Neurotoxicology and Teratology 53 (2016) 19–23

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

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

Trans-generational transmission of neurobehavioral impairments produced by developmental methylmercury exposure in zebrafish (*Danio rerio*)

Xiaojuan Xu [a,*], Daniel Weber [b], Amanda Martin [a], Daniel Lone [a]

[a] Department of Psychology, Grand Valley State University, Allendale, MI 49401, United States
[b] Children's Environmental Health Sciences Center, University of Wisconsin-Milwaukee, Milwaukee, WI 53204, United States

ARTICLE INFO

Article history:
Received 9 July 2015
Received in revised form 25 October 2015
Accepted 9 November 2015
Available online 10 November 2015

Keywords:
Trans-generational
Active avoidance conditioning
Learning
Methylmercury
Zebrafish

ABSTRACT

Our previous study showed that embryonic exposures to methylmercury (MeHg) produced learning impairments in adult zebrafish. The present study investigated the persistency of learning impairments in the second (F2) and third (F3) generations of zebrafish developmentally exposed to MeHg as embryos using active avoidance conditioning as the behavioral paradigm. The results showed that the control zebrafish learned avoidance responses during training and significantly increased avoidance responses during testing. The F2 generation of zebrafish developmentally exposed to MeHg as embryos displayed no significant changes in avoidance responses from training to testing, showing persistent learning impairments, while the F3 generation of zebrafish developmentally exposed as embryos to only the higher concentration of MeHg showed persistent learning impairments. Results of the present study showed that learning impairments produced by embryonic MeHg exposure persisted for at least three generations, demonstrating trans-generational effects of embryonic exposure to MeHg.

1. Introduction

The developmental neurotoxicity of methylmercury (MeHg) first attracted wide attention in 1950s when children born to pregnant women who consumed highly contaminated seafood displayed severe neurological disabilities in Minamata, Japan. Children exposed to MeHg in utero exhibited impaired vision and hearing, ataxia, dysarthria, paresthesias, mental retardation, and cerebral palsy (Harada 1995). Since then, both human and animal studies have shown that early life exposure to lower doses of MeHg may give rise to long-term neurobehavioral impairments (Bisen-Hersh et al. 2014; Fox et al. 2012; Weber et al. 2008), including learning deficits (Lam et al. 2013; Reed et al. 2008; Smith et al. 2010). However, the impact of early life exposure to MeHg is potentially greater if the neurobehavioral impairments produced by early life exposure to MeHg are propagated across generation. At present, little is known about trans-generational effects of early life exposure to MeHg or environmental contaminants in general.

Longitudinal studies of human populations revealed that conditions individuals experienced as fetuses (F1 generation) could alter the birth characteristics and/or later life health of the individuals and their offspring (F2 generation) (Painter et al. 2008; Rickard et al. 2012). Animal studies with rodents have corroborated those findings that characteristics linked to early life experiences of the parents may be transmitted to subsequent generations (Benyshek et al. 2006; Franklin et al. 2010). Thus, the repercussions of conditions experienced during early development may not be limited to the organisms who experienced them first-hand, but may affect the generations to follow.

The zebrafish has become a useful organism for studying the toxic effects of environmental contaminants on the neurobehavioral development of an organism due to its ease of breeding, high numbers of eggs per female, and short generation times. Based on the behavioral method and findings of the active avoidance paradigm in goldfish (Xu et al., 2001 & 2003), active avoidance conditioning in zebrafish has become an established behavioral paradigm (Xu et al. 2007; Xu and Goetz 2012). Using active avoidance conditioning as the behavioral paradigm, our previous study showed that embryonic MeHg exposure produced learning impairments in adult zebrafish (Xu et al. 2012a). The present study aimed to determine whether embryonic MeHg exposure produced trans-generational effects by investigating the persistency of those learning impairments in the second (F2) and third (F3) generations of the zebrafish exposed to MeHg as embryos.

* Corresponding author at: Department of Psychology, Grand Valley State University, 1 Campus Drive, Allendale, MI 49401, United States.
E-mail address: XUX@GVSU.EDU (X. Xu).

http://dx.doi.org/10.1016/j.ntt.2015.11.003
0892-0362/

2. Material and methods

2.1. Breeding and egg collection

Adult zebrafish (Ekkwill Waterlife Resources, Gibsonton, FL) were acclimated for several weeks prior to the initiation of experiments. Fish were maintained at 26–28 °C on a 14-h light and 10-h dark cycle in a flow-through buffered, dechlorinated water system at the Aquatic Animal Facility of the University of Wisconsin–Milwaukee Children's Environmental Health Sciences Center. All experimental procedures were approved by the University of Wisconsin-Milwaukee Animal Care and Use Committee. Zebrafish were bred in 2-L plastic aquaria with a 1/8″ nylon mesh false bottom to protect fertilized eggs from being consumed by the adults. Eggs were collected ≤2 h post fertilization (hpf) and placed into metal-free, glass culture dishes (100 mm diameter × 50 mm depth) in E2 medium (each liter contains 0.875 g NaCl, 0.038 g KCl, 0.120 g $MgSO_4$, 0.021 g KH_2PO_4, and 0.006 g Na_2HPO_4).

2.2. Exposure regimen

Methylmercury (MeHg; >98% purity) was obtained from ICN Biomedicals (Aurora, OH). Collected eggs (<2 hpf) were rinsed twice in MeHg-free E2 medium (as determined by ICP-MS analysis) and transferred to metal-free glass dish (100 mm diameter × 50 mm depth) containing 100 ml of E2 medium with MeHg at 0.0, 0.01, or 0.10 μM MeHg. At 24 hpf the embryos were rinsed in MeHg-free E2 medium and then raised in MeHg-free E2 medium. Fry were fed vinegar eels twice each day starting at day 5 post hatch regardless of treatment until large enough to consume *Artemia* nauplii. Juveniles and adults were fed Aquarian™ flake food (Aquarium Pharmaceuticals, Inc., Chalfont, PA) in the morning and *Artemia* nauplii in the afternoon. Based upon this and previous studies, there are no significant differences in embryo, larval, juvenile, or adult mortality or number of developmental malformations at the stated concentrations of MeHg. The F2 generation, i.e., the offspring of embryonic MeHg exposed fish, were used in Experiment 1, while the F3 generation, i.e., the grand-offspring of embryonic MeHg exposed fish, were used in Experiment 2. Neither the F2 nor the F3 generations were exposed to MeHg.

2.3. Housing during avoidance conditioning

During behavioral experiments, the second and third generations of zebrafish hatched from the embryos described above were kept in individual compartments of partitioned tanks at 26 ± 1 °C with a 12 h light–dark cycle (0700–1900 light) at the fish laboratory of Grand Valley State University. The behavioral experiments were conducted during the light cycle and all experimental procedures were approved by the Grand Valley State University Institutional Animal Care and Use Committee.

2.4. Apparatus for avoidance conditioning

Zebrafish were trained and tested individually in two identical zebrafish shuttle-boxes connected to a programmer/shocker unit. The zebrafish shuttle-box consisted of a water-filled tank separated by an opaque divider into two equal compartments. The divider was raised 0.6 cm above the floor of the tank during trials allowing zebrafish to swim freely from one side of the tank to the other. The crossing movement of zebrafish was monitored by infrared light beams and their corresponding detectors located on the long sides of the tank. There was a light at each end of the tank and there were two stainless steel electrode plates at each of the long sides of each compartment.

2.5. Active avoidance paradigm

Zebrafish were placed in the shuttle-boxes for 5 min, and then a trial began with the onset of the light on the side of the fish's location and the manually raised divider 0.6 cm above the floor of the tank. After the light was on for 12 s, a repetitive mild electrical shock was administered, along with the light, for 12 s through the water by means of electrodes. At the end of 24 s or at a crossing response by zebrafish during the 24 s, the trial ended with both the light and electrical shock switched off and the divider lowered. After an intertrial interval (ITI) of 12 s, another trial began.

Zebrafish initially swam through the raised divider after receiving several shocks. The crossing response made after the onset of both light signal and electrical shock to escape the electrical body shock is defined as an escape response. During the training sessions, zebrafish gradually learned to swim from the lighted end of the shuttle-box to the dark end to avoid the electrical body shock. The crossing response made after the onset of the light signal, but before the onset of electrical shock to avoid the electrical body shock, is defined as an avoidance response. The time it takes for zebrafish to make the crossing response following the onset of the light signal is defined as crossing latency. Several days later, zebrafish were tested for avoidance responses. The measurements were the number of avoidances and escapes; and crossing latency. Except the manually raised dividers, all experiments were automated through the programmer/shocker unit and a Gateway 2000 P5-100 computer that programmed stimuli, monitored and recorded behavior of zebrafish.

Zebrafish were trained on Behavioral Experimental Day 1, and tested on Behavioral Experimental Day 3. The training session consisted of 30 trials, and the testing session consisted of 10 trials. Both increases in percentage of avoidance responses and decreases in crossing latency during testing were used as indicators of learning.

2.6. Experiment 1: the persistency of learning impairments produced by embryonic MeHg exposure in the F2 generation

This experiment examined whether the F2 generation of the zebrafish exposed to MeHg as embryos showed learning impairments. Adult zebrafish of the F2 generation of zebrafish developmentally exposed as embryos to no MeHg (control group), 0.01 μM (0.01 μM group) or 0.1 μM of MeHg (0.1 μM group) were trained on Behavioral Experimental Day 1 and tested on Behavioral Experimental Day 3 for avoidance responses.

Two-way ANOVAs with one between factor (different groups) and one repeated measure (testing vs. training sessions) on the results were carried out first to determine possible significant differences, followed by one-way ANOVAs to determine any significant differences among groups and correlated t-tests to determine any significant differences between testing and training.

2.7. Experiment 2: the persistency of neurobehavioral effects of embryonic MeHg exposure through generations

This experiment examined whether the F3 generation of the zebrafish exposed to MeHg as embryos showed learning impairments. Adult zebrafish of the F3 generation of the zebrafish developmentally exposed as embryos to no MeHg, 0.01 μM or 0.1 μM of MeHg were trained on Behavioral Experimental Day 1 and tested on Behavioral Experimental Day 3 for avoidance responses.

Two-way ANOVAs with one between factor (different groups) and one repeated measure (testing vs. training sessions) on the results were carried out first to determine possible significant differences, followed by one-way ANOVAs to determine any significant differences among groups and correlated t-tests to determine any significant differences between testing and training.