc cell degradation and apoptosis.

- A single-cell biophysical model consisting of 56 differential equations is used to simulate the processes involved in gradual degradation of SNc cells (**Fig 2**)
  - We aim to improve the model by implementing new equations to represent different cellular systems

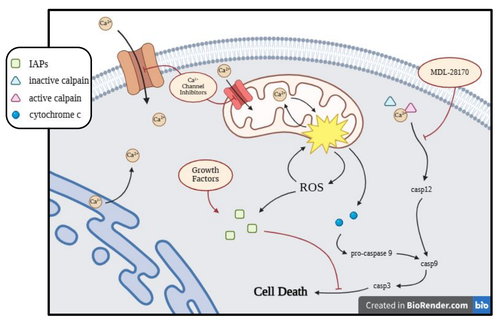

Figure 3: Schematic of the added processes in the apoptosis pathway and mechanisms of treatments

- Addition of new cell processes to the original model (**Fig 3**)
  - Signal for apoptosis decays over time
    - $d(apop)/dt = 10^{-6} |\ln(apop+1)| - 10^{-6}$
  - IAP is released at a rate that depends on ROS
    - $d(IAP)/dt \propto ROS$
  - Linear relationship between calcium and ROS beginning above a calcium threshold is added to the model
    - When $Ca_{mt} >$ threshold: $dROS/dt \propto Ca_{mt} - Ca_{mt^i}$
- Calcium channel inhibition using nimodipine
  - CCB inhibition is simulated through direct scalars in the differential equations that represent calcium ion channels
- Stimulation of IAP release by growth factor (GF)
  - Linear relationship between ROS and rate of IAP release is implemented with growth factor as a constant
- Introduction of calpain inhibitor MDL-28170
  - Concentration of active calpain is inversely related to the concentration of inhibitor; inhibitor potency remains constant
  - Based on competitive inhibition relationship
    - $d(calpain)/dt = (k4f * cai\_cal)/(1 + conc_{MDL}/K_i)$

Results

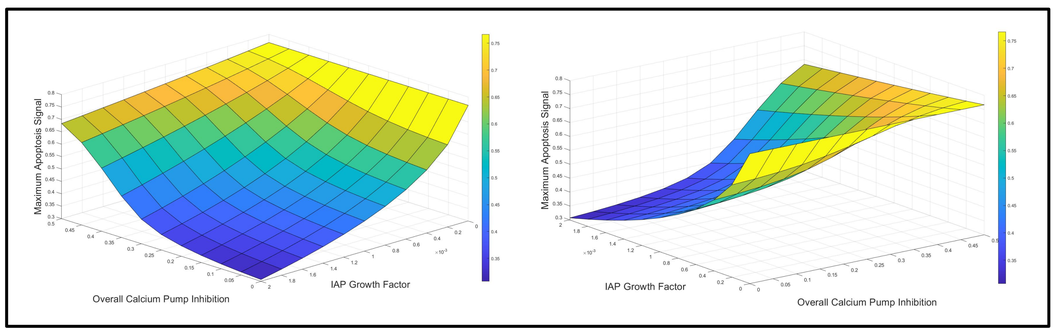

Figure 4: Combined effect of overall calcium inhibition and growth factor amplified IAP release on maximum apoptosis signal

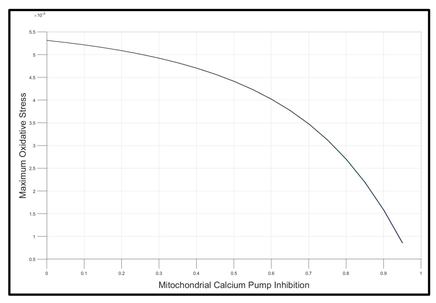

Figure 5: Effect of mitochondrial calcium inhibition on reactive oxidative species (ROS)

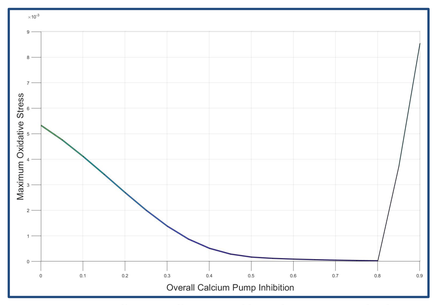

Figure 6: Effect of overall calcium inhibition on reactive oxidative species (ROS)

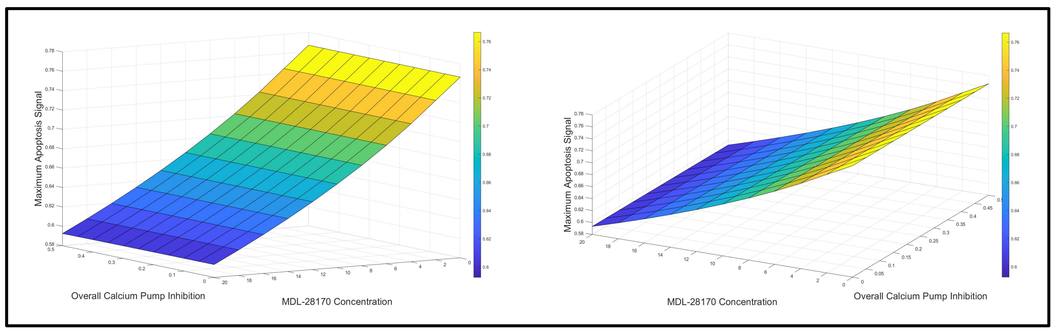

Figure 7: Combined effect of overall calcium inhibition and calpain inhibitor MDL-28170 concentration on maximum apoptosis signal

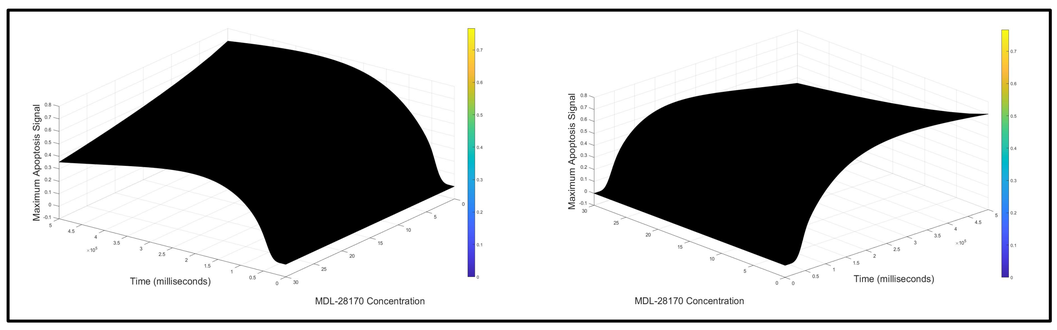

Figure 8: Effect of MDL-28170 concentration on how apoptosis signal evolves over time

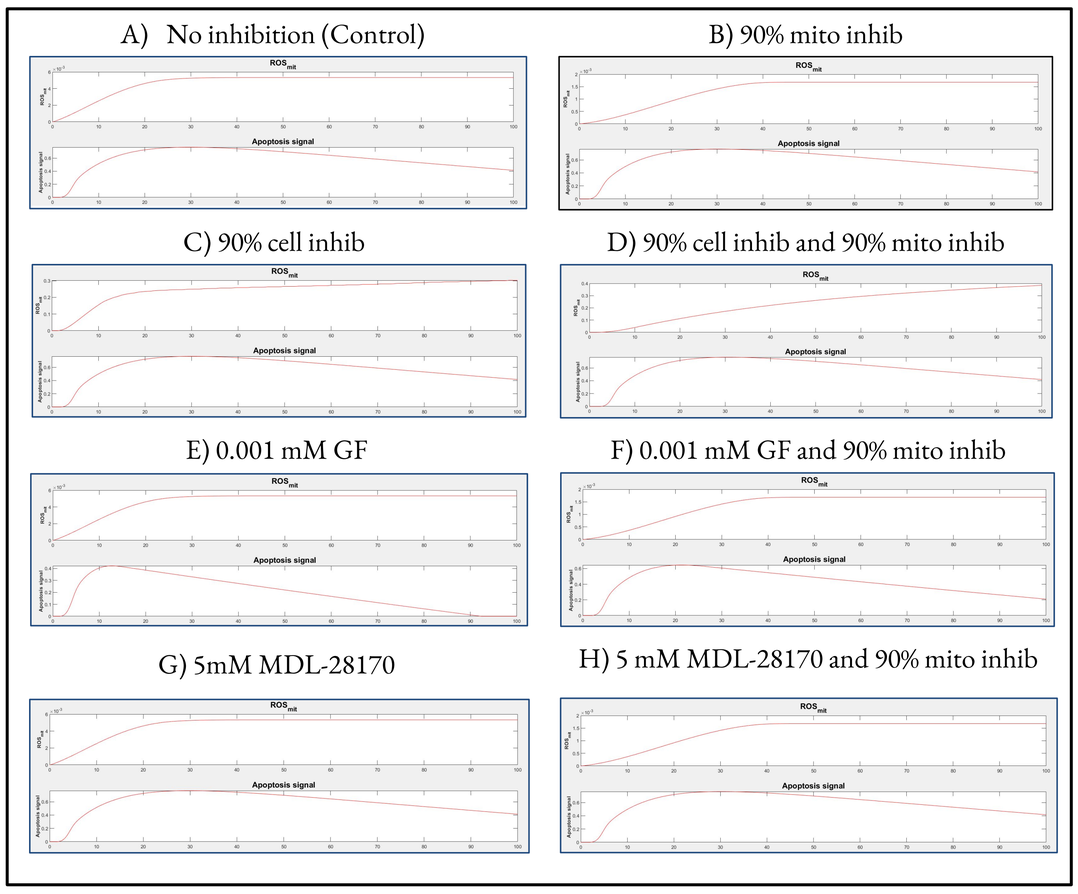

Figure 9: Reactive oxidative species (ROS_{mit}) in the mitochondria and quantity of apoptosis signaling over time in the single-cell model. With model additions, time graphs now simulate signal decay (**A**). Inhibition of mitochondrial protein channels (90% mito inhib) results in decreased ROS_{mit} and a slight delaying in of the onset of apoptosis signal (**B**). Inhibition of cell membrane protein channels (90% cell inhib) results in increased ROS_{mit} (**C, D**). Adding a concentration of general growth factor (0.001 mM GF) results in a lower peaking of apoptosis signal (**E, F**). Introducing MDL-28170 to inhibit calpain activation also decreases maximum apoptosis signal (**G, H**).

Discussion/ Conclusions

- The resulting changes due to implemented cell processes match realistic patterns, adding more general depth and plausibility to the base model
- Calcium inhibition **reduces oxidative stress** and delays the onset of apoptosis, supporting dihydropyridine CCBs such as nimodipine in cellular treatment of PD (**Fig 5**)
  - Blocking cell membrane calcium channels results in overall instability and increased ROS at higher inhibition, so specifically **targeting mitochondrial channels is optimal** (**Fig 6**)
  - However, too much inhibition of mitochondrial channels is detrimental, as it **prevents stress-induced IAP release** from occurring (**Fig 4**)
- Growth factor implication of IAP production and MDL-28170 inhibition of calpain **mitigate apoptosis signaling**
  - With presence of growth factor, maximum signal is reduced and the length of time in which the signal is present shortens (**Fig 9**)
  - MDL-28170 also reduces apoptosis signal though to a lesser degree than IAP release, but is not negatively affected by stress reduction (**Fig 7**)
- In conclusion, maximum combination of MDL-28170 and CCBs works well for mitigating both oxidative stress and apoptosis signals
  - Growth factor injection for IAP release is more effective in solely inhibiting apoptosis

Limitations

- Single-cell model limited ability to account for inputs from other neurons and lacks environmental context; downstream effects overlooked
- Assumed that inhibition of calpain by MDL-28170 primarily targeted calpain-2 and its neurodegenerative effects, producing oversimplified model of process
- Different concentrations for growth factor were based on trends seen in literature, not empirical data

Future Work

- Further improving plausibility of the model by altering equations according to dataset parameters
- Implementing more causational relationships between oxidative stress and apoptosis signal
- Using the model to simulate a network of SNc cells

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