



uOttawa

Homeostatic Synaptic Plasticity in Cortical and Enteric Neuronal Cultures



Eadan Farber¹, Sarah Schock², William Staines³

¹Undergraduate Research Opportunity Program, University of Ottawa, Ottawa, ON, ²Canada Children's Hospital of Eastern Ontario Research Institute, Ottawa, ON, Canada

³Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada

Introduction

Homeostatic synaptic plasticity (HSP) is the phenomenon of neurons regulating their activity to maintain axonal firing at a homeostatic level. If neuronal activity is increased due to an excitatory stimulation, increases in inhibitory mechanisms and decreases in excitatory mechanisms will follow. The opposite is true if neuronal firing is reduced. Many neurodevelopmental disorders show impairments in HSP including Rett syndrome, autism and epilepsy. This study primarily focused on mastering techniques of cell culture and immunohistochemistry in both rat cortical neurons and mouse enteric neurons in order to study HSP.

Rett Syndrome (RTT) is an X-linked neuroplasticity disorder that affects 1 in 10 000 young girls. Usually symptoms, which don't appear until 6-18 months of age, include impairments to motor control, severe intellectual disabilities and seizures. The majority of RTT cases can be attributed to mutations in the methyl CpG binding protein 2 (MeCP2) which is responsible for synaptogenesis during development and shows increased expression postnatally. It promotes neuronal communication in the central nervous system (CNS) and maintains the function of synapses and neuronal networks throughout the lifespan. Previous research has found expression of MeCP2 in the enteric nervous system (ENS) and that synaptic plasticity of enteric neurons are MeCP2 dependent.

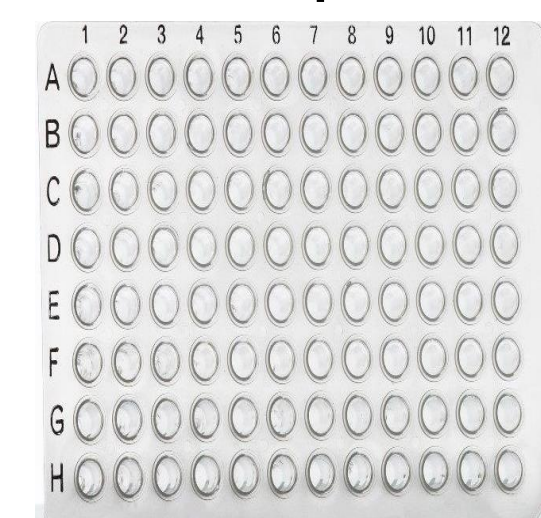
Methods

Cortical Cultures



Cryopreserved rat cortical neurons were thawed and plated (21-28 DIV)

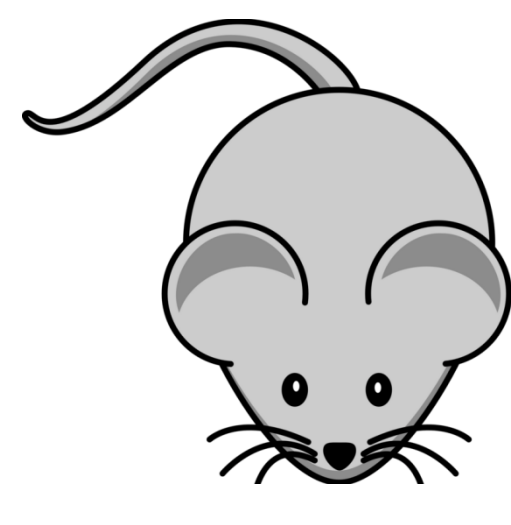
96 well plates



Drug Treatments

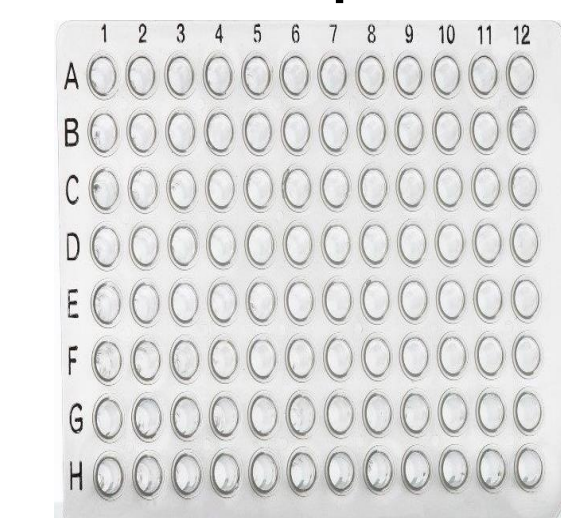
- KCl (45 mM)
- Bicuculline (20 μ M)
- TTX (1 μ g/ml)

Enteric Cultures



Freshly dissected adult mouse enteric neurons were plated (21 DIV)

96 well plates



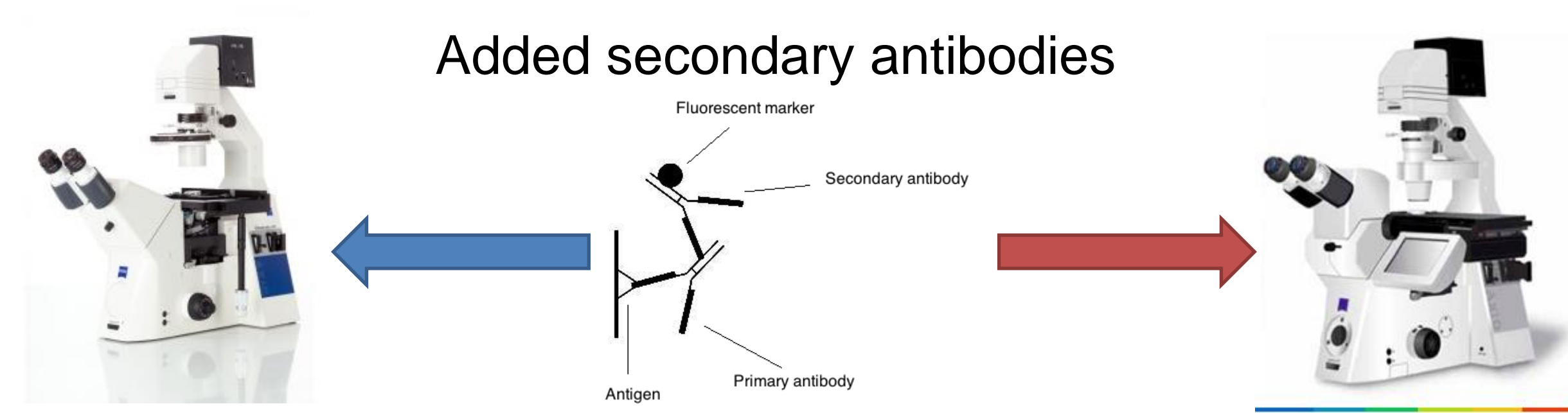
Drug Treatments

- KCl (45 mM)
- Acetylcholine (1 mM)
- Nialimide (1 μ M)
- 5-HT (10 μ M)

Media Changed every 3-4 days

Added primary antibodies

Added secondary antibodies



Zeiss Axio-observer D1
• Immunohistochemistry

Zeiss Axio-observer Z1
• Whole well scan
• Image J analysis

Results

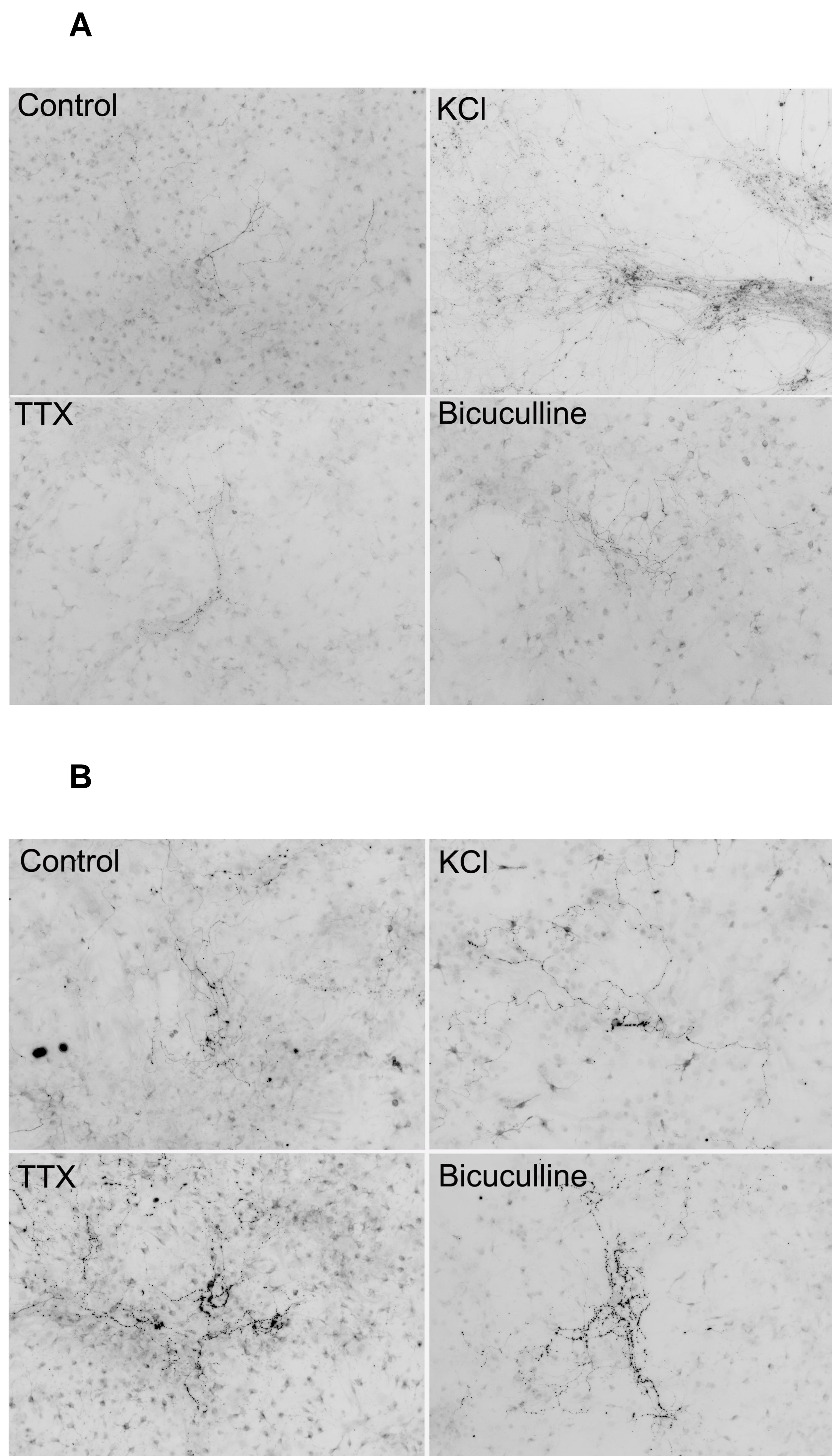


Figure 1. The vesicular marker synapsin following KCl (45mM), bicuculline (20 μ M) and TTX (1 μ g/ml) drug treatments lasting 48 hours in **A.** 21 DIV cortical neuron cultures and **B.** 28 DIV cortical neuron cultures. All three treatments show increased synapsin indicating HSP. A full study would employ quantification and statistical analysis.

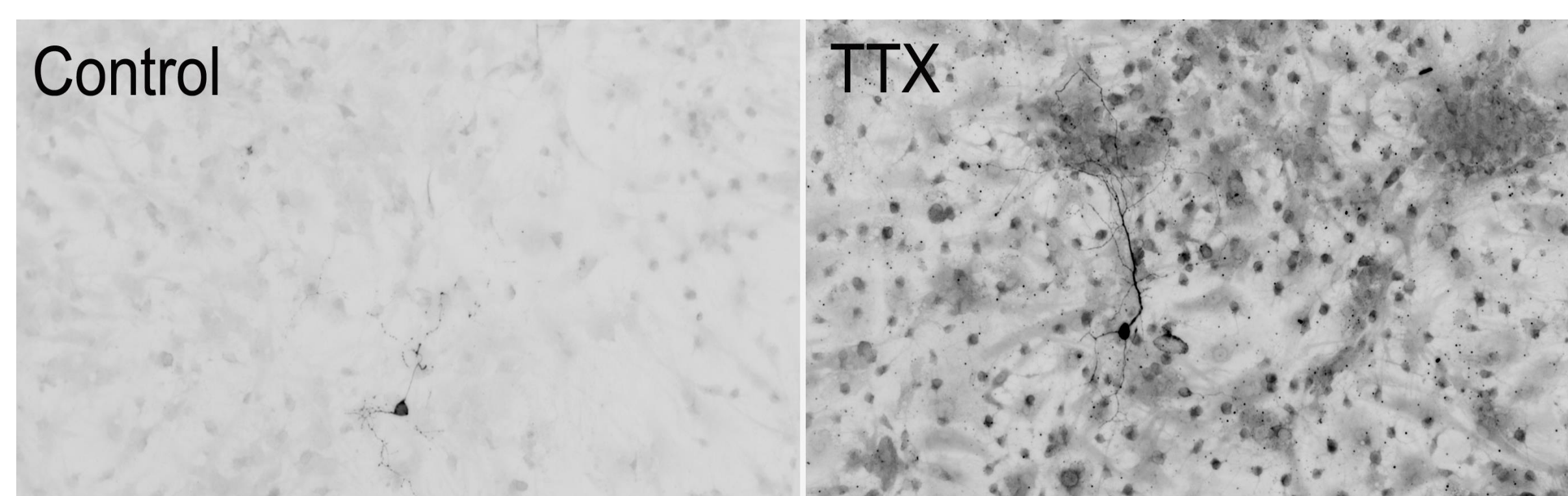


Figure 2. The up-regulation of the calcium binding protein calretinin following TTX (1 μ g/ml) drug treatment of 48 hours.

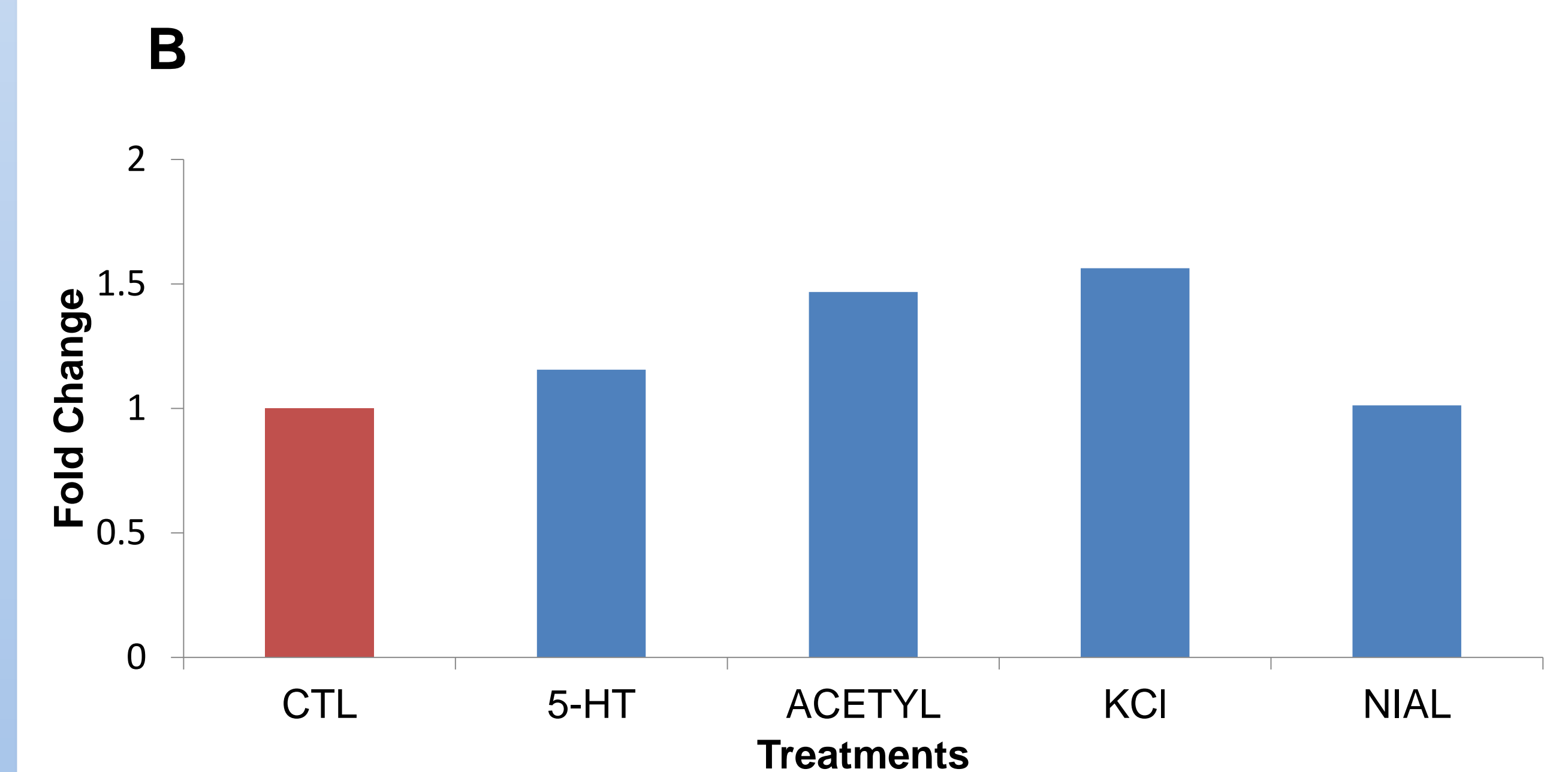
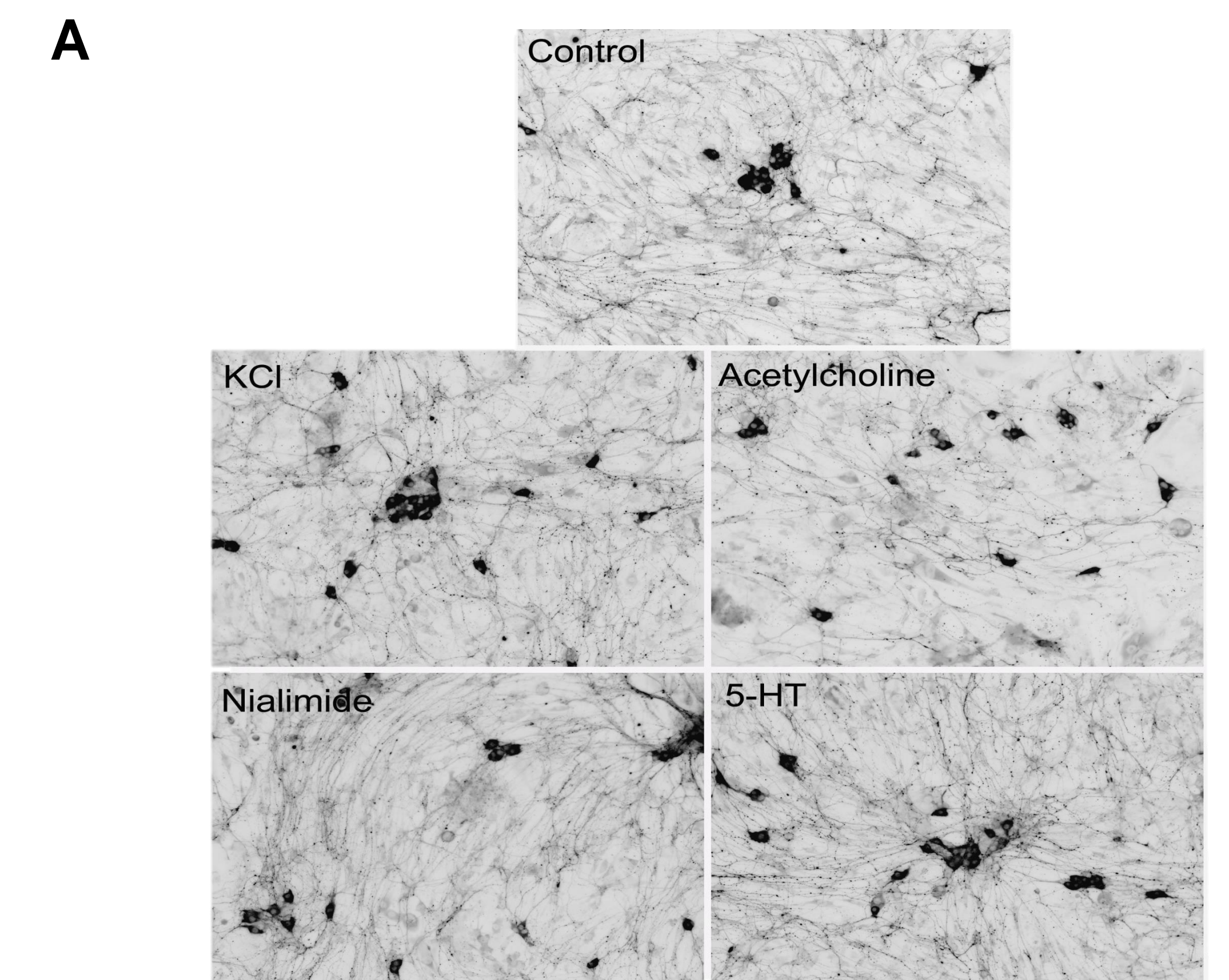


Figure 3. Immunohistochemical techniques performed on 21 DIV enteric cultures. **A.** NOS staining following KCl (45mM), acetylthiocholine iodide (1mM), nialimide (1 μ M) and 5-HT (10 μ M) drug treatments lasting 48 hours.

B. Acetylthiocholine iodide and KCl drug treatments each showed a 1.5-fold increase in NOS expression relative to control.

Conclusion

- Evidence of HSP was seen using a variety of different excitatory and inhibitory drug treatments in frozen cortical cultures.
- NOS levels in enteric cell cultures increase in response to acetylcholine and KCl drug treatments demonstrating the possibility of HSP in the ENS.

Future directions:

- Repeat procedure multiple times to achieve statistical significance.
- Show synaptic scaling changes with network electrophysiology using Multichannel-Electrode Arrays.

Acknowledgements

Funding for this study was provided by the Undergraduate Research Opportunity Program.

Many thanks to Dr. William Staines and Dr. Sarah Schock for sharing their expertise and guiding me throughout this project. Thank you to Dr. David Grynspan and George Wahba as well for all their help and support.

References:

1. Wahba, G., Schock, S. C., Claridge, E., Bettolli, M., Grynspan, D., Humphreys, P., & Staines, W. A. (2015). MeCP2 in the Enteric Nervous System. *Neurogastroenterology & Motility*, 27(8), 1156-1161.
2. Turrigiano, G. G. (2008). The Self-Tuning Neuron: Synaptic Scaling of Excitatory Synapses. *Cell*, 135(3), 422-435.