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Dopaminergic Synapse Loss in 6-OHDA Lesioned Zebrafish

Grace Stalions
Hope College

Heather Van Dort
Hope College

Gabriella Taylor
Hope College

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Grace Stalions, Gabriella Taylor, Heather Van Dort, and Erika Calvo-Ochoa
Departments of Biology and Neuroscience, Hope College, Holland, MI, 49423

Background

Parkinson's Disease (PD)

- PD is a neurodegenerative disorder that results from a loss of dopaminergic neurons in the substantia nigra of the midbrain.
- 90% of people with Parkinson's disease experience a loss in olfaction (smell). However, the relationship between PD's and olfactory loss is not well understood.

Zebrafish

- We share 70% of our genes with zebrafish, and they have an exposed olfactory system that is simpler and similar to humans.

Past Research

- The Calvo lab has found evidence of significant dopaminergic loss at 1 day post injection (dpi) with 6-OHDA.
- PET scans of human brains show that the loss of presynaptic terminals precedes dopaminergic loss (Chu et al., 2012).

We aimed to study the effects of 6-OHDA on dopaminergic synapses by studying colocalization with pre-synaptic markers.

Hypotheses

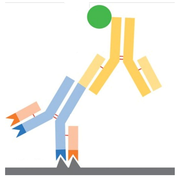
- We expected to observe a significant loss in dopaminergic neurons and synapses (TH+) in zebrafish following injection with 6-OHDA. This synaptic loss will be followed by a decrease in co-localization with an olfactory pre-synaptic marker. (SV2+)

Methods

1. Injection and tissue processing

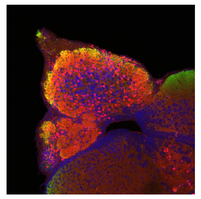
6-OHDA was injected into the ventricle space on the dorsal side of the zebrafish (*Danio rerio*) brain. Brains were dissected, embedded, and sectioned for immunohistochemical stainings.

2. Immunohistochemistry



- Dopaminergic neurons: rabbit anti-TH.
- Synapses: mouse anti-SV2.
- Tagged primary antibodies with fluorescent secondary antibodies.

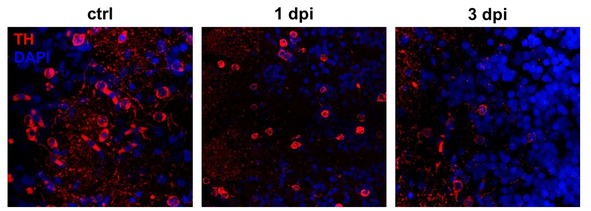
3. Confocal Microscopy



DAPI = Neurons
TH = Tyrosine Hydroxylase
SV2 = Synaptic Vesicles
Overlap = Dopaminergic Synapses

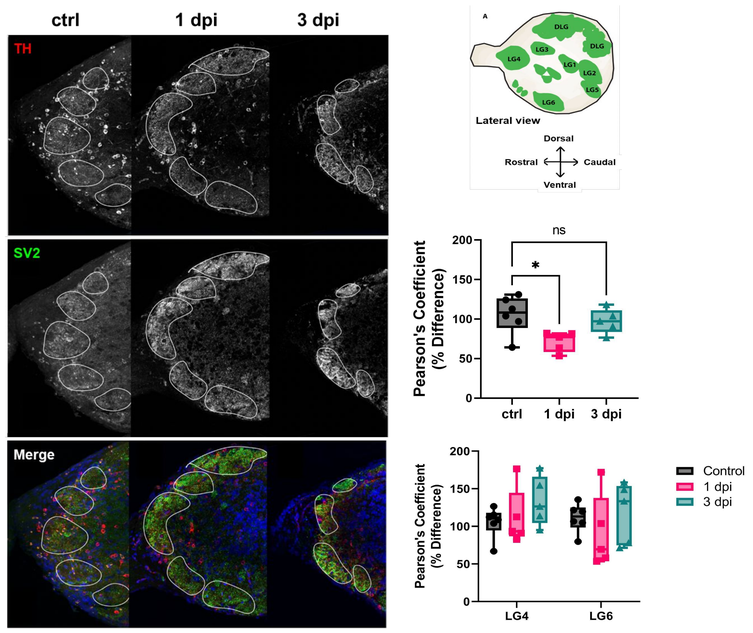
6OH causes dopaminergic neuron death

- The number of dopaminergic somata were counted.
- There was a significant loss of dopaminergic neurons at 1- and 3-days post-injection (dpi), showing that 6-OHDA effectively targets dopaminergic neurons.



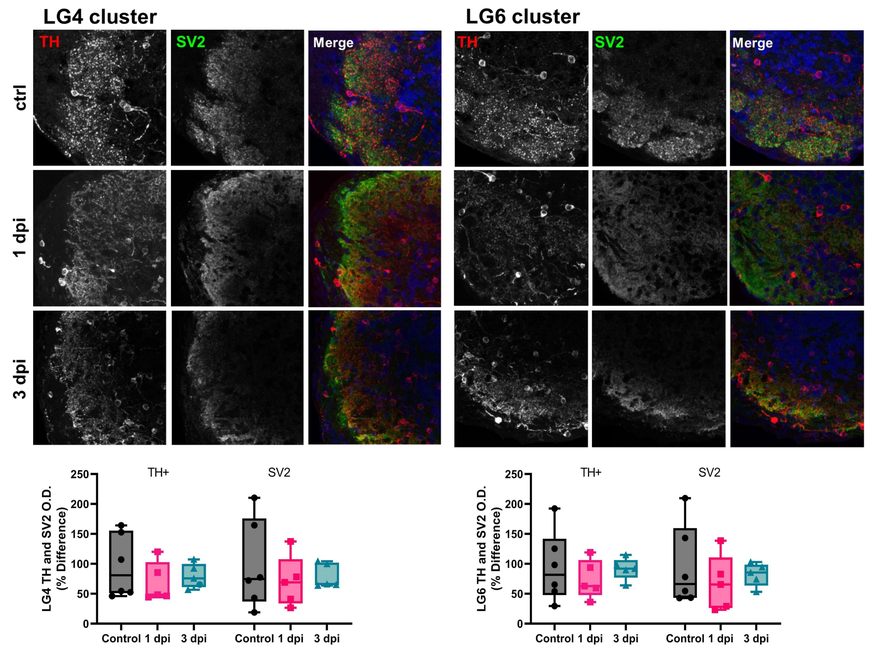
6-OHDA reduces dopaminergic overlap with olfactory synaptic terminals

- Pearson's Coefficient was used to analyze the amount of overlap between dopaminergic neurons and pre-synaptic vesicles to determine the relative amount of dopaminergic synapses.
- A significant whole-bulb decrease in overlap was found 1 dpi which indicates a decrease in dopaminergic synapses, no differences were found within individual glomeruli studied.



Dopaminergic loss disrupts glomerular morphology and localization

- Severe glomerular morphology disruption was found in the whole bulb and on the individual glomerular clusters selected, LG4 and LG6 after 6OHDA injection.
- No difference in optical density for TH or SV2 was found in neither glomeruli
- Both clusters localized in the direction of the olfactory nerve, suggesting changes to the olfactory epithelium and nerve



Conclusions

- There was a significant loss of dopaminergic neurons and synapses following 6OHDA injection.
- 6OHDA caused changes in the shape and location of glomerular clusters in the whole bulb.
- The glomeruli appeared to migrate in the direction of the nerve at 1 dpi and even more so at 3 dpi.
- We found a decrease in overlap of dopaminergic neurons and pre-synaptic terminals in the olfactory bulb. We hypothesize that synaptic terminals were reorganized following injection with 6-OHDA to cope with the stress of neuron loss due to injection. These data suggest that 6-OHDA may impact the axonal wiring or morphology within the olfactory bulb.

Acknowledgments

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References

- Chu et al., 2012, Brain