2017

The Effect of Temporary Anosmia on Olfactory Bulb Glomerular Neuron Activation

Kelsey E. Zuk

The Effect of Temporary Anosmia on Olfactory Bulb Glomerular Neuron Activation

Abstract
Neuroplasticity refers to changes in signaling and circuitry throughout the nervous system. The olfactory system is well known for its ability to induce neuroplasticity throughout the lifespan of the organism. Changes in olfactory sensory input are known to induce neuroplasticity. Neuron activation, due to olfactory sensory input, activates the immediate early gene c-fos in the olfactory bulb (OB). The presence of fos, the protein derived from c-fos gene expression, can be used to measure changes in OB circuit activity. In the present study, olfactory sensory input was disrupted in mice by intranasal irrigation with the detergent Triton X-100. This induces a temporary loss of the sense of smell, known as anosmia, and a decrease in OB dopamine (DA) concentration. We hypothesized that, due to a Joss of inhibitory DA, Triton X-100 treated mice would have an increase in fos immunopositive cells after odor exposure when compared to control (phosphate buffered saline treated) mice. To test for anosmia induction by Triton X-100, mice underwent a behavioral habituation/dishabituation task twenty-four or forty-eight hours after the final treatment. Both control mice and forty-eight hour recovered Triton X-100 mice displayed normal investigative behavior, indicating they were not anosmic. However, twenty-four hour recovered Triton X-100 mice displayed behavior indicative of anosmia. A separate group of mice were exposed to odor stimulation forty-eight hours after the final treatment, and the OBs were processed for immunohistochemical detection of fos protein. Tissue analysis showed no significant difference in fos immunopositive cell counts between treatment groups.

Degree Type
Open Access Senior Honors Thesis

Department
Biology

First Advisor
Thomas G. Mast

Second Advisor
Aaron Liepman

Keywords
Ofaction, Recoverable anosmia, Triton X-100, IEG, c-fos, Habituation/Dishabituation

Subject Categories
Biology

THE EFFECT OF TEMPORARY ANOSMIA ON OLFACTORY BULB GLOMERULAR NEURON ACTIVATION

By

Kelsey E. Zuk

A Senior Thesis Submitted to the

Eastern Michigan University

Honors College

in Partial Fulfillment of the Requirements for Graduation

with Honors in Biology

Approved at Ypsilanti, Michigan, on April 20, 2017

Table of Contents

Abstract

Neuroplasticity refers to changes in signaling and circuitry throughout the nervous system. The olfactory system is well known for its ability to induce neuroplasticity throughout the lifespan of the organism. Changes in olfactory sensory input are known to induce neuroplasticity. Neuron activation, due to olfactory sensory input, activates the immediate early gene c-fos in the olfactory bulb (OB). The presence of fos, the protein derived from c-fos gene expression, can be used to measure changes in OB circuit activity. In the present study, olfactory sensory input was disrupted in mice by intranasal irrigation with the detergent Triton X-100. This induces a temporary loss of the sense of smell, known as anosmia, and a decrease in OB dopamine (DA) concentration. We hypothesized that, due to a loss of inhibitory DA, Triton X-100 treated mice would have an increase in fos immunopositive cells after odor exposure when compared to control (phosphate buffered saline treated) mice. To test for anosmia induction by Triton X-100, mice underwent a behavioral habituation/dishabituation task twenty-four or forty-eight hours after the final treatment. Both control mice and forty-eight hour recovered Triton X-100 mice displayed normal investigative behavior, indicating they were not anosmic. However, twenty-four hour recovered Triton X-100 mice displayed behavior indicative of anosmia. A separate group of mice were exposed to odor stimulation forty-eight hours after the final treatment, and the OBs were processed for immunohistochemical detection of fos protein. Tissue analysis showed no significant difference in fos immunopositive cell counts between treatment groups.

Introduction

Background:

Neurodegenerative and neuronal deficit disorders, such as Parkinson's disease and stroke, directly affect over 50 million Americans each year (Brown et al., 2005). There are many medical treatments designed to prevent permanent damage from neuronal disorders. Often, these treatments involve medications that attain a homeostatic level of some biological chemical, such as neurotransmitters, hormones, or growth factors (Wieloch, 1985; Sakanaka et al., 1998). However, once these neuronal disorders have caused damage, there are very few treatments available to repair it. The ability to induce neuroplasticity could help to correct this issue.

Neuroplasticity refers to changes in neuronal pathways and synapses resulting from a variety of stimuli, including environmental, psychological, and physiological changes (Demarin and Morović, 2014). Examples of neuroplasticity include recovery from brain trauma, learning, memory formation, and the recovery or onset of behavioral disorders. Neuroplasticity can be further broken down to changes in the growth and differentiation of neurons, neuron excitability, and chemical signaling (Huttenlocher, 2009). Although these forms of neuroplasticity aid in the modification of neuronal circuitry, because they act in a synapse-specific manner, they often destabilize the overall activity in a circuit (Turrigiano et al., 1998). Another form of neuroplasticity, known as homeostatic plasticity, works to stabilize neuronal circuit activity.

Although neuronal systems require change, they also need stability. As implied, homeostatic plasticity works to maintain some level of homeostasis over a range of

cortical networks (Turrigiano, 2012). Homeostatic plasticity has two main mechanism groups: global mechanisms, which act on all synapses of a single neuron, and local mechanisms, which act on individual synapses of a single neuron (Turrigiano, 2008; Yu and Goda, 2009). These mechanisms often function through a diversity of within-neuron feedback loops (Turrigiano, 2012). That is, the neuron can detect changes in its own activity and respond accordingly to move firing rates closer to the homeostatic set-point. Because neuron activation causes changes in cellular calcium levels, calcium-dependent pathways are often involved in this type of feedback loop (Seeburg et al., 2008; Hu et al., 2010; Fu et al., 2011). The balance between destabilizing neuroplasticity and regulatory homeostatic plasticity is required for proper cortical adaptation (Turrigiano, 2012). By studying the natural mechanisms that induce plasticity in the brain, we may better understand the mechanisms that could be used to treat brain and spinal cord injuries and diseases.

Neuroplasticity and Olfaction:

The olfactory bulb (OB) and its surrounding neuronal network are known for their ability to regenerate neurons and create new neuronal connections (Mandairon and Linster, 2009; Huart et al., 2013). Unlike most neurons, olfactory sensory neurons (OSNs) can be destroyed and then regenerate through the proliferation and differentiation of basal stem cells (Graziadei and Monti-Graziadei, 1979; Calof and Chikaraishi, 1989; Schwob, 2002). This feature, along with the rapid annual turnover rate of about 50% of neurons throughout the rodent OB, makes this system an excellent model for studying neuroplasticity (Devor, 1975; Graziadei and Monti-Graziadei, 1979; Imayoshi et al., 2008).

Olfactory Network Overview:

Odor detection and discrimination begins in the olfactory epithelium, where odor molecules bind to receptors on the OSNs. These OSNs then relay odorant information to the OBs. The OBs are composed of five main layers (listed from superficial to deep): the glomerular layer, the external plexiform layer, the mitral/tufted cell layer, the internal plexiform layer, and the granular layer. OSNs synapse with mitral and tufted cells (MTCs) in the glomerular layer. These MTCs then synapse with a diversity of OB interneurons and send information to the olfactory cortex for higher processing (Huart et al., 2013). A simplified version of this pathway and the surrounding structures is shown in Figure 1.

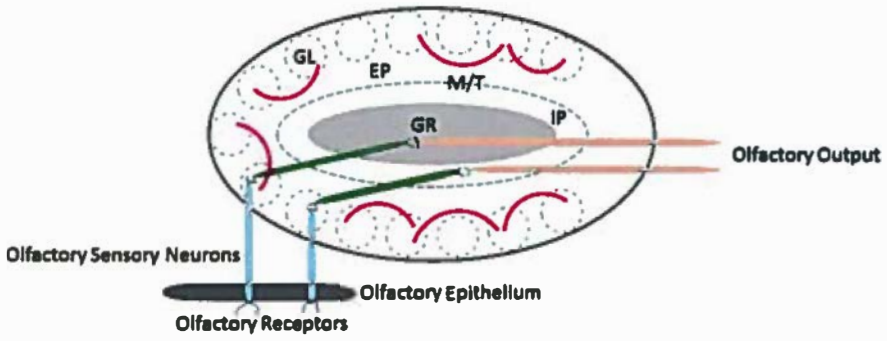

Figure 1. Simplified olfactory circuit, sagittal view. Glomerular layer (GL), external plexiform layer (EP), mitral/tufted cell layer (M/T), internal plexiform layer (IP), and granular layer (GR). Dashed circles represent glomerular organization. Light blue lines represent the olfactory sensory neurons innervating the OB at the glomeruli. Dark green lines represent the mitral/tufted cells. Red semi-circles represent glomerular interneurons. Orange lines represent the main olfactory output to higher processing areas.

Odor information results from the dynamic communication between OB cell types. In mice, each OSN has only one type of olfactory receptor (for review see Mombaerts, 2004). Multiple OSNs of the same receptor type project their axons into a

glomerulus and each glomerulus receives information only from OSNs of the same receptor type (Kay and Stopfer, 2006) (Fig. 2). These OSNs synapse with multiple MTCs in a glomerulus and their signals are regulated by many interneurons before projecting to the olfactory cortex (for review see Nagayama et al., 2014).

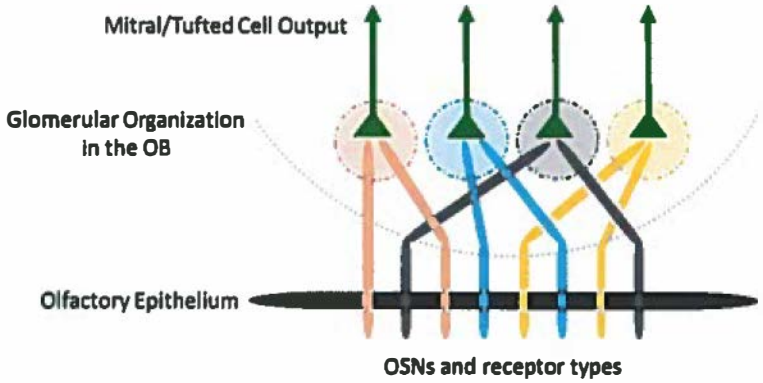

Figure 2. OSN receptor specific organization. Color coding refers to specific receptor types and the glomerulus in which that information is processed. Dotted semi-circle represents the OB. Each glomerulus processes information from only one receptor type, however there is more than one glomerulus dedicated to each receptor type.

Interneurons mainly function in an inhibitory manner. There are two broad types of interneurons found in the main OB: granule cells (GCs), which are found in the granular layer, and juxtaglomerular cells (JGCs), which are found in and around the glomerular layer (Nagayama et al., 2014). JGCs can be further broken down into short axon cells (SACs), external tufted cells (ETCs), and periglomerular cells (PGCs) (Nagayama et al., 2014). These interneurons employ a variety of neurotransmitters to aid in olfactory information processing, such as inhibitory γ-aminobutyric acid (GABA), inhibitory dopamine (DA), and excitatory glutamate (Glu) (Ennis et al., 2001; Heinbockel et al., 2007). MTCs are glutamatergic, while GCs are GABAergic, PGCs and SACs are dopaminergic/GABAergic, and ETCs are GABAergic/glutamatergic (Nagayama et al.,

2014). PGCs and ETCs most often project to a single glomerulus (Nagayama et al., 2014). There, PGCs function to inhibit the glomerulus, while ETCs function to excite the glomerulus (Nagayama et al., 2014). SACs often project to multiple glomeruli and promote inhibition across those glomeruli, also referred to as lateral inhibition (Nagayama et al., 2014). These SACs are thought to be the main homeostatic plasticity contributing cells (Turrigiano, 2012). Their ability to manipulate inhibitory DA levels across multiple glomeruli contributes to this hypothesis. In contrast, GCs project directly onto multiple MTCs in an inhibitory manner (Nagayama et al., 2014).

Each of these neuron types receives inputs from and sends outputs to multiple other neuron types. MTCs receive their main input from OSNs (Nagayama et al., 2014). Outside of the OB, they send outputs to the olfactory cortex (Nagayama et al., 2014). PGCs receive inputs from ETCs, other PGCs, SACs, MTCs and OSNs (Nagayama et al., 2014). They send their outputs to MTCs, other PGCs, and OSNs (Nagayama et al., 2014). SACs receive inputs from PGCs, other SACs, and ETCs that are found in the same glomerulus (Nagayama et al., 2014). They send their outputs to PGCs, other SACs, MTCs, and ETCs found in other glomeruli (Nagayama et al., 2014). ETCs receive inputs from OSNs and other ETCs (Nagayama et al., 2014). They send their outputs to PGCs, SACs, MTCs, and ETCs in the same glomerulus (Nagayama et al., 2014). GCs receive input from and send outputs to MTCs (Nagayama et al., 2014).

Measuring Olfactory Neuron Activation:

Odors induce a response in OB neurons, which activates immediate early genes (IEGs) and induces expression of their protein products (Bepari et al., 2012). IEG proteins regulate gene transcription—both increasing and decreasing gene expression

(Perez-Cadahia et al., 2011). The transcription regulation induced by IEGs plays a role in many cellular processes, including neuron growth and differentiation (Perez-Cadahia et al., 2011). Importantly, IEGs, including c-fos, may also regulate long-term changes in neuronal connections and synaptic function (Leslie and Nedivi, 2011). c-fos expression is upregulated rapidly after neuron activation in the OB. The presence of c-fos protein (fos) can be used as an indication of recent neuronal activity (Guthrie et al., 1993; Hoffman et al., 1993). Therefore, experimental treatments that alter OB function can be investigated by measuring and comparing fos immunopositive neurons. One such experimental treatment is sensory deprivation.

Inducing Anosmia:

Sensory deprivation, in this case, refers to a loss of sensory input through the OSNs into the OB. The loss of the sense of smell is referred to as anosmia. Since OB processing relies mostly on input from the environment, disrupting this input can have dramatic effects on the OB (Margolis et al., 1974). Anosmia can be induced using a diluted detergent (Triton X-100) solution (Baker et al., 1983). Inducing anosmia in this manner is cost effective and requires very little time, compared to potential surgical methods, such as naris occlusion. The detergent solution degrades the nasal epithelium, and the OSNs in the olfactory epithelium, for a period that is dependent on the treatment schedule and concentration (Cummings et al., 2000; Iqbal and Jacobs, 2010). During the period of sensory deprivation, the environment within the OB will change dramatically due to a lack of stimuli. Known changes in the OB resulting from anosmia include a decrease in tyrosine hydroxylase (TH) (Jin et al., 1996; Mast and Fadool, 2012), the rate-

limiting enzyme of DA synthesis (Molinoff and Axelrod, 1971; Daubner et al., 2011) leading to a decrease in inhibitory DA (Fig 3).

Case 1- Before anosmia

+TH → +DA → -Neuron activation

Case 2- After anosmia

-TH → -DA → +Neuron activation

Figure 3. Simplified overview of the activation pathway. Before inducing anosmia, tyrosine hydroxylase (TH) will be at basal levels, leading to a basal presence of dopamine (DA) and inhibition, which will dampen overall neuron activation. After recovery from anosmia, we hypothesize that TH levels will remain low enough to decrease the level of DA, therefore lowering inhibition and increasing neuron activation.

Since DA inhibits neuron activation in the OB (Hsia et al., 1999; Davila et al., 2003; Escanilla et al., 2009), this decrease in inhibitory DA should allow the OB to be more easily excited (Hsia et al., 1999) after epithelial recovery. Our lab has shown that we can study the changes within the OB as early as forty-eight hours after the last Triton X-100 application, as evidenced by behavioral testing. At that time, the olfactory epithelium has sufficiently recovered to allow stimuli to enter the OB via OSNs, enabling the mouse to smell, but there is not complete recovery of the OB. Because the OB circuits are not fully recovered, the animal is not expected to process odors in the same manner as a control animal. This is thought to be caused by the decrease in DA production from SACs, which are cells found in the glomerular layer that aid in glomerular communication. Loss of DA from SACs is caused by sensory deprivation (Jin et al., 1996). Since the presence of fos indicates recent neuronal activity, we hypothesize that we will be able to identify neuron activity changes in the OB caused by sensory deprivation, based on the location and amount of fos immunopositive cells present. The

goal of this study is to better understand the effect of sensory deprivation on OB glomerular neuron activation by measuring fos expression.

Materials and Methods

Subjects:

A total of 25 C57BL6 mice (Jackson Laboratories), 12 females and 13 males, were utilized in this experiment. Mice were housed in the Eastern Michigan University (EMU) vivarium on a reverse 12-hour light cycle in a temperature and humidity-controlled room. Housing consisted of polycarbonate cages with cob bedding and free access to food (Purina 5001) and tap water. All experiments were carried out under the approval of the EMU Institutional Animal Care and Use Committee (IACUC), protocol #2014-060.

Induction of Temporary Anosmia:

All reagents used were from Sigma-Aldrich or Fisher Scientific, unless otherwise specified. Intranasal irrigation was used to induce temporary anosmia. Triton X-100 (Electron Microscopy Sciences) in phosphate buffered saline (PBS) was diluted to a final concentration of 0.1% (v/v). PBS lacking Triton X-100 was used as a control. Unanesthetized mice were held in a supine position while 10 µl of 0.1% Triton X-100 or PBS was delivered to each nostril with a micropipette. A supine position was maintained for approximately one minute after treatment. Mice were then returned to home cages. Treatments took place on alternating days for five days (i.e. day one, day three, day five).

Habituation/Dishabituation Task:

Fifteen mice, seven females and eight males, were utilized to test anosmia. Twenty-four hours (i.e. day six) after PBS (n=5) or Triton X-100 (n=6) treatment and forty-eight hours (i.e. day 7) after Triton X-100 (n=4) treatment, mice underwent a behavioral habituation/dishabituation task. This task measures odor investigation time, allowing the researcher to determine whether the animal is anosmic. Polycarbonate cages with cob bedding were utilized as odor test chambers. Mice were presented with a cotton swab scented with 10µl of mineral oil for four consecutive trials. In the final test trial, a cotton swab scented with 10µl of acetophenone (99% purity) diluted 1:1,000 (v/v) in mineral oil was presented. Each odor was presented for one minute with an intertrial interval of three minutes. Odor investigation time was identified by rearing near the cotton swab, within approximately one square inch, and recorded. Investigation times were normalized, per animal, to the initial mineral odor investigation time. The average, normalized investigation time per treatment group is reported.

Odor Exposure to Induce c-fos Expression:

Ten mice, five females and five males, were utilized to test c-fos expression. Forty-eight hours (i.e. day seven) after the final treatment of either PBS or Triton X-100, mice were exposed to acetophenone (99% purity) diluted 1:1,000 (v/v) in mineral oil. Acetophenone dilutions were prepared daily. Odor exposure was conducted using an automated liquid-dilution olfactometer (Vulintus, Dallas TX), a housing chamber that allows for the control of odorant exposure through a pneumatic system. The olfactometer airflow had an output of 400 mL/min. Mice were first given 10 minutes to habituate to the olfactometer environment, then were exposed to acetophenone for a total of five odor

trials, lasting two minutes each. The intertrial interval lasted three minutes. The total run-time for the program, including the habituation period, was thirty-two minutes.

Tissue Preparation:

Approximately fifty minutes after the start of odor exposure, mice were euthanized by an overdose with urethane (3,000 mg/kg) and perfused using paraformaldehyde (PFA) (Acros Organics) dissolved in 5mM ethylenediaminetetra-aceticacid (EDTA) in PBS (4% PFA/PBS-EDTA). OBs were dissected and post fixed in 4% PFA/PBS-EDTA for 24 hours. OBs were then cryoprotected by incubation in a 30% sucrose/PBS solution at 4°C for 48 hours. OBs were vacuum-embedded in optimal cutting temperature compound (Sakura Finetek) before sectioning. Sixteen micrometer coronal sections were cut on an IEC minotome (International Equipment Company) cryostat, placed onto gelatin-coated slides (Fisher Scientific), and stored at -20°C for immunohistochemistry.

Immunohistochemistry:

All procedures were conducted at room temperature. Frozen sections were air-dried for 45 minutes and rehydrated with PBS. A 2.5% normal donkey serum (NDS) in 0.3% Triton X-100 blocking solution was applied for 90 minutes. Anti-cfos from Santa Cruz Biochem (#SC-52) was diluted 1:1,000 in blocking solution and applied overnight. Biotinylated donkey anti-rabbit secondary antibody from Jackson Immunoresearch (#711-055-152) was diluted 1:200 in 0.3% Triton X-100 and applied for 90 minutes. An avidin-biotin complex (ABC) kit (Vector Laboratories) was used in combination with the Enzmet HRP detection kit (Nanoprobes) to visually label the fos immunopositve cells by

depositing silver chloride ions. Coverslips were mounted using Vectamount (Vector Laboratories). A no primary antibody treatment was used as a negative control. Brightfield micrographs were taken with a Nikon Eclipse E400 microscope equipped with a Nikon Sight camera. Digital images were captured with NIS Elements software and stored on a personal computer.

Data Analysis:

Images of the dorsal, ventral, lateral, and medial surfaces were taken with a 10x objective at a resolution of 2560x1920. Dorsal and ventral images were used for analysis. ImageJ was used to process the images for maximum clarity. Immunopositive cells were manually counted using the "Cell Counter" plugin on ImageJ. The Analyze>Measure function was also used to find the total glomerular area being analyzed. Immunopositive cell count was standardized per unit area. Outliers were removed from treatment groups, following a standard statistical outlier analysis, leaving the groups with four animals each. A Student's T-Test was used to determine significant differences between treatment groups.

Results

Habituation/Dishabituation Task:

Mice were treated with either PBS or Triton X-100 on alternating days for five days to induce anosmia. After twenty-four (D6) or forty-eight (D7) hours of recovery, mice completed a behavioral habituation/dishabituation task where they were exposed to mineral oil (MO) for four trials and acetophenone (ACET) in a fifth trial. This task verified the effectiveness of our Triton X-100 treatments by analyzing the animals'

ability to smell. The behavioral data show that PBS and Triton X-100 D7 mice were not anosmic, as evidenced by their investigative behavior during the ACET trial, and that Triton X-100 D6 mice were anosmic, as evidenced by their lack of investigative behavior during the ACET trial.

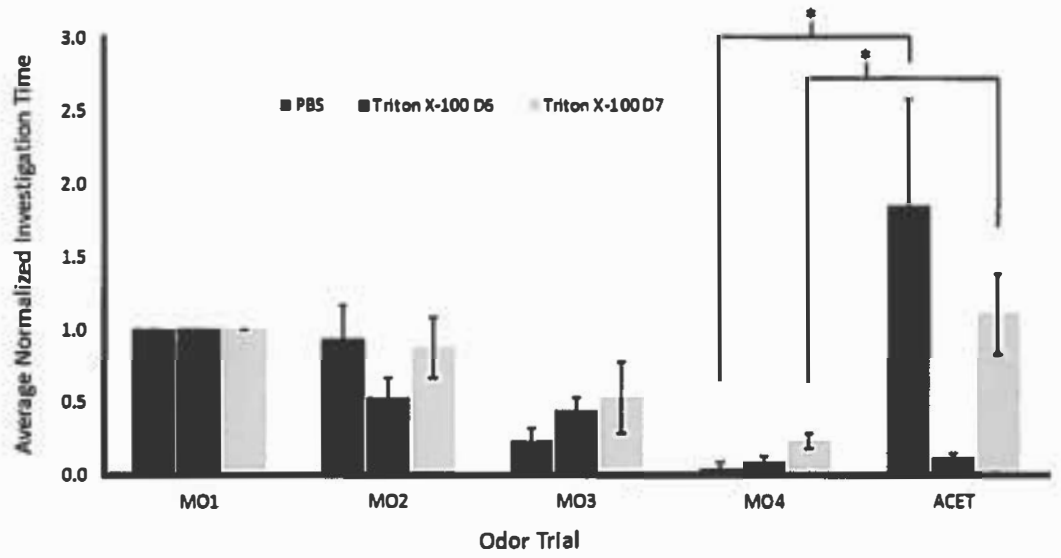

Figure 4. Habituation/dishabituation behavior. PBS and Triton X-100 D6 mice underwent behavioral testing 24 hours after the last treatment. Triton X-100 D7 mice underwent behavioral testing 48 hours after the last treatment. Four mineral oil (MO) presentations preceded the final 1:1,000 acetophenone (ACET) presentation. Dishabituation was measured by a significant ($p<0.05$) increase in investigative behavior between MO4 and ACET exposure. No significant difference ($p=0.18$, $n=6$) was found for the Triton X-100 D6 mice, indicating anosmia. A significant difference was found for both PBS ($p=0.038$, $n=5$) and Triton X-100 D7 ($p=0.033$, $n=4$) mice, indicating they were not anosmic.

c-fos Expression:

Once we determined that our treatments induced anosmia as expected, we began looking at changes in neuron activation after recovery from anosmia. Mice were treated with PBS or Triton X-100 on alternating days for five days to induce anosmia. After forty-eight hours of recovery, mice were exposed to acetophenone to induce c-fos

expression, which leads to production of the fos protein. Fos labeling could then be used as an indication of neuron activation throughout the OB. We were specifically interested in changes in the glomerular layer of the OB because OSN signal is first processed in this region. Staining was consistent among all tissue samples, providing a robust labeling of fos immunopositive cells in both the granular and glomerular regions, as seen in Figure 5 (A, B, D, E).

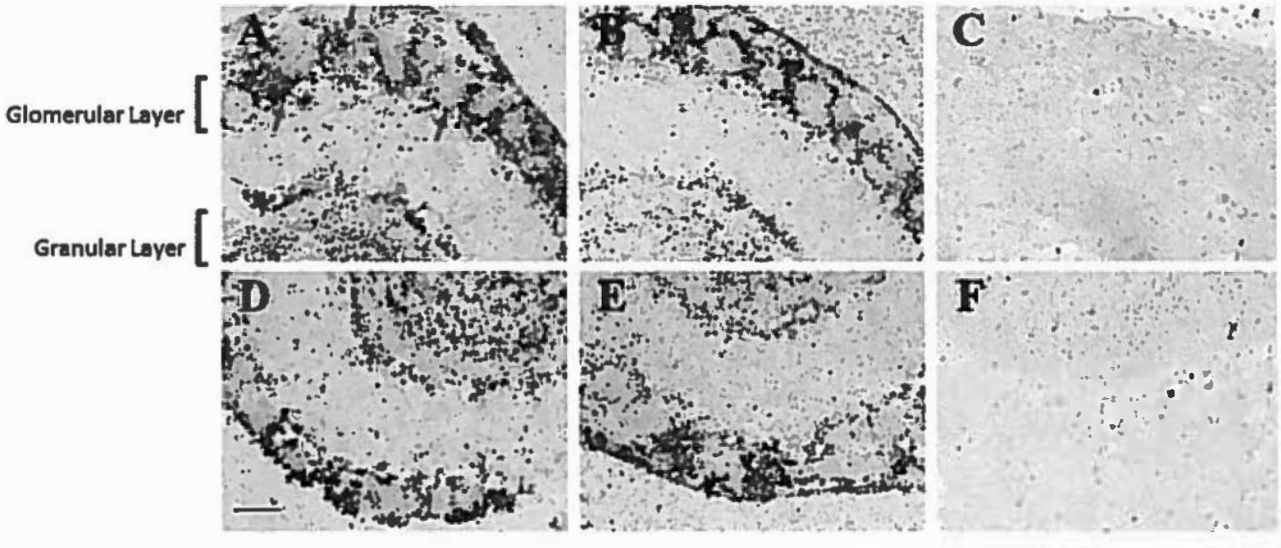

Figure 5. Comparison of dorsal and ventral regions for both PBS and Triton X-100 treated mice. Arrows indicate fos immunopositive cells. (A) Dorsal region of a PBS treated female. (B) Dorsal region of a Triton X-100 treated female. (C) Dorsal region of a PBS treated female, no primary negative control. (D) Ventral region of a PBS treated female. (E) Ventral region of a Triton X-100 treated female. (F) Ventral region of a PBS treated female, no primary negative control. Scale bar represents 100μm.

Although it was hypothesized that Triton X-100 treated mice would have an increase in fos immunopositive cells, statistical analysis showed no significant difference between Triton X-100 treated mice and PBS treated mice (Figure 6, $p>0.05$, $n=4$ per treatment group). Based on these data, there was no difference in overall OB neuron activation after a five-day temporary anosmia induced by Triton X-100, compared to a PBS control treatment. This suggests that Triton X-100 did not alter odor processing.

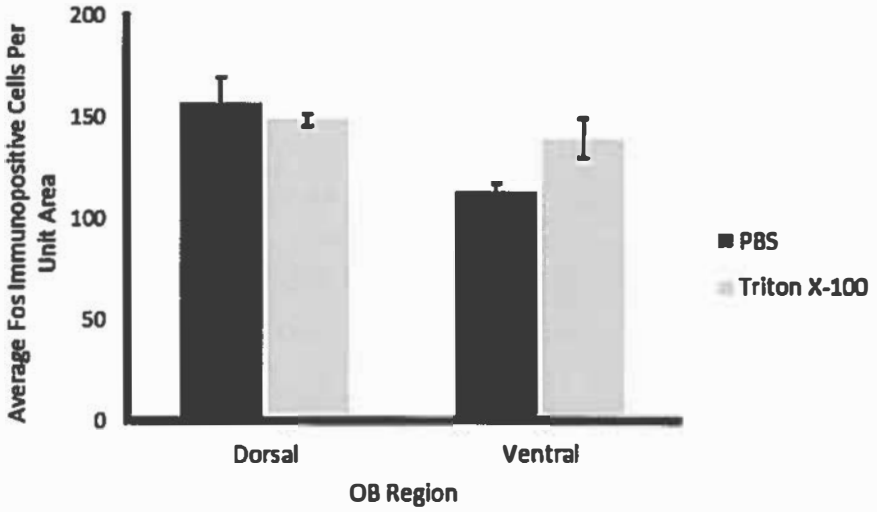

Figure 6. Average fos immunopositive cells per unit area, per region and treatment. No significant difference was found between treatment groups or regions ($p>0.05$, $n=4$ per treatment group after removing outliers).

Discussion

In this study, we aimed to identify changes in OB odor processing after a temporary disruption and recovery of olfactory inputs. The olfactory system has often been used as a model to study neuroplasticity (Devor, 1975; Schwob, 2002; Mandairon and Linster, 2009; Huart et al., 2013; Demarin and Morović, 2014). Many have studied how the OBs adapt after a permanent blocking of olfactory sensory input or damage (Alberts and Galef Jr., 1971; Edwards et al., 1972; Brunjes, 1994) and, thus, did not look at the recovery mechanisms employed by the OBs. By identifying what OB changes occur during recoverable treatments, we can better understand how the CNS copes with abrupt changes in signaling.

We know that DA acts as an inhibitory neurotransmitter in the glomerular layer of the OB (Hsia et al., 1999). We also know that sensory deprivation causes a decrease in both TH and DA in the OB (Molinoff and Axelrod, 1971; Jin et al., 1996). The source of

this DA is the SAC, an interneuron of the glomerular layer (Burton et al., 2016). It is hypothesized that these SACs adjust their DA release depending on OSN signaling; too much signaling will increase DA, while too little signaling will decrease DA. This change in inhibitory DA release is a key mechanism in homeostatic plasticity (Nelson and Turrigiano, 2008). We originally hypothesized that overall neuron activation in the main OB glomerular layer would be increased after a temporary disruption in signaling, suggestive of odor processing changes, due to the lower levels of DA. Lower levels of DA would lead to more sensitive neurons, which would be highly active when exposed to acetophenone. However, based on the results of this study, we now hypothesize that the lack of difference in fos expression is the result of homeostatic plasticity in action.

Homeostatic plasticity maintains the firing rate of neurons within a certain range; too little or too much activation can lead to neuron cell death (Turrigiano, 2012). In this study, we found that after a five-day recoverable anosmia, there was no significant difference in neuron activation between treatment groups. Although these data contradict the original hypothesis, they support the overall idea of homeostatic plasticity. By inducing sensory deprivation, we greatly reduce the signals coming into the OBs through the OSNs. In reaction to this, SACs reduce the amount of DA released, decreasing the level of inhibition within the OBs (Burton et al., 2016). After forty-eight hours, OSNs have mostly recovered and allow sensory input into the OBs. Even though the olfactory environment is still disrupted at that time, we found that the overall activation remains the same, possibly signifying homeostatic plasticity processes.

There are other possibilities that could explain our observations, as well. Relative to other studies that have utilized intranasal irrigation with Triton X-100, the total amount

delivered and the concentration of Triton X-100 utilized in this study was lower. Commonly, 100 μl of 0.7% Triton X-100 is used, leading to much longer recovery times of a week or more (Kream and Margolis, 1984; Cummings et al., 2000; Iqbal and Jacobs, 2010). It is possible that to disrupt the overall activation of olfactory neurons, a higher concentration of Triton X-100 must be used. This may explain the inconsistency between the results of this study and our original hypothesis. Because the concentration of Triton X-100 used in this study was lower and the recovery time was shorter, the OB may be able to compensate for the loss of signal more efficiently than previously expected. A future study could include the same treatment schedule and volume, but utilize a higher concentration of Triton X-100, which is expected to further disrupt the chemical environment of the OBs.

It is also possible that the changes in the OB environment may not affect the overall number of activated neurons. For example, neurons that were previously inactive may become more excitable, while those that were active may lose their excitability. Evidence from re-opened naris occluded mice has shown that MTC spontaneous activity, as well as the number of odor responsive cells, dramatically changes after naris re-opening (Guthrie, Wilson, and Leon, 1990). To test for these types of changes, electrophysiology studies can be conducted. Using electrophysiology, we can study the differences in activation of single neurons or groups of neurons in real time.

Our study of fos expression also utilized a small sample size of only ten mice. Recently in the neuroscience field, there has been a call for changes in data sampling and reporting to increase repeatability and reliability (Button et al., 2013). It is possible that a larger sample size is required for more reliable statistical data.

The present study aimed to determine whether there were neuron activation changes that reflected changes in odor processing after a temporary, recoverable anosmia. According to our results, an alternating five-day treatment with 0.1% Triton X-100 in PBS is sufficient to induce anosmia, which is recoverable after forty-eight hours. Although this type of anosmia is known to induce changes in the olfactory environment, we saw no significant difference in fos immunopositive cell counts, and therefore neuron activation, between anosmia-recovered mice and control mice. Because there was no difference in neuron activation, this suggests similar odor processing in Triton X-100 and PBS treated mice. Increasing sample size and implementing electrophysiology would help us ensure that our data is representative. Thus far, our data may suggest that the OB environment is more stable than originally hypothesized.

Literature Cited

Alberts, J.R., & Galef Jr., B.G. 1971. Acute anosmia in the rat: A behavioral test of a peripherally-induced olfactory deficit. *Physiology & Behavior.* 6:619–621.

Baker, H., Kawano, T., Margolis, F.L., & Joh, T.H. 1983. Transneuronal regulation of tyrosine hydroxylase expression in olfactory bulb of mouse and rat. *The Journal of Neuroscience.* 3(1), 69-78.

Bepari, A.K., Watanabe, K., Yamaguchi, M., Tamamaki, N., & Takebayashi, H. 2012. Visualization of Odor-induced Neuronal Activity by Immediate Early Gene Expression. *BMC Neuroscience.* 13:140-56.

Brown, R.C., Lockwood, A.H., & Sonawane, B.R. 2005. Neurodegenerative Diseases: An Overview of Environmental Risk Factors. *Environmental Health Perspectives.* 113(9), 1250–1256.

Brunjes, P.C. 1994. Unilateral naris closure and olfactory system development. *Brain Research Reviews.* 19:146–160.

Burton, S.D., LaRocca, G., Liu, A., Cheetham, C.E.J., & Urban, N.N. 2016. Olfactory bulb deep short-axon cells mediate widespread inhibition of tufted cell apical dendrites. *J. Neurosci.* 2880–16.

Button, K.S., Ioannidis, J.P.A., Mokrysz, C., Nosek, B.A., Flint, J., Robinson, E.S.J., & Munafò, M.R. (2013). Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci 14,* 365–376.

Calof A.L., & Chikaraishi, D.M. 1989. Analysis of neurogenesis in a mammalian neuroepithelium: proliferation and differentiation of an olfactory neuron precursor in vitro. *Neuron.* 3: 115-27.

Cummings, D.M., Emge, D.K., Small, S.L., & Margolis, F.L. 2000. Pattern of olfactory bulb innervation returns after recovery from reversible peripheral deafferentation. *Journal of Comparative Neurology.* 421(3), 362-373.

Daubner, S.C., Le, T., & Wang, S. 2011. Tyrosine hydroxylase and regulation of dopamine synthesis. *Archives of Biochem. And Biophysics.* 508:1-12.

Davila, N.G., Blakemore, L.J., & Trombley, P.Q. 2003. Dopamine modulates synaptic transmission between rat olfactory bulb neurons in culture. *Journal of Neurophyiol.* 90(1):395-404.

Demarin, V., & Morović, S. 2014. Neuroplasticity. *Periodicum biologorum.* 116.2: 209-211.

Devor, M. 1975. Neuroplasticity in the sparing or deterioration of function after early olfactory tract lesions. *Science.* 190:998–1000.

Edwards, D.A., Thompson, M.L., & Burge, K.G. 1972. Olfactory bulb removal vs peripherally induced anosmia: Differential effects on the aggressive behavior of male mice. *Behavioral Biology.* 7:823–828.

Ennis, M., Zhou, F.M., Ciombor, K.J., Aroniadou-Anderjaska, V., Hayar, A., Borrelli, E.,et al. 2001. Dopamine D2 receptor-mediated presynaptic inhibition of olfactory nerve terminals. *J. Neurophysiol.* 86, 2986–2997.

Escanilla, O., Yuhas, C., Marzan, D., & Linster, C. 2009. Dopaminergic Moduclation of Olfactory Bulb Processing Affects Odor Discrimination Learning in Rats. *Behavioral Neuroscience.* 1234: 828-33.

Fu, A.K., Hung, K.W., Fu, W.Y., Shen, C., Chen, Y., Xia, J., Lai, K.O., & Ip, N.Y. 2011. APC(Cdh1) mediates EphA4-dependent downregulation of AMPA receptors in homeostatic plasticity. *Nat Neurosci* 14: 181 – 189.

Graziadei, P.P.C., & Monti-Graziadei, G.A. 1979. Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons. *J. Neurocytology.* 8: 1-18.

Guthrie, K.M., Anderson, A.J., Leon, M., & Gall, C. 1993. Odor-induced Increases in C-fos MRNA Expression Reveal an Anatomical "unit" for Odor Processing in Olfactory Bulb. *Proceedings of the Nat. Acad. of Science of the USA.* 80: 3329-333.

Guthrie, K.M., Wilson, D.A., & Leon, M. 1990. Early unilateral deprivation modifies olfactory bulb function. *J. Neurosci. 10*, 3402–3412.

Heinbockel, T., Hamilton, K.A., and Ennis, M. 2007. Group I metabotropic glutamate receptors are differentially expressed by two populations of olfactory bulb granule cells. *J. Neurophysiol.* 97, 3136–3141.

Hoffman, G.E., Smith, M.S., & Verbalis, J.G. 1993. C-Fos and Related Immediate Early Gene Products as Markers of Activity in Neuroendocrine Systems. *Frontiers in Neuroendocrinology.* 14:173-213.

Hsia, A.Y., Vincent, J.D., & Lledo, P.M. 1999. Dopamine depresses synaptic inputs into the olfactory bulb. *Journal of neurophysiology.y* 82.2: 1082-1085.

Hu, J.H., Park, J.M., Park, S., Xiao, B., Dehoff, M.H., Kim, S., Hayashi, T., Schwarz, M.K., Huganir, R.L., & Seeburg, P.H. 2010. Homeostatic scaling requires group I mGluR activation mediated by Homer1a. *Neuron.* 68: 1128 – 1142.

Huart, C., Rombaux, P., & Hummel, T. 2013. Plasticity of the Human Olfactory System: The Olfactory Bulb. *Molecules.* 18: 11586-1600.

Huttenlocher, P.R. 2009. Neural plasticity: the effects of environment on development of the cerebral cortex. *Harvard University Press.* Print.

Imayoshi, I., Sakamoto, M., Ohtsuka, T., Takao, K., Miyakawa, T., Yamaguchi, M., Mori, K., Ikeda, T., Itohara, S., & Kageyama, R. 2008. Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci.* 11:1153–1161.

Iqbal, T., & Jacobs, C.B. 2010. "Rapid degeneration and regeneration of the zebrafish olfactory epithelium after triton X-100 application." *Chemical senses.* 35.5: 351-361.

Jin, B.K., Franzen, L., & Baker, H. 1996. Regulation of c-Fos mRNA and fos protein expression in olfactory bulbs from unilaterally odor-deprived adult mice. *International journal of developmental neuroscience.* 14(7), 971-982.

Kay, L.M., & Stopfer, M. 2006. Information processing in the olfactory systems of insects and vertebrates. *Seminars in Cell & Developmental Biology.* 17, 433–442.

Kream, R.M., & Margolis, F.L. 1984. Olfactory marker protein: turnover and transport in normal and regenerating neurons. *J. Neurosci.* 4:868–879.

Leslie, J.H., & Nedivi, E. 2011. Activity-regulated genes as mediators of neural circuit plasticity. *Progess in Neurobiology.* 94:223-237.

Mandairon, N., & Linster, C. 2009. Odor Perception and Olfactory Bulb Plasticity in Adult Mammals. *Journal of Neurophysiology.* 101:2204-209.

Margolis, F.L., Roberts, N., Ferriero, D., & Feldman, J. 1974. Denervation in the primary olfactory pathway of mice: Biochemical and morphological effects. *Brain Research* 81, 469–483.

Mast, T.G., & Fadool, D.A. 2012. Mature and Precursor Brain-Derived Neurotrophic Factor Have Individual Roles in the Mouse Olfactory Bulb. *PLoS ONE.* 7: e31978.

Molinoff, P.B., & Axelrod, J. 1971. Biochemistry of catecholamines. *Annual review of biochemistry.* 40: 465-500.

Mombaerts, P. 2004. Odorant receptor gene choice in olfactory sensory neurons: the one receptor–one neuron hypothesis revisited. *Current Opinion in Neurobiology* 14, 31–36.

Nagayama, S., Homma, R., & Imamura, F. 2014. Neuronal organization of olfactory bulb circuits. *Front. Neural Circuits* 8.

Nelson S.B., Turrigiano G.G. 2008. Strength through diversity. *Neuron.* 60: 477 – 482.

Perez-Cadahia, B., Drobic, B., & Davie, J.R. 2011. Activation and Function of Immediate-early Genes in the Nervous System. *Biochem. Cell Biol.* 89: 61-73.

Sakanaka, M., Wen, T.C., Matsuda, S., Morishita, E., Nagao, M., & Sasaki, R. 1998. In vivo evidence that erythropoietin protects neurons from ischemic damage. *Proc. Natl. Acad. Sci.* 95:4635-4640.

Schwob, JE. 2002. Neural regeneration and the peripheral olfactory system. *Anat Rec.* 269:33–49.

Seeburg, D.P., Feliu-Mojer, M., Gaiottino, J., Pak, D.T., & Sheng, M. 2008. Critical role of CDK5 and Polo-like kinase 2 in homeostatic synaptic plasticity during elevated activity. *Neuron.* 58: 571 – 583.

Turrigiano, G.G. 2012. Homeostatic Synaptic Plasticity: Local and Global Mechanisms for Stabilizing Neuronal Function. *Cold Spring Harb Perspect Biol. 4*-a005736.

Turrigiano, G.G., Leslie, K.R., Desai, N.S., Rutherford, L.C., & Nelson, S.B. 1998. Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature. 391,* 892–896.

Turrigiano, GG. 2008. The self-tuning neuron: Synaptic scaling of excitatory synapses. *Cell.* 135:422– 435.

Wieloch, T. 1985. Hypoglycemia-induced neuronal damage prevented by an N-methyl-D-aspartate antagonist. *Science.* 230:681–684.

Yu, L.M., & Goda, Y. 2009. Dendritic signaling and homeostatic adaptation. *Curr Opin Neurobiol.* 19: 327 – 335.