Physiology & Behavior 157 (2016) 67–71

Contents lists available at ScienceDirect

Physiology & Behavior

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

The smell of "anxiety": Behavioral modulation by experimental anosmia in zebrafish

Murilo S. Abreu [a], Ana C.V.V. Giacomini [a,b], Allan V. Kalueff [d,e,f,g,h], Leonardo J.G. Barcellos [a,b,c,h,*]

[a] Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria (UFSM), Av. Roraima, 1000, Cidade Universitária, Camobi, Santa Maria, RS 97105-900, Brazil
[b] Universidade de Passo Fundo (UPF), BR 285, São José, Passo Fundo, RS 99052-900, Brazil
[c] Programa de Pós-Graduação em Bioexperimentação, Universidade de Passo Fundo (UPF), BR 285, São José, Passo Fundo, RS 99052-900, Brazil
[d] Research Institute for Marine Drugs and Nutrition, College of Food Science and Technology, Guangdong Ocean University (GDOU), Zhanjiang, Guangdong 524025, China
[e] Institute of Translational Biomedicine, St. Petersburg State University, St. Petersburg 199034, Russia
[f] Institutes of Chemical Technology and Natural Sciences, Ural Federal University, Ekaterinburg, 620002, Russia
[g] ZENEREI Institute, 309 Palmer Court, Slidell, LA 70458, USA
[h] The International Zebrafish Neuroscience Research Consortium (ZNRC), Slidell 70458, USA

HIGHLIGHTS

- Intact and sham-anosmic fish showed similar behaviors and whole-body cortisol levels.
- Fluoxetine exposed fish displayed a robust anxiolytic-like effect.
- There were no differences in all parameters analyzed in the anosmic fish.
- Anosmia triggers anxiety-like behaviors in zebrafish.
- Experimentally-evoked anosmia modulates anxiety-like behaviors in adult zebrafish.

ARTICLE INFO

Article history:
Received 24 December 2015
Received in revised form 22 January 2016
Accepted 23 January 2016
Available online 25 January 2016

Keywords:
Olfactory sense
Fluoxetine
Stress
Novel tank
Food odor

ABSTRACT

Olfaction is strongly involved in the regulation of fish behavior, including reproductive, defensive, social and migration behaviors. In fish, anosmia (the lack of olfaction) can be induced experimentally, impairing their ability to respond to various olfactory stimuli. Here, we examine the effects of experimental lidocaine-induced anosmia on anxiety-like behavior and whole-body cortisol levels in adult zebrafish (*Danio rerio*). We show that experimentally-induced anosmia reduces anxiolytic-like behavioral effects of fluoxetine and seems to interact with anxiogenic effect of stress also paralleling cortisol responses in zebrafish. These findings provide first experimental evidence that temporary anosmia modulates anxiety-like behaviors and physiology in adult zebrafish.

1. Introduction

Neuroanatomically confined within the ancient rhinencephalon, olfactory circuits play an important role in modulating behaviors and brain functions [1]. In humans, which are microsmatics, scents perceived during traumatic experiences can trigger pathological stress [2], whereas impaired olfaction (by increasing uncertainty or reducing hedonic behaviors) may cause affective disorders, such as anxiety and depression [3]. Olfactory deficits are also are linked to many other psychiatric disorders, including dementia, Parkinson's disease and schizophrenia [4–5].

Rodents are nocturnal macrosmatic animals that rely on olfaction more heavily than humans [6]. In rodents, olfaction plays a critical role in modulating their behavior, including sexual and social interactions, olfaction-induced fear responses as well as anosmia-induced anxiety/depression-like phenotypes [7–8]. Olfaction is also involved in the regulation of many zebrafish behaviors, including reproduction, defense, foraging, social interaction and migration [9–10]. While olfactory deprivation causes anosmia (the loss of the ability to respond to olfactory stimuli) in fish, their acute anosmia can be induced experimentally (e.g., as an experimental procedure used to study fish chemosensory responses) [11].

* Corresponding author at: Programa de Pós-Graduação em Bioexperimentação, Universidade de Passo Fundo (UPF), BR 285, São José, Passo Fundo, RS, Brazil, 99052-900, Brazil.
E-mail address: lbarcellos@upf.br (L.J.G. Barcellos).

http://dx.doi.org/10.1016/j.physbeh.2016.01.030
0031-9384/

Studies using zebrafish (*Danio rerio*) as animal models have recently increased exponentially in genetics, developmental biology and behavioral neuroscience [12–14]. Multiple studies have comprehensively evaluated the effects of stressors, drugs (e.g., [15]), chemicals (e.g., [16]) and environmental manipulations (e.g., [17,18]) on zebrafish anxiety-like behavior and endocrine responses. Analyzing zebrafish affective behaviors, the novel tank test represents one of the most commonly used models to access anxiety-like phenotypes in zebrafish [12]. Based on the fish innate protective 'diving' response, this aquatic model provides an efficient test for anxiety-like behaviors in zebrafish, highly sensitive to various experimental manipulations [14, 19]. Given the importance of olfaction in the regulation of animal and human behavior, here we examine the effects of experimentally induced transient anosmia on anxiety-like behavior in adult zebrafish. This study aimed to better understand the role of olfactory modulation in zebrafish affective behaviors, in an attempt to parallel these findings with clinical evidence related to human behavioral regulation by the olfactory system.

2. Materials and methods

2.1. Animals

A stock population of 192 mixed-sex (~50:50 males and females) 1-year old adult wild-type short-fin (SF) zebrafish was housed (1 fish/L) in 100-L tanks equipped with biological filters, under constant aeration and a natural (14 h light:10 h dark) photoperiod (lights on at 20:00 pm). Water temperature was maintained at 27 ± 1 °C, with pH = 7.0 ± 0.1, dissolved oxygen at 6.0 ± 0.2 mg/L, total ammonia at <0.01 mg/L, total hardness at 6 mg/L, and alkalinity at 22 mg/L $CaCO_3$.

2.2. Experimental design

To examine whether anosmia modulates anxiety-like behavior in zebrafish, the three cohorts of fish (intact, anosmic and sham-anosmic zebrafish) were used, as shown in Fig. 1.

In the control group, zebrafish remained in an aquarium for 17 min and then tested in the novel tank task for 6 min. In the odor group, zebrafish were exposed to the food odor for 17 min (see [20] for details) prior to behavioral testing in the novel tank. The FLU group was exposed to fluoxetine (50 μg/L, Daforin® EMS São Paulo, Brazil) for 17 min prior to the novel tank testing. In the stressed group, fish remained in the aquarium for 15 min and then were exposed to an acute stressor (chasing with a net for 2 min) prior to behavioral analyses in the 6-min novel tank test.

2.3. Experimental procedures

2.3.1. Anosmia protocol

The transient experimental anosmia was induced in zebrafish by application of lidocaine gel (Lidocaína Gel, EMS, São Paulo, Brazil) in the nares and olfactory surface, as described in [21]. Briefly, after zebrafish were captured and placed individually on a wet sponge, we applied a cotton ball soaked in lidocaine gel (50 mg/g) into the nares. In the sham fish, we repeated the same procedure but using only saline solution. Intact control fish were experimentally naïve and did not undergo any experimental manipulation or treatment.

2.3.2. Behavioral and cortisol analysis

In all cohorts, fish were transferred individually to a glass transparent test tank (24 × 8 × 20 cm; width × depth × height) and filmed for 6 min using a Logitech HD Webcam C525 camera (Logitech, Romanel-sur-Morges, Switzerland). The test tank was divided into two virtual zones (upper and bottom halves). The videos were then analyzed using ANY-maze® software (Stoelting Co, USA), scoring the following behavioral parameters: the number of upper zone entries, time spent (s) in the upper zone, the number of bottom zone entries, time spent (s) in the bottom zone, absolute turn angle, mean swimming speed (m/s), the number of crossings between the tank zones, and total distance travelled (m), according to the Zebrafish Behavioral Catalog (see [22]). In addition, we assessed the whole-body cortisol concentration in anosmic zebrafish, extracted and determined using the method described previously [23]. This study was approved by the Ethics Commission for Animal Use of the Universidade de Passo Fundo, UPF, Passo Fundo, RS, Brazil (Protocol #10/2014), and performed in full compliance with the guidelines of Conselho Nacional de Controle de Experimentação Animal (Concea).

2.4. Statistics

The effects of four treatments were compared in intact, anosmic or sham-anosmic zebrafish using the one-way ANOVA followed by post-hoc Tukey's test or Kruskal-Wallis test, depending on the data normality (assessed by the Bartlett's test), for significant effects. In all experiments, P was set at <0.05.

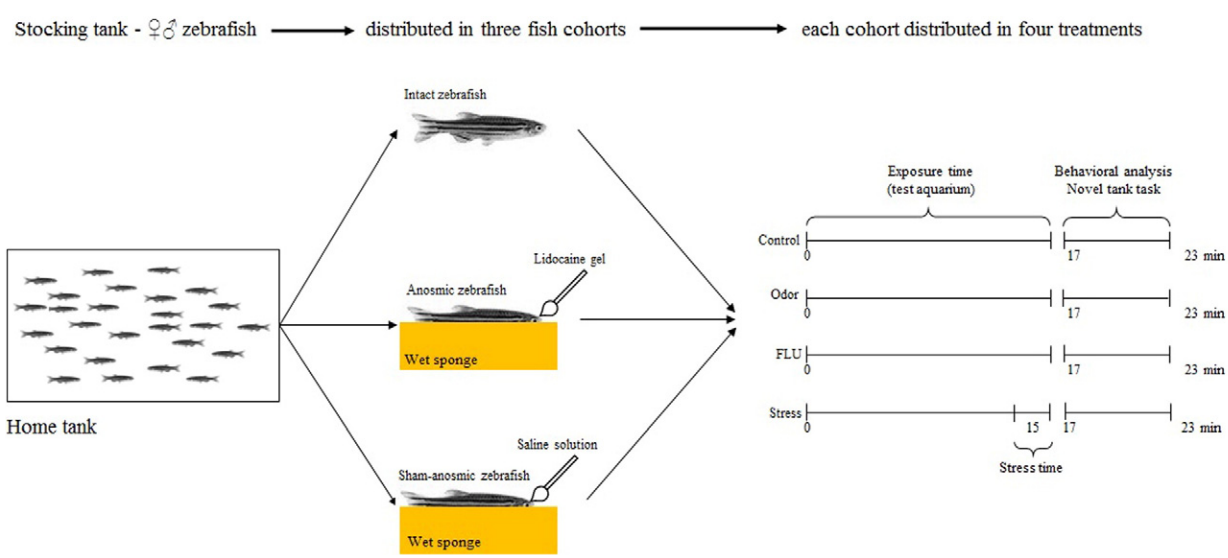

Fig. 1. Schematic representation of experimental design.