Simulating Mitochondrial Failure in a Dopaminergic Neuron Upon Exposure to Increasing Dosages of MPP+ Neurotoxin

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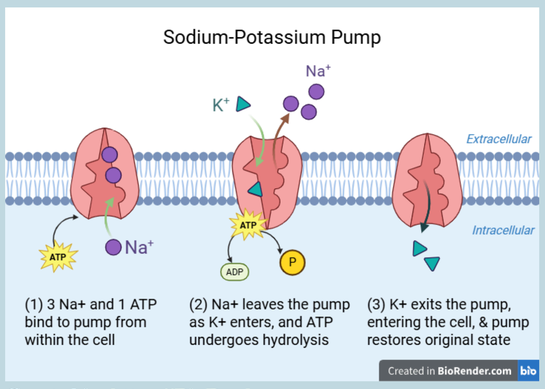
Introduction

- MPP+ is a neurotoxin that damages neurons responsible for motor control by binding to dopamine transporters.
- Often used in Parkinson's disease (PD) models due to how similarly MPP+-affected neurons and PD-affected neurons act.
- MPP+ induces mitochondrial dysfunction by inhibiting complex I of the electron transport chain, leading to ATP depletion and Na+/K+ pump failure.
  - Leads to sustained excessive depolarization of the axonal membrane, resulting in excitotoxicity and cell death.



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- Previous research outlines how MPP+ causes neuron death but does not propose a model to represent underlying mechanisms and subsequent effects.

Objective: To model the effect of MPP+ and subsequent ATP depletion on sodium-potassium pump function, membrane voltage changes, and neuronal firing rate and to assess at what concentration MPP+ will cause cell death.

Methods

- Using NEURON, we modeled a Hodgkin-Huxley neuron and simulated the effects of ATP depletion by adjusting sodium channel conductance, passive leak conductance, and leak reversal potential.
- ATP levels were set to decay exponentially over time, with decay rate dependent on MPP+ concentration.
  - Sigmoid function was fit to two known data points (91% decrease in ATP at 0.06 mM, 67% decrease in ATP at 0.05 mM) to estimate ATP suppression at other doses of MPP+.
- Baseline ATP amount was calculated based on 10 Hz firing rate.
- Tested various MPP+ concentrations (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07 mM) and compared to a control (0.00 mM).
- Neuron was stimulated with repeating electrical pulses. Membrane voltage changes, Na+ and K+ transport rates, and neuronal firing rate were each recorded.
- Neuron was considered dysfunctional (neuron failure) if membrane voltage failed to depolarize above -36 mV for longer than 100 milliseconds.

Results

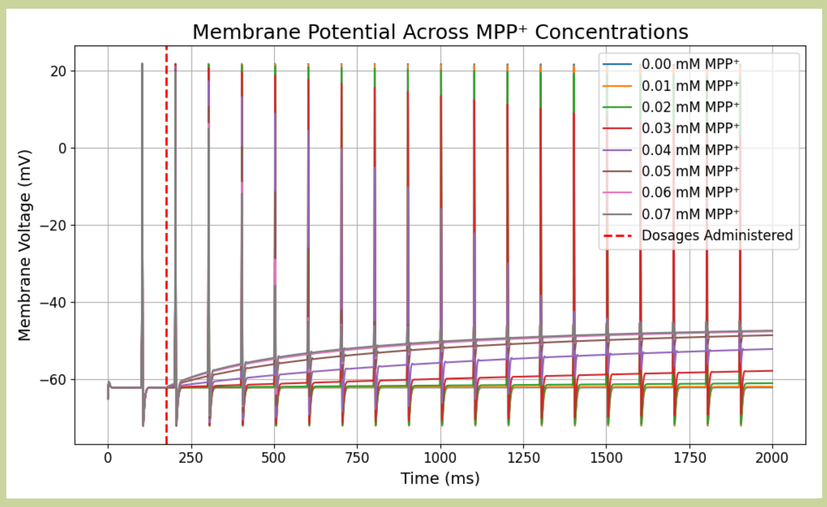
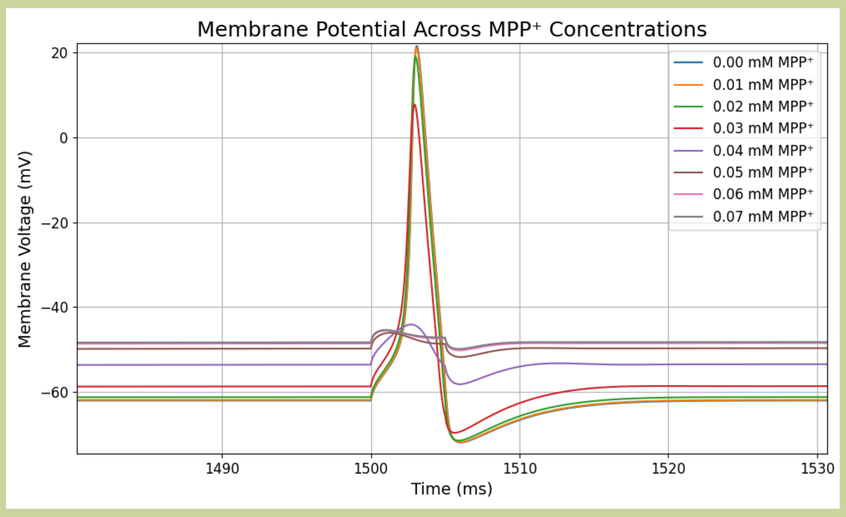


Figure 1

(on left) Graph depicts membrane voltage measured in millivolts (mV) of the dopaminergic neuron over time measured in milliseconds (ms). The red dashed line indicates initial dosage of MPP+. Resting membrane voltage increased over time for all dosages of MPP+ but not for the control. The most dramatic difference from the control was recorded in neurons exposed to dosages of MPP+ of 0.04 mM and more.

Figure 2

(on right) Graph of membrane potential in neurons over time in response to varying MPP+ concentrations, centered at 1480-1530 ms interval. At this time point, neurons exposed to concentrations of 0.04 mM and greater were unable to reach the threshold and fire an action potential. All neurons exposed to MPP+ also showed increased depolarization.



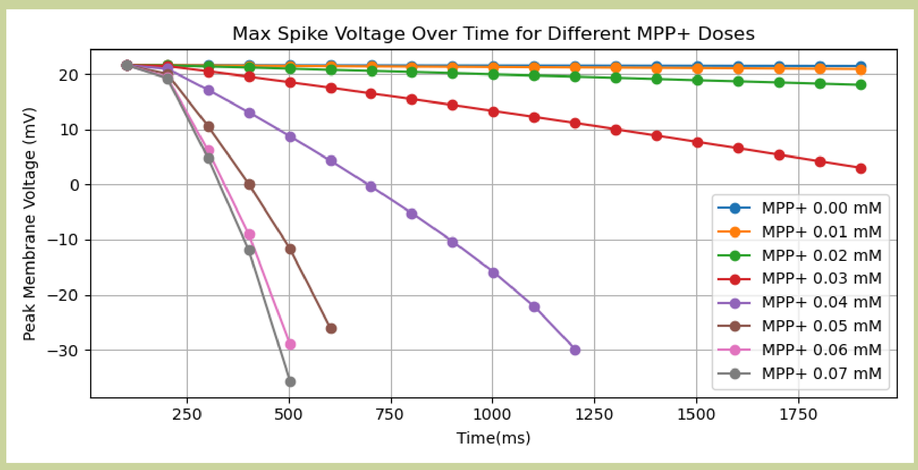


Figure 3

(on left) Graph of maximum spike voltage over time in response to varying MPP+ doses. Results indicate that as MPP+ dosage increases, maximum spike voltage decreases over time. At 0.04 mM, there is a noticeable drop-off that becomes more dramatic as MPP+ concentration increases, showing death.

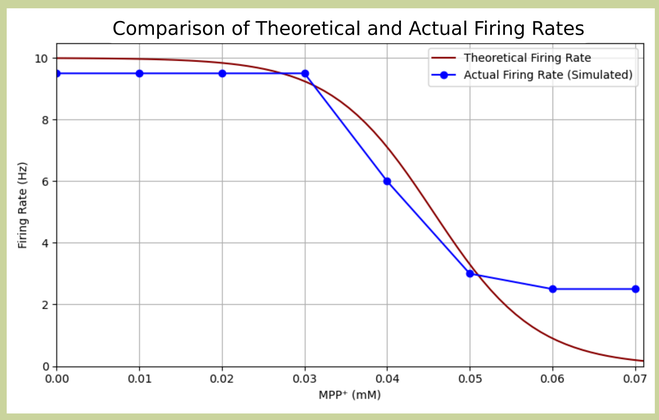


Figure 4

(above) Graph comparing theoretical and observed firing rates of modeled dopaminergic neurons, based on concentration of MPP+ exposure. Results show a disparity between theoretical and actual firing rate, prompting further investigation and refinement of model. However, the prediction that increased exposure to MPP+ would decrease firing rate held true, overall.

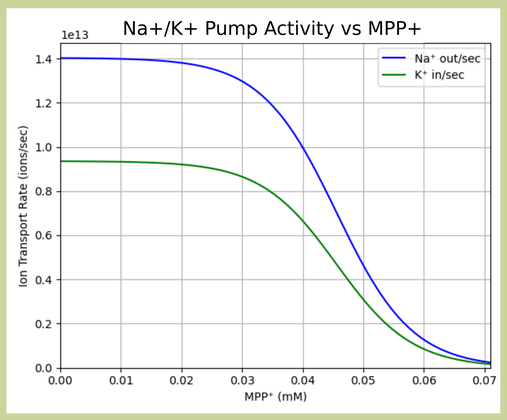


Figure 5

(above) Graph visualizing Na+ and K+ ion movement out of and into the neuron, respectively, for different MPP+ dosages. This represents Na+/K+ pump activity in the modeled neuron. Results indicate declining performance of the Na+/K+ pump as MPP+ dosage increases.

Analysis

- Neuron failure first occurred at 0.04 mM MPP+.
- Neurons exposed to MPP+ depolarized over time. The degree of change from the baseline resting potential increased with higher concentrations of MPP+.
  - Such depolarization also reduced the neuron's ability to hyperpolarize, reducing firing ability (as seen in the maximum spike voltage decrease).
- Overall, for all concentrations of MPP+ exposure, the neuron demonstrated decreased neuron firing, reduced ability to hyperpolarize, and reduced ability to transport Na+ and K+ ions.

Discussion

Impact

- Model serves as a predictive tool to quantify the MPP+ concentration at which cell failure occurs.
- Contributes to Parkinson's research by providing insight into ATP-driven neurodegeneration mechanisms on the cellular level.
- Acts as a cheap, convenient template to model the effects of varying doses of neurotoxins, like MPP+.

Limitations

- Hodgkin-Huxley model does not account for intracellular/extracellular Na+/K+ levels, which contributes to rising resting membrane potential
  - Hodgkin-Huxley also does not consider calcium influx as a factor in causing cell death.
- Relationship between MPP+ dosage and ATP depletion is based on limited data and a sigmoid function, which could contribute to discrepancies in accuracy
- Uses arbitrary parameters that are not entirely biologically accurate.
- Only models a single neuron; interactions in the scope of networks could hold more complexity.

Future Work

- Rerunning model with more specific parameters defining cell death.
- Collecting more data relating MPP+ concentrations and ATP depletion through wet lab experiments.
- Actual firing rates differed from theoretical firing rates. Future research may investigate the cause driving these variations.

Acknowledgements

We would like to thank Karla Montejo for her guidance, expertise, and insight on our research as well as the teaching fellows Rebecca Belisle, Heer Patel, Yichi Zhang, and Shahin Roozkhosh for their support throughout this whole process. We are also grateful to the RISE Program and Boston University for providing us with this wonderful opportunity and experience.

References

1. Dong, X.; Wang, Y.; Qin, Z. Molecular Mechanisms of Excitotoxicity and Their Relevance to Pathogenesis of Neurodegenerative Diseases. *Acta Pharmacologica Sinica* 2009, *30* (4), 379–387. https://doi.org/10.1038/aps.2009.24.
2. Pierpaolo Risiglione; Leggio, L.; Antonio, S.; Reina, S.; Paternò, G.; Marchetti, B.; Magrì, A.; Iraci, N.; Messina, A. High-Resolution Respirometry Reveals MPP+ Mitochondrial Toxicity Mechanism in a Cellular Model of Parkinson's Disease. *International Journal of Molecular Sciences* 2020, *21* (21), 7809–7809. https://doi.org/10.3390/ijms21217809.
3. Simms, Brett A.; Zamponi, Gerald W. Neuronal Voltage-Gated Calcium Channels: Structure, Function, and Dysfunction. *Neuron* 2014, *82* (1), 24–45. https://doi.org/10.1016/j.neuron.2014.03.016.