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Research report

Rest mutant zebrafish swim erratically and display atypical spatial preferences

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HIGHLIGHTS

- Adult *rest* mutants engage in erratic swimming and display atypical spatial preferences.
- *rest* mutant larvae are hypoactive when compared to sibling controls.
- Adult *rest* mutant males but not females show diminished reproductive success.

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ABSTRACT

The Rest/Nrsf transcriptional repressor modulates expression of a large set of neural specific genes. Many of these target genes have well characterized roles in nervous system processes including development, plasticity and synaptogenesis. However, the impact of Rest-mediated transcriptional regulation on behavior has been understudied due in part to the embryonic lethality of the mouse knockout. To investigate the requirement for Rest in behavior, we employed the zebrafish *rest* mutant to explore a range of behaviors in adults and larva. Adult *rest* mutants of both sexes showed abnormal behaviors in a novel environment including increased vertical swimming, erratic swimming patterns and a proclivity for the tank walls. Adult males also had diminished reproductive success. At 6 days post fertilization (dpf), *rest* mutant larva were hypoactive, but displayed normal evoked responses to light and sound stimuli. Overall, these results provide evidence that *rest* dysfunction produces atypical swimming patterns and preferences in adults, and reduced locomotor activity in larvae. This study provides the first behavioral analysis of *rest* mutants and reveals specific behaviors that are modulated by Rest.

1. Introduction

Regulation of chromatin landscapes is essential for proper transcriptional control of gene expression in numerous contexts. This is especially important in neurons, where functional diversity and complex activities require elaborate mechanisms to modulate transcription. One key transcriptional regulator, which regulates chromatin structure to broadly influence neural gene expression is *rest* [RE1-silencing transcription factor; also known as Neuron-restrictive silencing factor (*nrsf*)]. Rest silences neural-specific genes in non-neural cells [1,2] and within the developing nervous system [3].

Rest is widely conserved in vertebrates, including zebrafish [4,5] and interacts with the ~23 bp RE1/NRSE element that is associated with hundreds of neural genes [5,6]. Rest modifies chromatin by recruiting a diverse series of chromatin modifying complexes. The N-terminal domain of Rest interacts with Sin3, and recruits a range of repressor complexes that include MeCP2 and HDACs1/2 [7,8]. The C-terminal domain of Rest interacts with CoREST to convene chromatin modifying complexes that included HDACs 1/2 and histone H3 K9 methyltransferases [6,9,10]. Misregulation of *rest* activity has been implicated as a molecular cause in an assortment of human diseases that impact nervous system function including Alzheimer's disease, Huntington's Disease, Down's syndrome and X-linked mental retardation [11–14].

We have previously shown that while *rest* is required to modulate early gene expression, it is not essential for viability in

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zebrafish. *rest* is broadly expressed in the developing nervous system [4] and it appears to fine tune expression of target genes during early development [15]. In contrast, the mouse *rest* mutant is an early lethal [16], which has hindered analysis of the role of *rest* in behavior. While previous behavioral studies have employed murine tissue specific knockouts to investigate the role of *rest* in seizure progression in epilepsy [17] and hypersensitivity to the locomotor effects of MPTP treatment [18], it remains unknown how Rest influences other complex behaviors. The zebrafish *rest* mutant provides unique access to study changes in complex behaviors in the context of the complete elimination of Rest. In this study, we demonstrate that adult *rest* mutants present with atypical swimming behaviors consisting of frequent vertical swimming, sharper turning angles and enhanced wall preferences. In addition, sex-specific effects on reproductive success were identified. *rest* mutant larva display hypoactivity, but normal responses to light and acoustic stimuli. Together, these results reveal dynamic roles for Rest-mediated transcriptional regulation on complex behaviors in zebrafish.

2. Materials and methods

2.1. Fish maintenance

Adult zebrafish strains and embryos were obtained from natural crosses and were maintained at 28.5 °C under a 13:11 h light:dark cycle. Adult fish were fed twice a day with a combination of artemia and flake food. The *rest*^{sbu29} mutation was maintained in a hybrid wild-type background consisting of Tuebingen long-fin crossed to Brian's wild-type [19] and the fish used for these studies were created by incrossing *rest* heterozygotes. This creates a thoroughly controlled population where siblings are genetically related and environmental conditions are controlled for. Larval assays were performed on multiple clutches derived from different parents to minimize effects of genetic background. Behavioral test were conducted at 6 days post fertilization (dpf) and at 4–6 months of age from 1 to 5 in the afternoon unless stated otherwise. The care and the experimental procedures were approved by the Stony Brook University IACUC.

2.2. Housing and genotyping

Fish embryos were raised in a density of less than 100 per 150 mm dish until 24 hpf in egg water (6 g of synthetic sea salt, 20 ml of methylene blue (1 g/L) solution in to 20 L of water pH 7). Embryos were then transferred to a 24 well plate, one embryo per well in egg water. The embryos were raised in the dishes, tested and genotyped as previously described using primers 181 (5′-CTGAGGGGAAGCAGATGATG-3′) and 184 (5′-TGTCCATGCTGTATCTCACGA-3′) [15]. Adult fish were raised in groups of 8–10 in 1.8 L tanks, but were individually housed during genotyping and behavioral assays in 1-L tanks.

2.3. Behavioral testing apparatus

2.3.1. Adult behavioral apparatus

Adult behaviors were recorded and tracked in an xyZfish tracker/software system [20–22]. All testing was done in an eight-liter observation container (25 cm × 25 cm × 18 cm, l × w × h; filled with water to a depth of 13.5 cm) that had two white walls and a white bottom, and two walls that were transparent. A camera recorded the front and top views (via an angle mirror over the tank) simultaneously at a speed of approximately 40 frames/s. This enabled continuous monitoring of the *X* (perpendicular to the camera), *Y* (parallel to the camera) and *Z* (vertical) coordinate locations of each fish. LED lights above the container provided illumination at approximately 800 lux and a dark green curtain enclosed the recording chamber. The data were imported into a software filter to reduce noise and analyzed in the Data Processing Module of the xyZfish software.

2.3.2. Larvae behavioral apparatus

Larvae behaviors were recorded using a Zebrabox imaging system (Viewpoint Life Sciences, France) constantly illuminated by infrared light and tracked with automated video-tracking software (Zebralab; Viewpoint Life Sciences, France). Movement thresholds were set at 4 mm/s for "small movements" and 8 mm/s for "large movements" that are characteristic of a startle/escape behavior.

2.4. Behavioral paradigms

2.4.1. Adult testing

2.4.1.1. Novel environment. Adult zebrafish were gently poured into the center of the observation container from the 1-L home tank. Recording started immediately after the fish were poured into the cube and continued for 15 min.

2.4.1.2. Reproductive success. Natural crosses were set up with one male and one female per tank. Each cross consisted of a *rest* mutant or sibling wild-typed mated to a rotating stock of unrelated wild-type fish. Crosses were set up during the late afternoon with a divider separating the fish until 9:15 AM. The fish were allowed to breed for four hours and embryos were collected every two hours. Later that day the embryos were counted and classified into fertilized (normally developing embryos), unfertilized eggs or bad embryos (an embryo that developed abnormally or showed necrosis) [23].

2.4.2. Larval tests

2.4.2.1. Visual-motor-behavioral assay. Larvae were placed in the Zebrabox illuminated with white light on and allowed to acclimate for 20 min. Spontaneous movements were tracked for an additional 15 min. Then the light was turned off at 35 min and the behavior was recorded for an additional minute in the dark using IR illumination.

2.4.2.2. Acoustic stimuli assay. Larvae were placed in the Zebrabox in the dark and allowed to acclimate for 20 min. Spontaneous movements were then measured for an additional 15 min using IR illumination. Then 50 min in to the assay, larvae were exposed to a single sound pulse (3 ms/1000 Hz) delivered though a speaker touching the plate containing the larvae. Behavior was recorded for an additional minute.

2.5. Statistics

2.5.1. Adult behaviors

Statistical analyses were conducted using SPSS, version 21 and Graphpad software. The novel environment data collected over the testing period were first compared using a multivariate analysis of variance (MANOVA) to identify main effects of sex and/or genotype and significant interactions between the two. A two 2-way ANOVA with repeated measures design was also used to compare within-sex data collected in 1-min bins across the 15 min testing period. For these analyses, the genotype served as the independent factor and time served as the repeated measure; Mauchly's Test of Sphericity was applied and the Greenhouse–Geisser Correction was used as needed. All allowed post hoc comparisons used Least Squared Difference (LSD) analysis.

Fertility was analyzed by the use of a 2-way ANOVA to probe for main effects of sex and genotype and significant interactions between the two; post hoc comparisons used LSD analysis, Within