Effects of Calcium Mishandling in Astrocytic Mitochondria: The Unexplored Powerhouse

Undergraduate Research Scholars
LAUNCH: UNDERGRADUATE RESEARCH


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Abstract

Astrocytes are the most populous cell type in the central nervous system (CNS) and are vital in neuron development, regulating blood flow, and maintaining communication between neurons. Astrocytic control on the CNS is due in part to their complex morphology, consisting of a circular soma extending into primary branches that further divide into smaller branchlets and leaflets. The morphology of astrocytes allows calcium (Ca^{2+}) to function as their main communicator, much like electrical signals in neurons. Increased Ca^{2+} in astrocytes causes gliotransmitter release, leading to regulation of synaptic transmission in neurons. The majority of Ca^{2+} signals in astrocytes originate in mitochondria, even within the nanometer sized branchlets and leaflets. Recent studies show Ca^{2+} mishandling in neuronal mitochondria leads to dysfunction and is associated with early neurodegeneration in Alzheimer's and Parkinson's disease. However, effects of Ca^{2+} mishandling in astrocytic mitochondria are less understood. Considering this, we will disrupt Ca^{2+} signaling specifically in astrocytic mitochondria using an adeno-associated virus (AAV) overexpressing neuronal calcium sensor 1 (NCS1), a ubiquitous Ca^{2+} buffering protein. Under normal conditions, NCS1 functions in neurotransmitter release, cell growth and survival. However, NCS1 is upregulated in schizophrenia, bipolar disorder, and autism as well as Parkinson's disease. In order to verify disruption of Ca^{2+} signaling via NCS1, our construct will first be assessed in the astrocytoma cell line, SMA-560. By assessing changes in morphology and respiration, as a result of Ca^{2+} mishandling, we hope to progress our understanding of the role this unexplored powerhouse plays in neurodegenerative diseases.

Astrocytic Mitochondrial Calcium

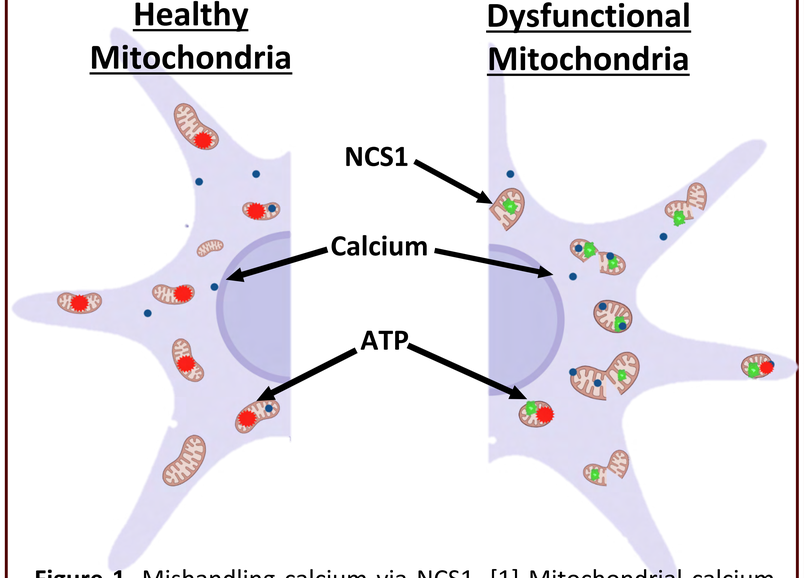


Figure 1. Mishandling calcium via NCS1. [1] Mitochondrial calcium plays pivotal roles in physiological processes such as neuronal homeostasis, ATP production and induction of apoptosis. Calcium dysfunction can cause inflammation and neurodegeneration. [2,3]

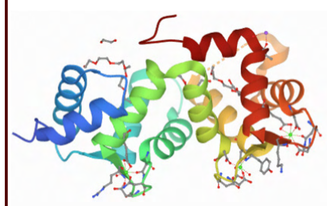


Figure 2. Structure of NCS1 [4]. Upregulation of NCS1 to observe changes in astrocytic mitochondria to understand the role calcium places in neurodegenerative diseases. [5]

Methods

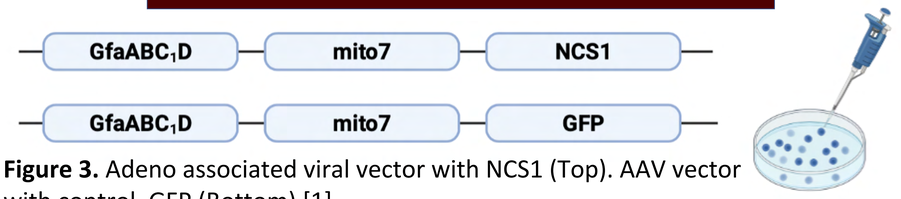


Figure 3. Adeno associated viral vector with NCS1 (Top). AAV vector with control, GFP (Bottom) [1].

1) Analyzing structure and morphology using confocal imaging & MitoGraph
2) Analyzing mitochondrial respiration using Seahorse XFe96 Analyzer

MitoGraph Analysis

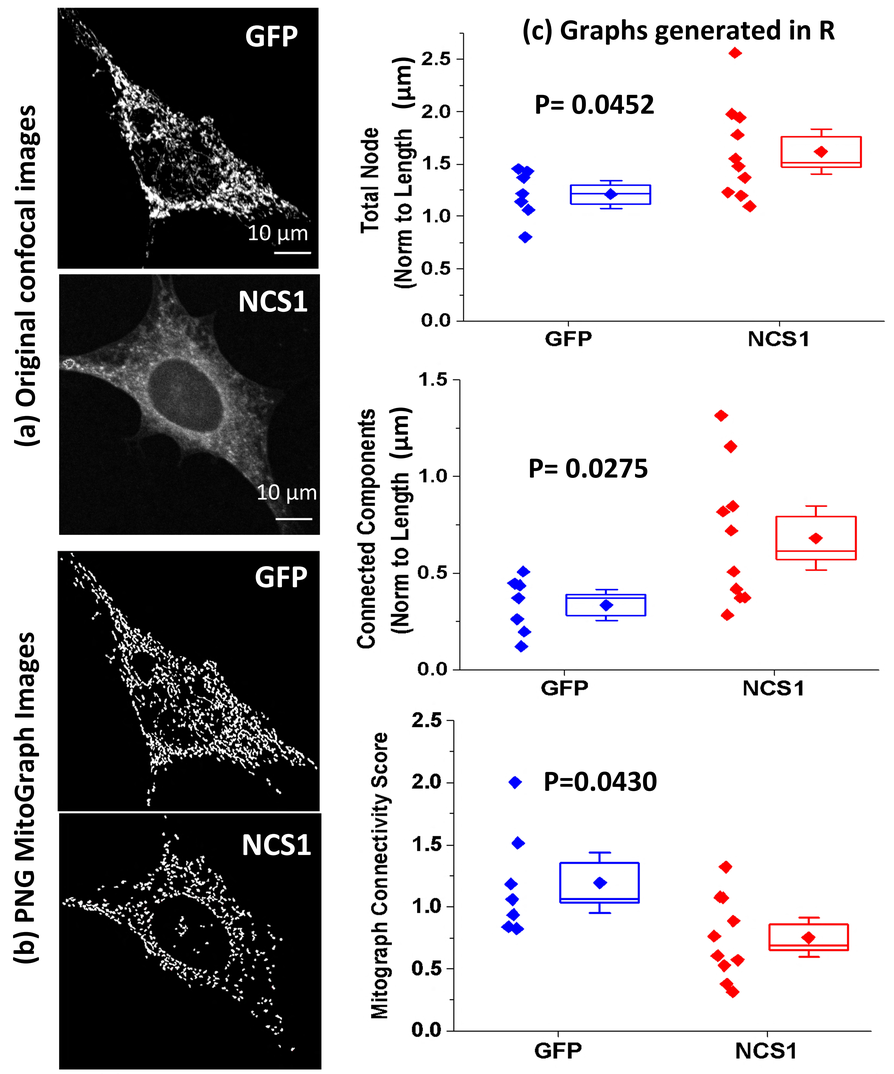


Figure 4. (a) Original confocal image. GFP targeted to fluoresce astrocytic mitochondria. (b) PNG binary image generated from MitoGraph shows mitochondria network. (c) Graphs created in R Studio comparing mitochondrial morphology in control cells and NCS1 upregulated cells.

Seahorse XFe96: Mito Stress Test

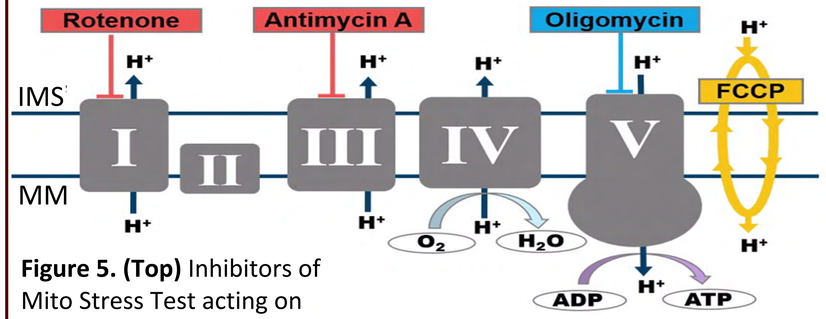
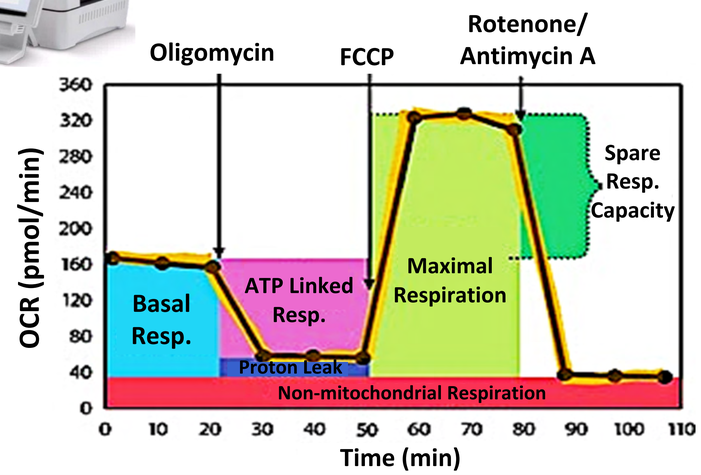


Figure 5. (Top) Inhibitors of Mito Stress Test acting on Electron Transport Chain. [6]

Figure 6. (Left) Seahorse XFe96 Analyzer. **(Bottom)** Mito Stress Test Profile: Showing changes in Oxygen Consumption Rate. [6]



Conclusion & Future Directions

MitoGraph:

- Overall decreases in mitochondrial network connectivity when NCS1 was upregulated this is apparent given the increase in total node length and connecting components

Seahorse:

- Preliminary results indicate a change oxygen consumption rate and extracellular acidification rate with overexpression of NCS1.

Future Directions:

- More experiments upregulating NCS1 *in vitro* and eventually test of NCS1 construct *in vivo*

Acknowledgments & References

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[1] Image Created with BioRender.com
[2] Giorgi C, Danese A, Missiroli S, Patergnani S, Pinton P. Calcium Dynamics as a Machine for Decoding Signals. *Trends in Cell Biology*. 2018;28(4):258-273. doi:10.1016/j.tcb.2018.01.002
[3] Giorgi C, Marchi S, Pinton P. The machineries, regulation and cellular functions of mitochondrial calcium. *Nature Reviews Molecular Cell Biology*. 2018;19(11):713-730. doi:10.1038/s41580-018-0052-8
[4] PDB: 1G8I
[5] Gong, Yehong & Zhu, Yuzhen & Zou, Yu & Ma, Buyong & Nussinov, Ruth & Zhang, Qingwen. (2016). Human Neuronal Calcium Sensor-1 Protein Avoids Histidine Residues To Decrease pH Sensitivity. The Journal of Physical Chemistry B. 121. 10.1021/acs.jpcb.6b11094.
[6] Agilent Technologies. Seahorse XF Cell Mito Stress Test Kit User Guide. 2019;2:20.