



### **DOPAMINERGIC SIGNALING WITHIN THE OLFACTORY BULB**

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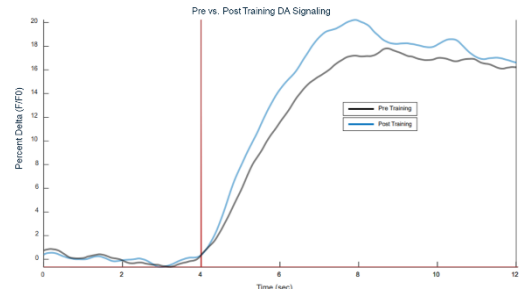
**Background:** When an animal inhales an odorant, an action potential forms and travels through the axons of the receptor cells to the glomeruli structures within the olfactory bulb (OB). Dopamine (DA) is a neural transmitter that is released in the Short Axon Cells (SACs) located on the surface of the glomeruli. DA is synthesized by the enzyme Tyrosine Hydroxylase (TH) and regulates inputs and outputs of the olfactory bulb through exciting or inhibiting the neurons. Glomerular signals are received by OB output mitral and tufted cells, which send an action potential to higher order brain regions [1]. Previous studies have shown that TH expression, and therefore DA signaling, in the OB is stimulated by odor exposure and decreased during odor deprivation [2]. Additionally, decreased DA expression has been correlated with memory loss and the inability to control fine movements, common in Parkinson's and Alzheimer's disease. [3,4] In our project, we sought to determine what effect odor enrichment and odor learning has on the DA signaling in the OB of a mouse model. The OB is an excellent model for DA signaling because of the ability to easily manipulate inputs and image in-vivo. Two paradigms were used to determine if simple odor enrichment had a different effect than odor learning. We hypothesized that odor evoked activity would lead to an increase in DA signaling. Similarly, we hypothesized odor enrichment would also heighten DA signaling.

**Methods:** In this project, we tested DA signaling in two different paradigms: learned behavior and passive exposure. In the learned behavior paradigm, mice were injected with GRABda, a receptor-activation optical sensor, into the left olfactory bulb. [5] After recovery, mice were imaged with 2-photon microscopy to gather a baseline odor evoked DA signal. Then the mice were trained to perform a lick, no-lick task to recognize a specific odor. After both mice had at least 80% success rate with identifying the correct odor, we began post training imaging. In analysis, regions of interest were selected for the baseline and post training imaging and then compared for the difference of DA signaling. The mice were then perfused for anti-TH immunohistochemistry along with the control mice. The primary antibody Anti-TH AB152 from Millipore Sigma and secondary antibody Goat anti-rabbit IgG Alexa fluor 488 from Thermo Fisher Scientific were used. The control group slices and learned behavior group slices were then examined using epifluorescence microscopy and average fluorescence was analyzed and compared.

In the passive exposure paradigm, we exposed three mice to 2-Hydroxy Acetophenone odor in a tea ball. This odor was replaced every 24 hours for five days. The control group consisted of one mouse with an empty tea ball placed in the cage for five days. After five days, all mice were perfused for anti-TH immunohistochemistry. These slices were then examined using epifluorescence microscopy and average fluorescence was analyzed. The average was calculated by taking the sum of the gray values of all pixels in the selection divided by the number of pixels, using the Image J program [6]. This was done for each slice then the all values for each group were averaged.

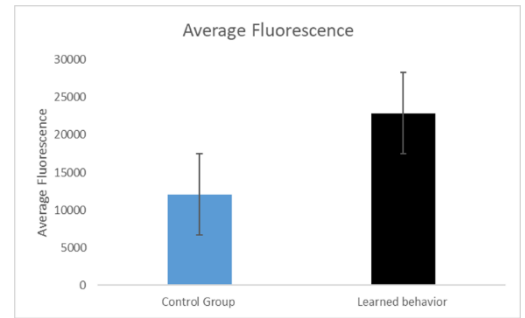
## Results:

**Graph 1: Pre and Post Training DA Signaling** In the learned behavior paradigm, analysis of the imaging data revealed there was no significant difference between the baseline and post training DA signaling. This graph depicts the mean fluorescence of selected Regions of Interest (ROIs) in a mouse (n=1) before and after training.



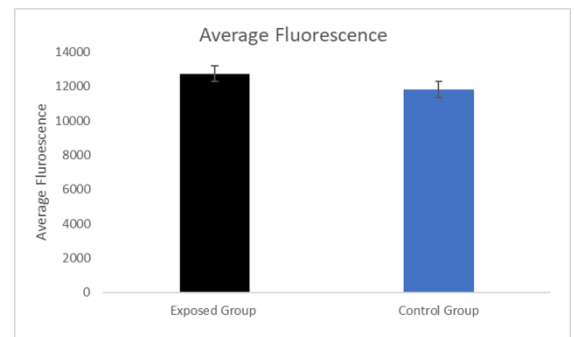
Graph 1: Pre and Post Training DA Signaling

**Graph 2: Average Fluorescence of Learned Behavior Paradigm** TH immunohistochemistry analysis revealed there was an increase in TH expression in the experimental group (n=64) when compared to the control group (n=42). A two-sample t test confirms that the difference is statistically significant ( $p = .007$ ).



Graph 2: Average Fluorescence of Learned Behavior Paradigm

**Graph 3: Average Fluorescence of Passive Exposure Paradigm** In the passive odor exposure paradigm, there was a small increase in TH expression in the exposed group (n=23) in response to the odor exposure when compared to the control group (n=12). A two-sample t test confirms the difference is statistically significant ( $p = .006$ ). In both the control slices and the experimental group, we observed heterogeneity in the TH labeling in different glomeruli in the olfactory bulbs.



Graph 3: Average Fluorescence of Passive Exposure Paradigm

**Discussion:** In our project, we found that the average fluorescence in the learned behavior paradigm was greater for the experimental group, indicating an increase in TH expression. Interestingly, there was no difference in the DA signaling from pre and post training imaging. Our results also suggest a small increase in TH expression in mice passively exposed to an odor.

A large increase in TH expression, as we had seen in learned behavior paradigm, is surprising, as it has not been reported before. A potential reason for not seeing an increase in DA signaling is the time frame of the experiment. Mice were trained for five days, which may not have been enough time for a significant change in DA signaling. For future experiments, extending the time between the baseline imaging and post training imaging may yield results that more accurately represent learned behavior DA signaling. In the passive exposure paradigm, reducing cage and bedding odor to prevent any effect on TH expression could allow a more precise detection of TH expression from environmental odors.

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