4-14-2023

Modeling Olfactory Dysfunction in Parkinson's Disease in Zebrafish

Nathaniel Vorhees
Hope College

Samantha Groenwold
Hope College

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Samantha Groenwold, Nathaniel Vorhees, and Dr. Erika Calvo-Ochoa

Hope College, Holland, MI

Background

Parkinson's Disease (PD), is a neurodegenerative disorder characterized by a loss of motor function and dopaminergic neurons in the brain.

Olfactory dysfunction is one of the earliest and most common indicators of PD due to the dopaminergic center found in the olfactory bulbs (OB).

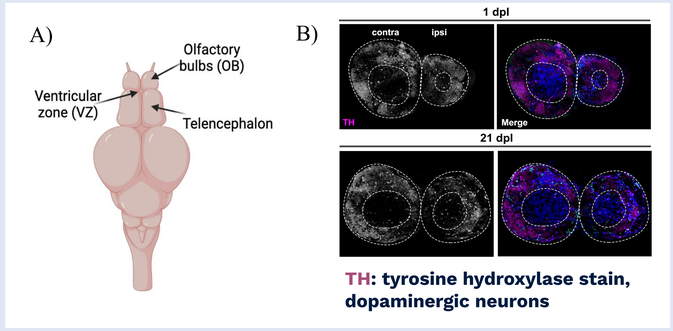


Fig. 1 A) The olfactory bulbs (OB) and telencephalic ventricular zone (VZ) are known neurogenic niches of the zebrafish. Thus providing a useful tool to study PD. **B)** Dopaminergic neuronal regeneration is seen within the the OB. This regeneration is seen from 1 to 21 days post lesion with the use of tyrosine hydroxylase (TH) staining.

Zebrafish are a valuable model for PD and other neurodegenerative diseases due to their remarkable regenerative capacity and high plasticity of the central nervous system. With this model we are able to study both neuronal degenerative and regenerative mechanisms.

Our goal is to establish a novel model of Parkinson's Disease in zebrafish to study olfactory neurodegeneration and dysfunction.

Hypotheses

Following injection of 6-Hydroxydopamine (6-OHDA) there will be dopaminergic neuronal loss and an increase in neuroinflammation, resulting in olfactory loss.

After the neurotoxin injection there will be a(n)...

a) Decrease in the number of (TH) stained neurons
b) Increase of GFAP expression showing astrocytic inflammation
c) Loss in olfaction shown during the behavioral assays

Methods

- Adult zebrafish were injected with 6-OHDA into the VZ and left to recover for either 1 or 3 days post injection (dpi). Olfactory and motor function behavioral analyses were performed.
- Brains were dissected and embedded in paraffin for immunohistochemistry and TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) assays
- Antibodies against an astrocyte marker, GFAP (glial fibrillary astrocytic protein), and a dopaminergic neuron marker, TH, were used. TUNEL assays were performed to assess cell apoptosis.
- Tissue was treated with fluorescent secondary antibodies and observed in a Nikon A1 confocal microscope.

Morphological Results

A 6-OHDA injection generates increased apoptosis in the olfactory bulb at 1 dpi. At 3 dpi apoptosis return to control levels.

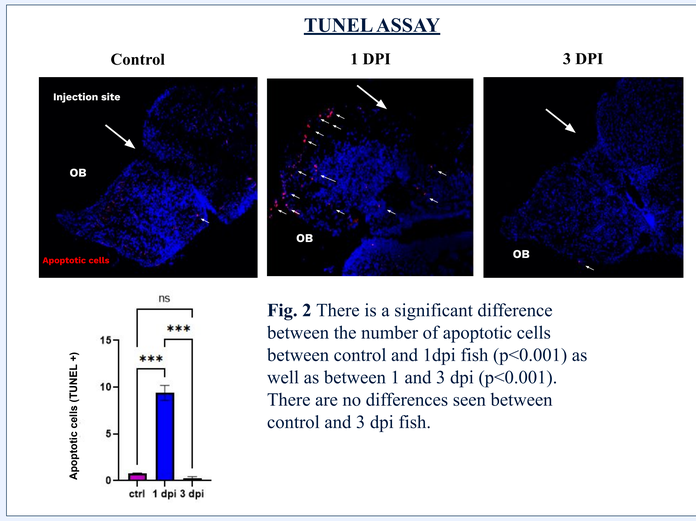


Fig. 2 There is a significant difference between the number of apoptotic cells between control and 1dpi fish ($p<0.001$) as well as between 1 and 3 dpi ($p<0.001$). There are no differences seen between control and 3 dpi fish.

A 6-OHDA injection leads to a decreased number of dopaminergic neurons in the OB.

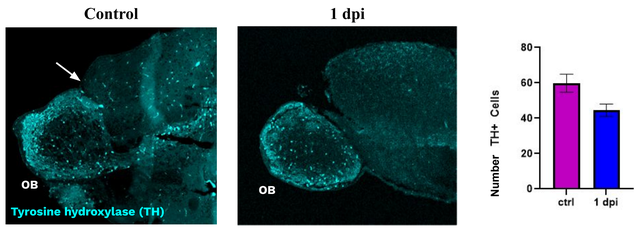


Fig 3. Our preliminary data (n=3) suggests that there is a decrease in the number of TH+ cells in the OB of the 1 dpi group.

Work in progress!

A 6-OHDA injection produces a strong inflammatory response shown by increased astrocytic activation.

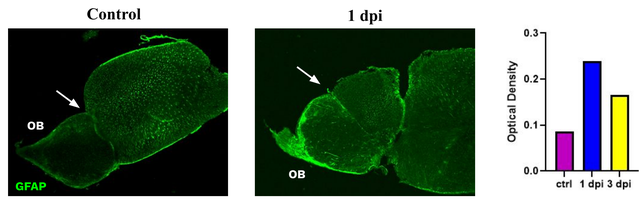
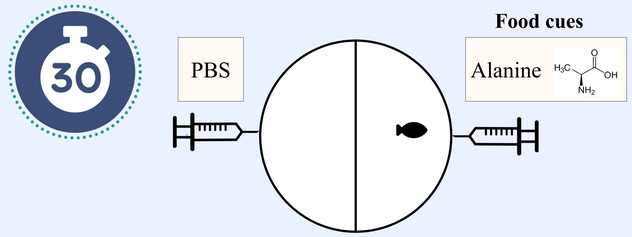


Fig 4. Our preliminary data (n=2) suggests that there is an increase in GFAP staining in the OB of the injected groups, indicating astrocytic activation.

Work in progress!

Experimental Design



After 30 minutes of acclimation, 3 mL of PBS and Alanine (a food cue) are injected into the behavioral chamber and the subject's responses are recorded. Data such as distance, speed, and location are recorded both before and after injection.

Behavioral Results

A 6-OHDA injection increases zebrafish locomotion

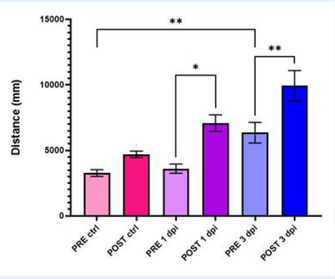


Fig. 5) Injected fish (3 dpi) swim significantly ($p > 0.01$) more than control fish before and after odorant delivery, indicating an effect on locomotion. The distance traveled also increased 1 and 3 dpi after the injection, indication that the fish can smell

Olfactory function is not affected by the injection

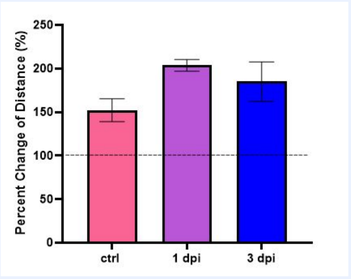


Fig. 6) All three groups swam a further difference on average after the alanine was injected to the behavioral chamber, indicating that the fish could smell. No significant difference was found between controls and 6-OHDA injected groups.

Conclusions

Our results show that following a 6-OHDA injection there is:

1. **An increase in cell apoptosis that subsides 3 dpi in the OB**
2. **A decrease in the number of dopaminergic cells in the OB**
3. **An increase in astrocytic activation that decreases at 3 dpi**
4. **An increase in locomotor activity, but limited olfactory deficits**

We are looking forward to continue to explore the degenerative properties, inflammatory response, and regenerative capabilities in our model of PD

Thank you to Hope College, the Ed and Ann Anderson Award, the Stewart family, and the Kenneth Campbell Foundation to help fund this research