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Unveiling Sensory Gaps: A Study on Zebrafish Alanine Taste Blindness and the Consequential Neurotoxic Impact on Dopaminergic Neurons in Motor Regions

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Unveiling Sensory Gaps: A Study on Zebrafish Alanine Taste Blindness and the Consequential Neurotoxic Impact on Dopaminergic Neurons in Motor Regions

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Basic Background

Parkinson's Disease (PD) is a neurodegenerative disorder characterized by deficits associated with Dopaminergic (DA) neuronal death. Smell loss is also associated with patients with PD as DA neurons are found in the Olfactory Bulb (OB), the brain structure that processes odor signals.

Previous research has used 6-OHDA zebrafish models, but no studies have researched the impact of these injections on other brain regions. An additional experiment was needed to test whether this model was targeting solely DA neurons in the OB, or if DA neurons in the motor nuclei were also affected.

Our study aims to focus on the behavioral repercussions of DA neuron loss in the OB of zebrafish. We intend to investigate the validity of an animal model of PD.

Morphological Background

In previous experiments, this lab found that this novel 6-OHDA lesion targeted the dopaminergic neurons in the Olfactory Bulb, resulting in significantly fewer Tyrosine Hydroxylase positive stained neurons.

However, studies performed by other labs found that motor nuclei were affected by a 6-OHDA injection in the ___ ventricle in the zebrafish brain, caudal of our novel injection.

Another experiment needed to be performed to assess whether the novel injection also affected the DA neurons in the motor nuclei.

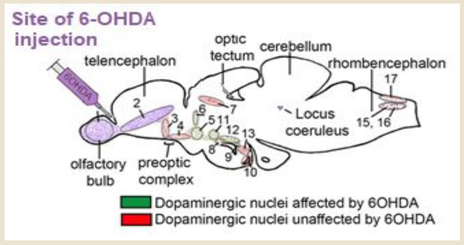


Fig. 1) A schematic sagittal section of an adult zebrafish brain with a 6-OHDA-resistant dopaminergic cell populations (red) and the vulnerable dopaminergic (green) and nonadrenergic populations (purple) in relation to the injection site in the third ventricle (Caldwell et al. 2019).

Results

6-OHDA injections increase swimming and speed distance before and after alanine exposure

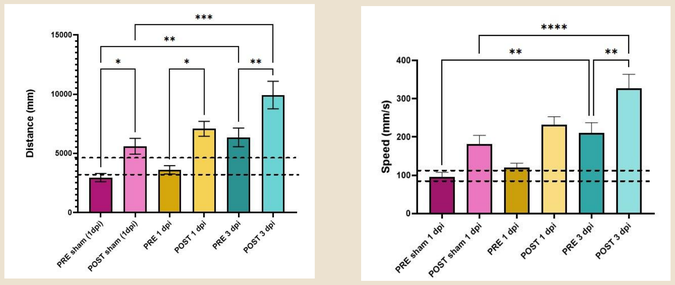


Fig. 7) Injected fish (3 dpi) swam significantly ($p > 0.01$) more and faster than control fish before and after odorant delivery, indicating an effect on locomotion. There is also evidence that these fish can smell due to the significantly ($p < 0.05$) further distance swam after odor delivery.

Zebrafish Cannot Taste Alanine

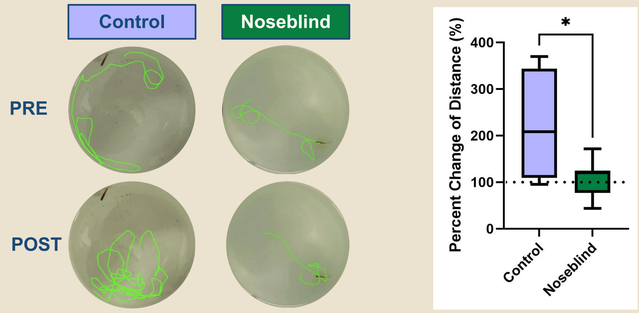


Fig. 8) Fish with their nostrils glued did not swim significantly more after odor delivery. This significantly ($p < 0.05$) contrasted with the control zebrafish group, which swam 200% further distance on average, after odorant delivery. Therefore, when the sense of smell was removed, alanine was not detected. Indicating that zebrafish cannot taste alanine.

Methods

- We injected adult zebrafish with 6-OHDA into the ventricular zone (Fig. 1) and left to recover for either 1 or 3 days post injection (dpi).
- Brains were dissected and embedded in paraffin for immunohistochemistry, using antibodies against a dopaminergic neuron marker, TH. Tissue was observed with a Nikon A1 confocal microscope.
- We performed motor and olfactory-mediated behavioral assays and analyzed data using an animal tracking software (Fig. 2)

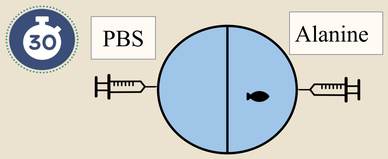
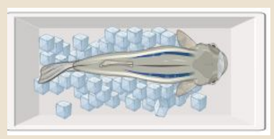


Fig. 2) After 30 minutes of acclimation, PBS and alanine (a food cue) are injected into the behavioral chamber and the subject's responses are recorded. Data including distance, speed, and location are recorded both before and after injection.

- To assess if zebrafish can taste alanine we performed a behavioral assay with a "noseblind" treatment group which had their nostrils glued shut

Fig. 3) illustrates the method behind our nares occlusion. Vetbond glue was used to occluded the nares (nostrils) of the zebrafish which was applied while the fish was anesthetized over with a lab brush.



Hypotheses

Following 6-Hydroxydopamine (6-OHDA) injection, the zebrafish brain will present:

1) **Reduced dopaminergic (DA) neurons in the olfactory Bulbs**
2) **Similar DA neurons in the motor regions**
3) **Disruption in olfactory-mediated responses to alanine**

Additionally, we hypothesize that zebrafish with their noses glued shut will not detect alanine in behavioral assays

Novel 6-OHDA injection location does not affect the dopaminergic neurons in the motor regions of the brain

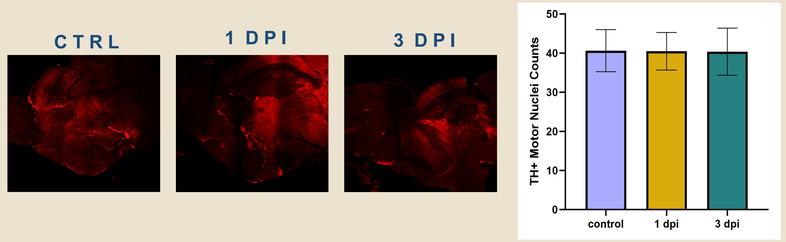


Fig. 5) There was not a significant difference between the number of Tyrosine Hydroxylase positive stained neurons in the motor regions of the zebrafish brain of either treatment groups or the control group. ($p > 0.05$)

Conclusions

Our results show that our novel 6-OHDA injections cause:

1. **A decrease in Dopaminergic neurons in the OB**
2. **No affect on number of Dopaminergic neurons in the motor regions**
3. **Increased locomotion**

Additionally, our Behavioral Assays were supported as zebrafish cannot taste alanine

We are looking forward to continue to explore the degenerative properties and regenerative capabilities in our model of Dopaminergic Neuronal death in the OB

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