Behavioural Brain Research 235 (2012) 293–301

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Reversible deafferentation of the adult zebrafish olfactory bulb affects glomerular distribution and olfactory-mediated behavior

Taylor R. Paskin, Christine A. Byrd-Jacobs*

Department of Biological Sciences, Western Michigan University, 1903 W Michigan Ave, Kalamazoo, MI 49008-5410, USA

HIGHLIGHTS

- Chronic intranasal detergent exposure causes reversible olfactory deafferentation.
- Medial glomeruli are severely affected, but lateral glomeruli retain innervation.
- Behavioral preference for bile salts is lost, but amino acid detection remains.
- Glomerular distribution in the olfactory bulb returns with olfactory organ recovery.
- Olfactory-mediated response to bile salts returns with glomerular reinnervation.

ARTICLE INFO

Article history:
Received 2 July 2012
Received in revised form 8 August 2012
Accepted 12 August 2012
Available online 17 August 2012

Keywords:
Chemical lesion
Triton X-100
Anti-keyhole limpet hemocyanin
Amino acid
Bile salts
Regeneration

ABSTRACT

The olfactory system is a useful model for studying central nervous system recovery from damage due to its neuroplasticity. We recently developed a novel method of deafferentation by repeated exposure of Triton X-100 to the olfactory organ of adult zebrafish. This long-term, reversible method of deafferentation allows both degeneration and regeneration to be observed in the olfactory bulb. The aim of the present study is to examine olfactory bulb innervation, glomerular patterns, and olfactory-mediated behavior with repeated Triton X-100 treatment and the potential for recovery following cessation of treatment. Olfactory bulbs of control, chronic-treated, and recovery animals were examined for the presence or absence of glomeruli that have been identified in the zebrafish glomerular map. Following chronic treatment, the number of glomeruli was dramatically reduced; however, partial innervation remained in the lateral region of the bulb. When animals were given time to recover, complete glomerular distribution returned. A behavioral assay was developed to determine if innervation remaining correlated with behavior of the fish. Chronic-treated fish did not respond to odorants involved with social behavior but continued to react to odorants that mediate feeding behavior. Following recovery, responses to odorants involved with social behavior returned. The morphological and behavioral effects of chronic Triton X-100 treatment in the olfactory system suggest there may be differential susceptibility or resistance to external damage in a subset of sensory neurons. The results of this study demonstrate the remarkable regenerative ability of the olfactory system following extensive and long-term injury.

1. Introduction

The olfactory system is unique among sensory systems due to its direct contact with the environment and continuous neuronal turnover. This provides the olfactory system with the remarkable capacity for recovery after injury to help maintain critical sensory function. Damage to the peripheral olfactory organ can result from a variety of factors including physical injury, chemicals found in the environment, and disease. In addition, neurons are added to the olfactory bulb, the part of the brain that responds to olfactory signals, throughout life [1–5]. This continuous degeneration and regeneration makes the olfactory system a good model for understanding the molecular mechanisms required for peripheral and central nervous system recovery.

To study the importance of sensory input to a central nervous system structure, various methods of deafferentation are commonly used. Cautery ablation of the olfactory organ in fish results in complete and permanent removal of afferent fibers [6,7]. Following ablation, it takes several weeks before deafferentation effects occur in the olfactory bulb. These changes include decreased

Abbreviations: OSN, olfactory sensory neuron; MS222, tricaine methanesulfonate; KLH, keyhole limpet hemocyanin; PBS, phosphate-buffered saline; DiI, 1,1′-dioctadecyl 3,3,3′,3′ tetramethylindocarbocyanine perchlorate.

* Corresponding author. Tel.: +1 269 387 5843; fax: +1 269 387 5609.
E-mail addresses: taylor.r.paskin@wmich.edu (T.R. Paskin), christine.byrd@wmich.edu (C.A. Byrd-Jacobs).

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2012.08.018

olfactory bulb volume, removal of olfactory sensory neuron (OSN) axons, and reduced sensory input, as indicated by decreased tyrosine hydroxylase-like immunoreactivity [6]. Chemical lesioning of the peripheral olfactory organ is another method of deafferentation used to examine degeneration and subsequent regeneration of the olfactory bulb. Exposure of the olfactory organ to chemicals such as copper [8,9], methyl bromide [10], zinc sulfate [11–15], or the detergent Triton X-100 [16–20] destroys OSNs and their axons, which reduces olfactory bulb weight and activity. The Triton X-100 technique causes only temporary removal of afferent input and, when given time to recover, there is reconstitution of the olfactory epithelium and subsequent olfactory bulb reinnervation as axons regenerate.

Previous studies from our laboratory have shown that one day following Triton X-100 application to the zebrafish nasal cavity, the number of OSNs is dramatically reduced and other morphological abnormalities are observed in the olfactory epithelium [19]. However, 5 days after treatment these changes are reversed and there is restoration of olfactory organ morphology and neuronal population. This rapid regeneration does not allow significant morphological and functional changes to occur in the olfactory bulb, since it takes several weeks for deafferentation effects to be observed following the removal of afferent input. Therefore, to examine degeneration and regeneration in the olfactory bulb, we used a method of deafferentation involving repeated exposure of the olfactory organ to Triton X-100 for several weeks. Following treatment, there is severe morphological disruption of the olfactory organ, decreased neuronal labeling in the olfactory epithelium, a reduction in olfactory bulb volume, and decreased tyrosine hydroxylase-like immunoreactivity in the bulb [20]. This technique also allows for regeneration; when the animals are given a survival period following the cessation of treatment, the olfactory organ recovers and olfactory bulb volume and tyrosine hydroxylase-like immunoreactivity return [20].

Teleosts possess three morphologically distinct types of OSNs: ciliated OSNs with long dendrites and somata located deep in the epithelium, near the basal lamina; microvillous OSNs with intermediate-length dendrites of variable thickness and spindle-shaped cell bodies found in the middle of the olfactory epithelium; and crypt OSNs with egg-shaped somata located in the upper third of the epithelium and no apparent dendrites [21]. In the olfactory organ, each OSN type expresses a specific chemosensory receptor, projects to a distinct region of the olfactory bulb, and produces a different physiological and behavioral response. In zebrafish, microvillous OSNs respond physiologically and behaviorally to amino acids and nucleotides, which mediate their feeding behavior, while ciliated OSNs are stimulated by bile salts and pheromones, which mediate social and reproductive behavior [22–24]. Although the receptor molecules and specific function of crypt neurons is not known, it has been suggested in the crucian carp that these OSNs may have a role in reproductive behavior by responding to sex pheromones [25]. An interesting observation seen after repeated Triton X-100 treatment is a differential effect on OSN subtypes. Following treatment, very few OSNs with long dendrites were observed and the majority of neurons remaining had ovoid-shaped somata that lacked dendrites or spindle-shaped somata with intermediate-length dendrites [20].

Individual OSNs each express a single odorant receptor molecule and project their axons to very specific glomeruli in the olfactory bulb [26,27]. The zebrafish olfactory bulb contains about 80 individual glomeruli, of which 22 have been identified and are highly stereotyped with similar morphology and distribution between animals, regardless of sex, and the rest residing in groupings such as the dorsal cluster, lateral chain, and anterior plexus [28]. In zebrafish, ciliated OSNs innervate glomeruli located in the dorsal and medial regions of the olfactory bulb while microvillous OSNs project to the lateral and ventrolateral regions of the bulb [23]. Physiological and behavioral studies have shown in several fish species, including zebrafish, the olfactory bulb is divided into functional zones with the medial regions processing social and reproductive behavior and the lateral regions processing feeding behavior [23,29–33].

Repeated exposure of Triton X-100 to the olfactory organ is a useful technique for examining both degeneration and regeneration in the zebrafish olfactory system. This long-term, partial deafferentation method reduces afferent input to the olfactory bulb, without completely eliminating it, for an extended period of time. The current study further investigates the effects of detergent treatment and recovery by examining the glomerular distribution patterns in the olfactory bulb as well as the behavioral response to specific odorants. The hypothesis is that repeated Triton X-100 treatment will reduce innervation, alter glomerular patterns, and change olfactory-mediated behavior, but these effects will be reversed with cessation of treatment.

2. Materials and methods

2.1. Glomerular assay

2.1.1. Animals

Both male and female adult zebrafish, *Danio rerio*, aged 5–18 months and approximately 4–5 cm in length, were obtained from a commercial vendor and used for this study. Animals were fed flake food twice daily and maintained in aerated, conditioned freshwater tanks at 28.5 °C. All experimental and animal care protocols were approved by the Institutional Animal Care and Use Committee.

2.1.2. Chemical lesioning

The olfactory organs of chronically treated fish were subjected to repeated chemical lesioning. Fish were anesthetized with tricaine methanesulfonate (0.03% MS222; Sigma) until they were no longer responsive to tail pinch then transferred to a clay dish. To prevent chemical contact with the contralateral olfactory organ, a thin strip of petroleum jelly was applied between the two nasal cavities. For detergent application, a Drummond wiretrol 50 μL micropipette (1.0 mm I.D.) was pulled with a WPI PUL-1 (World Precision Instruments) and the tip was broken to a diameter of about 30 μm. Using the pulled micropipette and wiretrol plunger, approximately 1 μL of a solution of 0.7% Triton X-100 and 0.005% methylene blue in 0.1 M phosphate buffered saline (PBS) was applied to the right nasal cavity. The clay dish was put on ice for 2 min before the fish was returned to its housing tank to provide sufficient exposure to the detergent and ensure the fish remained anesthetized. The same procedure was repeated every 2–3 days for 3 weeks. The recovery fish were treated as described above but these animals were allowed 3 weeks of survival following the 3 weeks of repeated chemical lesioning. In both chronic and recovery groups, the left nasal cavity was not treated to serve as an internal control. Control fish did not receive any chemical exposure.

2.1.3. Tissue processing

All fish were euthanized with 0.03% MS222, perfused transcardially with PBS, and fixed with 4% paraformaldehyde for 2 h at room temperature or 24 h at 4 °C. Whole heads were dissected and decalcified with 0.5 M ethylenediaminetetraacetic acid disodium salt dihydrate (pH 7.4, made soluble with sodium hydroxide pellets and heat) for 30 h at 4 °C. Heads were then dehydrated in an ascending ethanol series and xylenes, embedded in paraffin, and 10 μm semi-serial sections were mounted onto positively charged slides and left at 37 °C overnight.

2.1.4. Immunocytochemistry

To identify OSN axonal projections in the olfactory bulb, an antibody to keyhole limpet hemocyanin (anti-KLH) was used. Mounted sections were dewaxed in xylenes and rehydrated in a descending ethanol series. Slides were subjected to antigen retrieval for 10 min in 10 mM sodium citrate solution at 100 °C. Slides were immersed for 1 h at room temperature with 1% bovine serum albumin in PBS to block nonspecific binding and incubated overnight at 4 °C with anti-KLH produced in rabbit (Sigma H0892; 1:1000) diluted in blocking buffer. Slides were then rinsed in PBS and incubated in biotinylated goat anti-rabbit antibody (Dako; 1:100) diluted in blocking solution for 1 h at room temperature. Following buffer rinses, sections were treated with a fluorescent avidin (Alexafluor 488 avidin, 1:200, Molecular Probes) in PBS for 1 h at room temperature, rinsed in PBS, coverslipped with glycerol and PBS (1:1), and viewed on a Nikon E600 microscope.

2.1.5. Analysis of glomerular patterns

The first group consisted of control fish ($n = 3$) and was used to observe the staining patterns of anti-KLH. Glomerular distribution was determined by examining serial horizontal sections of the olfactory bulb and comparing the anti-KLH