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PLASTICITY OF GLOMERULI AND OLFACTORY-MEDIATED BEHAVIOR IN ZEBRAFISH FOLLOWING DETERGENT LESIONING OF THE OLFACTORY EPITHELIUM

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Abstract—The zebrafish olfactory system is a valuable model for examining neural regeneration after damage due to the remarkable plasticity of this sensory system and of fish species. We applied detergent to the olfactory organ and examined the effects on both morphology and function of the olfactory system in adult zebrafish. Olfactory organs were treated once with Triton X-100 unilaterally to study glomerular innervation patterns or bilaterally to study odor detection. Fish were allowed to recover for 4–10 days and were compared to untreated control fish. Axonal projections were analyzed using whole mount immunocytochemistry with anti-keyhole limpet hemocyanin, a marker of olfactory axons in teleosts. Chemical lesioning of the olfactory organ with a single dose of Triton X-100 had profound effects on glomerular distribution in the olfactory bulb at 4 days after treatment, with the most significant effects in the medial region of the bulb. Glomeruli had returned by 7 days post-treatment. Analysis of the ability of the fish to detect cocktails of amino acids or bile salts consisted of counting the number of turns the fish made before and after odorant delivery. Control fish turned more after exposure to both odorants. Fish tested 4 and 7 days after chemical lesioning made more turns in response to amino acids but did not respond to bile salts. At 10 days post-lesion, these fish had regained the ability to detect bile salts. Thus, the changes seen in bulbar innervation patterns correlated to odorant-mediated behavior. We show that the adult zebrafish brain has the capacity to recover rapidly from detergent damage of the olfactory epithelium, with both glomerular distribution and odorant-mediated behavior returning in 10 days.

Key words: olfactory sensory neuron, chemical lesion, Triton X-100, anti-keyhole limpet hemocyanin, teleost, plasticity.

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Abbreviations: KLH, keyhole limpet hemocyanin; OSN, olfactory sensory neuron; PBS, phosphate-buffered saline; PBS-T, phosphate-buffered saline blocking solution; ZBC, Zebrafish Behavior Catalog.

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INTRODUCTION

The olfactory system is a useful model for studies on neuroplasticity because of its ability to recover from lesion, in part due to the inherent neuronal turnover seen in the olfactory organ. Various methods of chemical lesioning have been used to examine the mechanisms by which the olfactory system responds to damage. Exposure of the olfactory epithelium to a variety of chemicals can eliminate the sensory input to the olfactory bulb by destroying the olfactory sensory neurons (OSNs). The olfactory epithelium can replenish itself, reinnervate the olfactory bulb, and restore function (Schwob et al., 1995, 1999; Herzog and Otto, 1999; Paskin and Byrd-Jacobs, 2012). While a number of toxic chemicals have been used, Triton X-100 application is a common technique in studies examining the degeneration and regeneration of the olfactory system. Application of the detergent to the nasal cavity destroys OSNs, which temporarily reduces afferent input to the olfactory bulb (Nadi et al., 1981; Baker et al., 1983; Cummings et al., 2000).

A number of studies have examined the effects of chemicals on the fish olfactory system, due to concerns about pollution and toxins in the aquatic environment (Tierney et al., 2010). Application of Triton X-100 to the olfactory organ of catfish damages the olfactory epithelium to various extents, depending on the concentration (Cancalon, 1982, 1983). Low doses of the detergent affect only the superficial portions of the cells of the olfactory epithelium, while high doses destroy both sensory and non-sensory regions of the olfactory organ. In zebrafish, intranasal infusion of Triton X-100 causes immediate disruption of the olfactory epithelium (Iqbal and Byrd-Jacobs, 2010). One day post-lesion the olfactory epithelium is significantly thinner and has an apparent loss of most OSNs. The thickness of the epithelium progresses with return of epithelial depth and density of OSNs by 5 days post-lesion, and rosette morphology returns to near control levels within 7 days. This time course is more rapid than in mammals (Verhaagen et al., 1990; Cummings et al., 2000) and larger fish (Cancalon, 1983). Chronic treatment with Triton X-100 severely disrupts rosette morphology and removes most of the OSNs, although some subsets of OSNs appear more affected than others (Paskin et al., 2011; Paskin and Byrd-Jacobs, 2012).

Zebrafish possess three physiologically distinct OSNs, which are dispersed throughout the olfactory

epithelium (Hansen and Zieske, 1998). In general, ciliated OSNs detect bile salts and pheromones (Koide et al., 2009), microvillous OSNs detect amino acids and nucleotides (Lipschitz and Michel, 2002), and crypt OSNs appear to detect pheromones, although these cells are much less understood (Germana et al., 2004; Hamdani et al., 2008). Interestingly, chronic Triton X-100 exposure appears to affect ciliated OSNs primarily, while some microvillous and crypt neurons survive the treatment (Paskin et al., 2011; Paskin and Byrd-Jacobs, 2012).

The axons of the OSNs project to the olfactory bulb in the brain, where they relay sensory information to projection neurons and interneurons in discrete glomeruli. Adult zebrafish have approximately 140 glomeruli per olfactory bulb, a subset of which are highly stereotyped and distinguishable (Baier and Korsching, 1994; Braubach et al., 2012). Glomeruli in the olfactory bulb contain the axonal projections of a single OSN subtype and group in functional zones (Li et al., 2005; Sato et al., 2007; Yaksi et al., 2007). Ciliated OSNs project to the dorsal and medial regions of the olfactory bulb, microvillous OSNs project to the lateral and ventro-lateral regions of the bulb (Friedrich and Korsching, 1997; Sato et al., 2005), and crypt neurons project to a single glomerulus in the dorsomedial group (Ahuja et al., 2013). Consequently, the medial bulb regions process social and reproductive odors (Li et al., 2005; Yaksi et al., 2007) whereas the lateral region of the olfactory bulb tend to process feeding behavior (Li et al., 2005; Yaksi et al., 2007; Koide et al., 2009). Thus, the three zebrafish OSN subtypes are distinct in anatomy, physiology, and behavior.

The olfactory bulb is also affected by detergent application to the peripheral olfactory organ, since its afferent input is reduced. Following chronic application of Triton X-100 over 3 weeks, there is a reduction in olfactory bulb volume (Paskin et al., 2011). The glomeruli in the olfactory bulb that receive innervation from ciliated OSNs are lost, while those containing the axons of the other OSN subtypes show less damage (Paskin and Byrd-Jacobs, 2012). These fish lose the ability to detect bile salts but retain the ability to perceive amino acids. It is unclear if these results are due to accumulated damage from repeated exposure to the detergent.

In the current study, we examined whether a single intranasal infusion with Triton X-100 would yield results similar to the chronic exposure to the detergent. We hypothesized that the axonal projections of ciliated OSNs, and the glomeruli they innervate, would be most affected by detergent exposure, with degradation and recovery within a week. We also wanted to see if regeneration of the OSNs would result in regeneration of glomeruli in the same place and with the same morphology. From this, we also hypothesized that if chemical lesioning causes glomerular disruption, olfactory-mediated behavior would also be affected, with responses to odors mediated by ciliated OSNs most affected. Our work provides a model for rapid degeneration and regeneration of olfactory innervation patterns and of odorant-mediated behavior in a vertebrate.

EXPERIMENTAL PROCEDURES

Adult zebrafish of both sexes were generously donated by R. Warga and D. Kane. Fish were maintained in 10–15 gallon aquaria and fed flake food twice daily. All experimental procedures were approved by the WMU Institutional Animal Care and Use Committee.

Chemical lesioning of the olfactory epithelium

Adult zebrafish were anesthetized in tricaine (0.03% MS222, Sigma, St. Louis, MO, USA), a barrier of petroleum jelly was placed between the two nasal cavities to prevent crossover of the chemical to the contralateral organ, and the olfactory organ was exposed to a solution of 0.7% Triton X-100 and 0.005% methylene blue in phosphate-buffered saline (PBS) for 2 min. For glomerular analysis, the detergent solution was applied to the right nasal cavity, while the left nasal cavity was not treated to serve as an internal control. For the behavior study, both nasal cavities were exposed to the detergent solution. Treated fish were allowed to survive for either 4, 7, or 10 days. Untreated, control fish were also processed. Fish were euthanized with an overdose of tricaine, perfused transcardially with PBS, and incubated in 2% paraformaldehyde for 24 h at 4 °C.

Whole mount immunocytochemistry

Dissected brains were labeled with an antibody to keyhole limpet hemocyanin (KLH) to identify the projections of OSN axons in the olfactory bulb, using a method similar to Braubach et al. (2012). Briefly, whole brains were washed in PBS, put in a PBS blocking solution (PBS-T; 0.25% Triton X-100, 2% dimethyl sulfoxide, 1% bovine serum albumin, and 1% goat serum in PBS), and incubated in anti-KLH (Sigma; 1:200 in PBS-T) for 14 days at 4 °C. Brains were then washed in PBS-T, soaked in Alexa Flour 594-conjugated anti-rabbit secondary antibody (Invitrogen, Carlsbad, CA, USA; 1:200 in PBS-T) for 3–14 days, and rinsed thoroughly in PBS before being stored in a 3:1 glycerol–Tris solution containing 2% propyl gallate until viewing.

Glomerular analysis

Antibody-labeled brains were mounted in 1.5% agar between two coverslips and imaged using a Zeiss LSM 510 laser scanning confocal microscope. Z-stacks of 50–150 μm were collected from dorsal and ventral views and examined as single slices or as image projections. The olfactory axon component of glomeruli was identified from previously established anatomical and spatial glomerular maps in zebrafish (Baier and Korsching, 1994; Braubach et al., 2012). All glomeruli were individually examined in multiple views and at multiple magnifications from control (n = 3), 4 day post-treatment (n = 6), and 7 day post-treatment (n = 6) fish. Data analysis entailed rating glomeruli based on presence and completeness. The glomerular nomenclature of Braubach et al. (2012) was used.