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Comparison of Neurobehavioral Effects of Methylmercury Exposure in Older and Younger Adult zebrafish (*Danio rerio*)

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ABSTRACT

It is widely recognized that the nature and severity of responses to toxic exposure are age-dependent. Using active avoidance conditioning as the behavioral paradigm, the present study examined the effect of short-term methylmercury (MeHg) exposure on two adult age classes, 1- and 2-year-olds to coincide with zebrafish in relatively peak vs. declining health conditions. In Experiment 1, 2-year-old zebrafish were randomly divided into groups and were exposed to no MeHg, 0.15% ethanol (EtOH), 0.01, 0.03, 0.1, or 0.3 μM of MeHg (in 0.15% ethanol) for 2 weeks. The groups were then trained and tested for avoidance responses. The results showed that older zebrafish exposed to no MeHg or EtOH learned and retained avoidance responses. However, 0.01 μM or higher concentrations of MeHg exposure impaired avoidance learning in a dose-dependent manner with 0.3 μM of MeHg exposure producing death during the exposure period or shortly after the exposure but before the avoidance training. In Experiment 2, 1-year-old zebrafish were randomly divided into groups and were exposed to the same concentrations of MeHg used in Experiment 1 for 2 weeks. The groups were then trained and tested for avoidance responses. The results showed that younger zebrafish exposed to no MeHg, EtOH, or 0.01 μM of MeHg learned and retained avoidance responses, while 0.1 or 0.3 μM of MeHg exposure impaired avoidance learning in a dose-dependent manner. The study suggested that MeHg exposure produced learning impairments at a much lower concentration of MeHg exposure and more severely in older adult compared against younger adult zebrafish even after short exposure times.

1. Introduction

It is widely recognized that the nature and severity of responses to toxic exposure are age-dependent. Animal studies show that early life history stage, especially embryonic, exposures to toxic chemicals are extremely deleterious due to the high sensitivity of actively developing organ systems (Şişman, 2010, 2011; Gu et al., 2010; Meier et al., 2010; Jezierska et al., 2009; Barry et al., 1995). As animals get older, progressive degeneration of tissue and loss of organ function have been observed in animals (Anchelin et al., 2011; Di Cicco et al., 2011; Durán et al., 2010). Thus, age-related loss of organ function and structural integrity, e.g., DNA hypomethylation, neurodegeneration, immunodeficiencies, tissue degeneration and decreases in biochemical activity related to metabolic detoxification, may also potentiate the harmful effects of chemical contaminant exposures (Madrigano et al., 2011; Risher et al., 2010; Bollati et al., 2009; Scheuplein et al., 2002; Moser, 1999; Cory-Slechta, 1990; Lin et al., 1975; Sansar et al., 2011; Lemire et al., 2010; Peters et al., 2010; Ostachuk et al., 2008; López-Diazguerrero et al., 2005; Moser, 1999; Barnett, 1997).

Mercury (Hg^{2+}) compounds including methylmercury (MeHg) induce neurodegeneration, oxidative stress, alterations in gene expression and declines in immune function, processes that are often associated with the aging process in aquatic animals and humans (Lushchak, 2011; Cambier et al., 2010; Houston, 2007; Monnet-Tschudi et al., 2006; Berntssen et al., 2003; Schmechel et al., 2006). Several studies have demonstrated that adult exposures to Hg^{2+} compounds induce alterations in learning and memory in humans (Hilt et al., 2009; Yokoo et al., 2003; Smith et al., 1983). However, there are no data in which there is a direct comparison on the effects of short-term, adult MeHg exposures vs. behavioral outcomes influenced by the normal aging process in any vertebrate species, especially as it relates to learning and memory. Therefore, due to its continued presence in fish- and

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seafood-based diets, it is important to investigate the potential role of MeHg, the organic and most common environmental form of Hg, plays in age-related behavioral effects.

Zebrafish (*Danio rerio*) have become a widely used vertebrate model system for examining learning and memory (Gómez-Laplaza and Gerlai, 2010; Sison and Gerlai, 2010; Xu et al., 2007; Salas et al., 2006; Williams et al., 2002; Xu and Goetz, 2012). The regions in the teleost brain responsible for directing those behaviors have been shown to be the dorsolateral telencephalon, critical for spatial learning (Salas et al., 1996; Dodson, 1988), and dorsomedial telencephalon, critical for avoidance learning (Portavella and Vargas, 2005; Xu et al., 2003, 2009). Therefore, zebrafish were used to study the following question: are older adult fish more vulnerable to waterborne MeHg exposure than younger adult fish? Since zebrafish live to approximately 2 years of age in the wild (Spence et al., 2008), the present study examined the effect of short-term MeHg exposure on two adult age classes, 1- and 2-year-olds to coincide with fish in relatively peak vs. declining health conditions (Kanuga et al., 2011). Since life-time accumulation of environmental toxicants can be a confounder in age-related effects, as well as a result of the aging process (Bunton et al., 1987), this study used adult exposures only on test subjects who had previously been raised in a MeHg-free environment. Our previous work with zebrafish showed that embryonic exposure to MeHg induced learning deficits when tested at 4–8 months of age in a spatial alternation task that involves the dorsolateral telencephalon (Smith et al., 2010), and an active avoidance task that involves the dorsomedial telencephalon (Xu et al., 2012). To extend those investigations, the present study investigated the neurobehavioral effects of late- vs. early-stage adult zebrafish exposures to MeHg using active avoidance conditioning as the behavioral paradigm.

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 in. nylon mesh false bottom to protect fertilized eggs from being consumed by the adults. Eggs were collected $\leq$2 h post fertilization and placed into glass culture dishes (100 mm diameter $\times$ 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) with 0.0 μM MeHg. Fry were fed vinegar eels twice each day 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.

2.2. Exposure regimen

Methylmercury (MeHg; >98% purity) was obtained from ICN Biomedicals (Aurora, OH) and dissolved in 0.15% ethanol (EtOH). Fish were raised in MeHg-free and dechlorinated water for 12 or 24 months at which time they were exposed to a daily pulse of 0.0, 0.01, 0.03, 0.10, or 0.30 μM MeHg or the vehicle 0.15% EtOH for 2 weeks. Each exposure group was visibly healthy (no aberrant swimming styles, normal eating patterns, normal respiration activity as monitored by gill opercular movements, no visible surface fungal growth, etc.) at the start of the exposure.

2.3. Housing during avoidance conditioning

During behavioral experiments, adult zebrafish 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 (18 cm in length $\times$ 7.5 cm in width $\times$ 10 cm in height) separated by an opaque divider (7.5 cm in width $\times$ 10 cm in height) 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 (6.5 cm in length $\times$ 4 cm in height) 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, the conditioned stimulus (CS), 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 (0.73 V/cm AC, pulsed 100 ms on and 1400 ms off), the unconditioned stimulus (US), 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) ranging from 12 to 36 s, another trial began.

Zebrafish initially swam through the raised divider only 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 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 taken by zebrafish to make the crossing response following the onset of the light signal is defined as crossing latency. 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. Percentage of avoidance responses and crossing latency were used as indicators of learning.

2.6. Experiment 1: the effects of MeHg exposure in 2-year-old zebrafish

This experiment examined the neurobehavioral effects of MeHg exposure in older adult zebrafish. Adult zebrafish of 2-year old

were randomly divided into groups and were exposed to 0.01 μM, 0.03 μM, 0.1 μM, or 0.3 μM of MeHg for 2 weeks. One control group was exposed to neither MeHg nor the vehicle, while a vehicle control group was exposed only to the vehicle 0.15% EtOH for 2 weeks. Two to three weeks after the completion of the exposure, the groups were trained and then tested for avoidance responses. Percentage of avoidance responses and crossing latency were used as indicators of learning.

Two-way ANOVAs with one between factor (different groups) and one repeated measure (training vs. testing) 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 training and testing.

2.7. Experiment 2: the effects of MeHg exposure in 1-year-old zebrafish

This experiment examined the neurobehavioral effects of MeHg exposure in younger adult zebrafish. Adult zebrafish of 1-year old were randomly divided into groups and were exposed to the same concentrations of MeHg used in Experiment 1, i.e., 0.01 μM, 0.03 μM, 0.1 μM, or 0.3 μM of MeHg for 2 weeks. One control group was exposed to neither MeHg nor the vehicle, while a vehicle control group was exposed only to the vehicle 0.15% EtOH for 2 weeks. Two to three weeks after the completion of the exposure, the groups were trained and then tested for avoidance responses. Percentage of avoidance responses and crossing latency were used as indicators of learning.

Two-way ANOVAs with one between factor (different groups) and one repeated measure (training vs. testing) 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 training and testing.

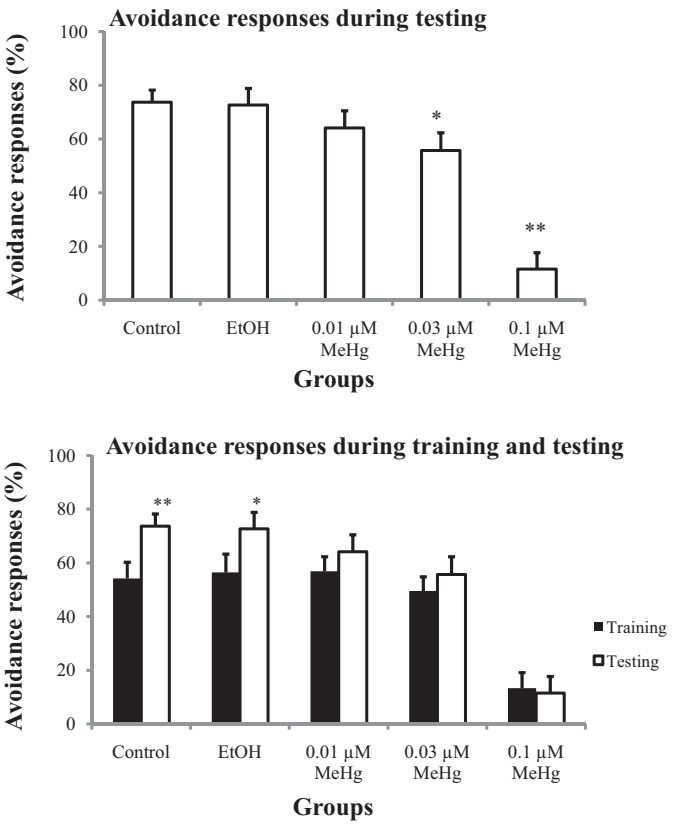

Fig. 1. Avoidance responses of older adult zebrafish exposed to no MeHg or various levels of MeHg. Each bar represents the mean percentage of avoidance responses ±SE for 13–21 fish. Upper panel shows avoidance responses during testing. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the vehicle control group. Lower panel shows avoidance responses during both training and testing. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the training session of the same group.

3. Results

3.1. Results of 2-year-old zebrafish in Experiment 1

All 2-year-old zebrafish that were exposed to 0.3 μM died before the behavioral experiment started. Fig. 1 shows avoidance responses of five groups of 2-year-old zebrafish. A two-way ANOVA with one between factor (5 groups) and one repeated measure (2 sessions) on the avoidance responses indicated a significant group difference [$F(4, 80) = 13.727$, $p < 0.01$], and a significant session difference [$F(1, 80) = 14.731$, $p < 0.01$]. A one-way ANOVA with multiple comparisons on the avoidance responses of the groups during the training session showed that only the 0.1 μM MeHg group was significantly different from the vehicle control group [$F(4, 80) = 7.922$, $p < 0.01$], while another one-way ANOVA with multiple comparisons on the avoidance responses of the groups during the testing session showed significant differences between the vehicle control and MeHg groups in a dose-dependent manner [$F(4, 80) = 14.612$, $p < 0.01$]. There were no significant differences between the control group and the vehicle control group. However, compared with the vehicle control EtOH group, the 0.01 μM MeHg group showed lower avoidance responses, the 0.03 μM MeHg group showed significantly lower avoidance responses ($p < 0.05$), and the 0.1 μM MeHg group showed the lowest avoidance responses ($p < 0.01$) [Fig. 1: upper panel].

When comparisons between training and testing were made for each group, correlated *t*-tests on the avoidance responses of each group showed that the control fish learned avoidance responses during training and showed significant increases in avoidance responses during testing ($p < 0.01$), the EtOH group also learned avoidance responses during training and showed significant increases in avoidance responses during testing ($p < 0.05$), while zebrafish exposed to MeHg showed no significant increases in avoidance responses from training to testing [Fig. 1: lower panel].

The crossing latency results showed the similar pattern [Fig. 2]. A two-way ANOVA with one between factor (5 groups) and one repeated measure (2 sessions) on the crossing latency indicated a significant group difference [$F(4, 80) = 25.363$, $p < 0.01$], and a significant session difference [$F(1, 80) = 20.387$, $p < 0.01$]. A one-way ANOVA with multiple comparisons on the crossing latency of groups during the training session showed that only the 0.1 μM MeHg group was significantly different from the vehicle control group [$F(4, 80) = 19.579$, $p < 0.01$], while another one-way ANOVA with multiple comparisons on the crossing latency of groups during the testing session showed significant differences between the vehicle control and MeHg groups in a dose-dependent manner [$F(4, 80) = 22.448$, $p < 0.01$]. There were no significant differences between the control group and the vehicle group. However, compared with the vehicle control EtOH group, the 0.01 μM MeHg group showed longer crossing latency, the 0.03 μM MeHg group showed significantly longer crossing latency ($p < 0.05$), and the 0.1 μM MeHg group showed the longest crossing latency ($p < 0.01$) [Fig. 2: upper panel].

When comparisons between training and testing were made for each group, correlated *t*-tests on the crossing latency of each group showed that the control and EtOH groups learned avoidance responses during training and showed significantly shortened crossing latency during testing ($p < 0.05$), while zebrafish exposed

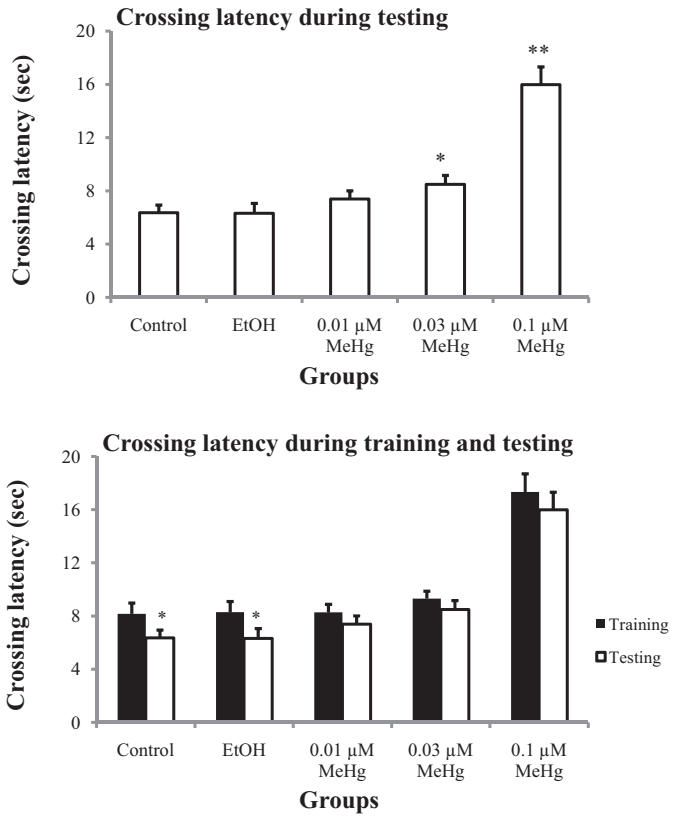

Fig. 2. Crossing latency of older adult zebrafish exposed to no MeHg or various levels of MeHg. Each bar represents the mean percentage of crossing latency ±SE for 13–21 fish. Upper panel shows crossing latency during testing. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the vehicle control group. Lower panel shows crossing latency during both training and testing. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the training session of the same group.

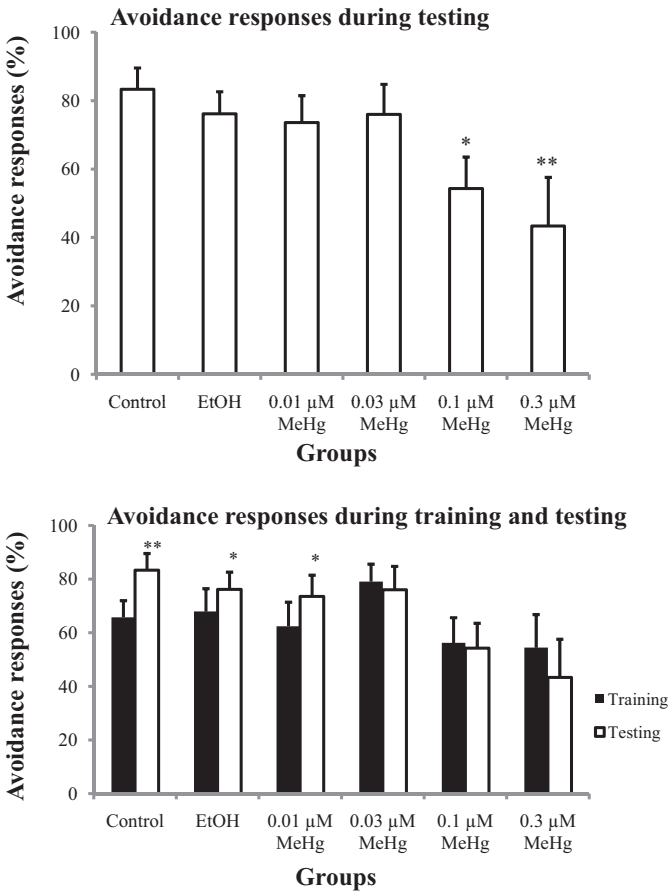

Fig. 3. Avoidance responses of younger adult zebrafish exposed to no MeHg or various levels of MeHg. Each bar represents the mean percentage of avoidance responses ±SE for 9–15 fish. Upper panel shows avoidance responses during testing. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the vehicle control group. Lower panel shows avoidance responses during both training and testing. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the training session of the same group.

to MeHg showed no significant changes in the crossing latency from training to testing [Fig. 2: lower panel].

There were no significant differences in escape responses during training among the five groups of 2-year-old zebrafish, although the 0.03 and 0.1 μM MeHg groups showed slightly more escape responses than other groups during training. Thus, 2-year-old zebrafish exposed to the levels of MeHg used in the study were able to perceive the shock and swim cross the divider to the dark side to escape the shock.

3.2. Results of 1-year-old zebrafish in Experiment 2

Fig. 3 shows avoidance responses of the six groups of 1-year-old zebrafish. A two-way ANOVA with one between factor (6 groups) and one repeated measure (2 sessions) on the avoidance responses indicated only the group × session interaction close to a significant level [$F(5, 68) = 2.009$, $p = 0.09$]. A one-way ANOVA with multiple comparisons on the avoidance responses of the groups during the training session showed no significant differences among groups, while another one-way ANOVA with multiple comparisons on the avoidance responses of the groups during the testing session showed significant differences between the vehicle control and MeHg groups in a dose-dependent manner [$F(5, 68) = 2.534$, $p < 0.05$]. There were no significant differences between the control group and the vehicle control group. However, compared with the vehicle control EtOH group, the 0.1 μM MeHg group showed significantly lower avoidance responses ($p < 0.05$), and the 0.3 μM MeHg group showed the lowest avoidance responses ($p < 0.01$) [Fig. 3: upper panel].

When comparisons between training and testing were made for each group, correlated *t*-tests on the avoidance responses of each group showed that the control group learned avoidance responses during training and showed significant increases in avoidance responses during testing ($p < 0.01$); the EtOH and the 0.01 μM groups also learned avoidance responses during training and showed significant increases in avoidance responses during testing ($p < 0.05$); while the 0.03 μM, 0.1 μM, and 0.3 μM MeHg groups showed no significant increases in avoidance responses from training to testing [Fig. 3: lower panel].

The crossing latency appeared to be a more sensitive indicator of learning [Fig. 4]. A two-way ANOVA with one between factor (6 groups) and one repeated measure (2 sessions) on the crossing latency indicated a significant group difference [$F(5, 68) = 2.488$, $p < 0.05$]. A one-way ANOVA with multiple comparisons on the crossing latency of the groups during the training session showed no significant differences among groups, while another one-way ANOVA with multiple comparisons on the crossing latency of the groups during the testing session showed significant differences between the vehicle control and MeHg groups in a dose-dependent manner [$F(5, 68) = 3.634$, $p < 0.01$]. There were no significant differences between the control group and the vehicle control group. However, compared with the vehicle control EtOH group, the 0.1 μM MeHg group showed significantly longer crossing latency ($p < 0.05$), and the 0.3 μM MeHg group showed the longest crossing latency ($p < 0.01$) [Fig. 4: upper panel].

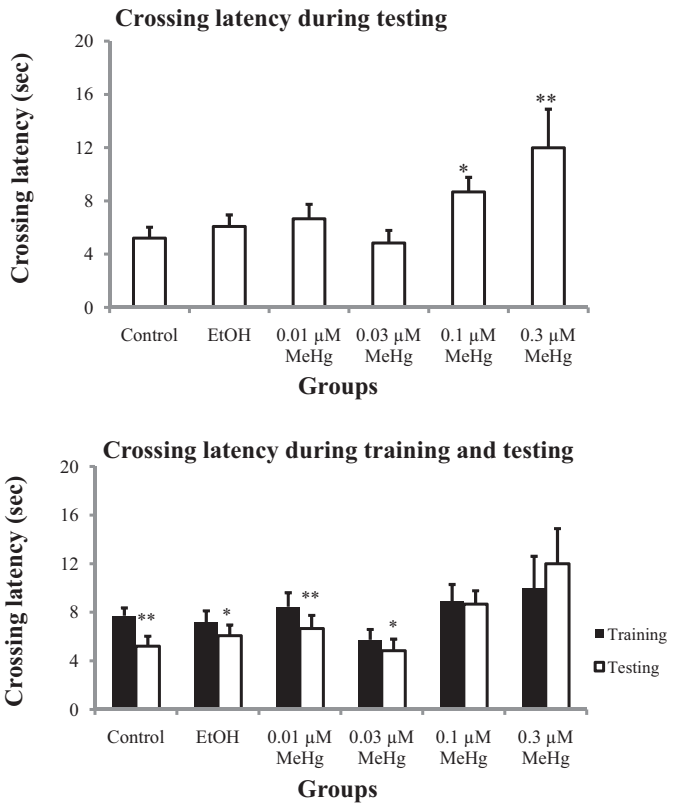

Fig. 4. Crossing latency of younger adult zebrafish exposed to no MeHg or various levels of MeHg. Each bar represents the mean percentage of crossing latency ±SE during testing for 9–15 fish. Upper panel shows crossing latency during testing. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the vehicle control group. Lower panel shows crossing latency during both training and testing. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with the training session of the same group.

When comparisons between training and testing were made for each group, correlated *t*-tests on the crossing latency of each group showed that the control and 0.01 μM MeHg groups learned avoidance responses during training and showed significantly shortened crossing latency during testing ($p < 0.01$); the EtOH and the 0.03 μM MeHg groups also showed significantly shortened crossing latency during testing ($p < 0.05$); while the 0.1 μM and 0.3 μM MeHg groups showed no significant changes in the crossing latency from training to testing [Fig. 4: lower panel].

There were no significant differences in escape responses during training among the six groups of 1-year-old zebrafish. Thus, 1-year-old zebrafish exposed to the levels of MeHg used in the current study were able to perceive the shock and swim cross the divider to the dark side to escape the shock.

4. Discussion

The results of Experiment 1 showed that older adult zebrafish exposed to no MeHg or EtOH learned and retained avoidance responses, while 0.01 μM or higher concentrations of MeHg exposure impaired avoidance learning in a dose-dependent manner with 0.3 μM of MeHg exposure producing death before the avoidance training started. The results of Experiment 2 showed that younger adult zebrafish exposed to no MeHg, EtOH, or 0.01 μM of MeHg learned and retained avoidance responses, while 0.1 or 0.3 μM of MeHg exposure impaired avoidance learning in a dose-dependent manner. Thus, the present study showed that MeHg exposure produced learning impairments at a much lower concentration of MeHg exposure and more severely in older adult compared against younger adult zebrafish.

The levels of MeHg exposures from 0.01, 0.03, 0.1 to 0.3 μM used in older zebrafish all produced impaired learning with 0.03 and 0.1 μM produced significant impairments and 0.3 μM produced death either during the MeHg exposure period or shortly after the MeHg exposure but before the avoidance training started. The two control groups learned to associate the CS of the light with the US of body shock during training and displayed their learning through increased avoidance responses and shortened crossing latency during testing. The 0.01 and 0.03 μM MeHg groups displayed slightly, but not significantly, increased avoidance responses from training to testing. The two groups also display slightly, but not significantly, shortened crossing latency from training and testing. The 0.1 μM MeHg group displayed significantly lower avoidance responses and longer crossing latency during training, and showed no significant changes in either avoidance responses or crossing latency from training to testing. This group of zebrafish displayed a slightly higher level of escape responses during training compared with the control and EtOH groups, indicating that this group of zebrafish were able to perceive the body shock and the opening under the divider, and were able to swim cross the divider to the dark side to escape the body shock. However, this group of zebrafish was sometimes lethargic and observed to swim upside down and backwards or floated on their sides in their home tanks and during the avoidance conditioning. Those behavioral deficits were not seen in younger zebrafish in the study. Thus, the 0.1 μM MeHg exposure also produced more generally and profoundly behavioral deficits in older zebrafish.

Among the levels of MeHg exposures from 0.01, 0.03, 0.1 to 0.3 μM used in younger zebrafish, only 0.1 and 0.3 μM MeHg produced impaired learning with three of the twelve 0.3 μM MeHg fish found died before the avoidance training started. The control groups and 0.01 μM MeHg group learned avoidance responses as showed by their increased avoidance responses and shortened crossing latency during testing. While the 0.03 μM MeHg group in Experiment 2 showed no significant changes in avoidance responses from training to testing, the group showed significantly shortened crossing latency from training to testing. The 0.03 μM MeHg group displayed much higher avoidance responses during training. It may not be possible for them to display further increases in avoidance responses during testing due to the ceiling effect. Thus, the group may have learned to associate the light with body shock and displayed learning through significantly shortened crossing latency during testing.

Learning and memory are sometimes inseparable, and both are reflected in improved performances. One cannot say that learning occurred unless the learner remembers what was learned. Nothing can be remembered unless it was learned in the first place. Therefore, any improved performance during testing over the prior training session reflects both learning and memory. Thus, zebrafish that showed significant increases in avoidance responses during testing learned and retained avoidance responses. However, a lack of improved performances during testing over the prior training session may be due to impaired learning or impaired memory (Xu, 2002; Xu et al., 2003, 2009). To determine whether a lack of improved performances is due to impaired learning or impaired memory, the same experimental treatment (such as, MeHg exposure) is often given to one group of animals before training and another group of animals immediately following training (Xu, 2002). If the experimental treatment produces a lack of improved performances when given only before but not after training, then the experimental treatment impairs learning but not memory. If the experimental treatment produces the same lack of improved performances when given either before or after training, then the experimental treatment impairs memory but not learning (Xu, 2002; Xu et al., 2003, 2009). However, the 2-week MeHg exposures used in the current study is

not useful in investigating its post-training effects, because the slow exposure process may parallel with memory decay over time and thus confounds the testing results. The training session is when learning occurs, but whether the results of the training session show the learning may be debatable (Xu, 1997). Nevertheless, when the results of the 30 trials during the training session were grouped into six blocks of five trials, both older and younger adult zebrafish exposed to MeHg did not show any increases in avoidance responses from the block of the first five trials to the block of the last five trials, providing no evidence of learning. Therefore, the lack of increased avoidance responses or shortened crossing latency during testing over the prior training session produced by MeHg exposure was most likely due to impaired learning.

Studies have showed that short-term exposure to high doses of MeHg or chronic mercury exposure produces sensory and motor deficits, including impaired color vision and general visual acuity (Barboni et al., 2009; Feitosa-Santana et al., 2010; Heath et al., 2010; Neghab et al., 2012). Thus, MeHg exposure in the present study might impair learning process specifically or by impairing sensory or motor processes that are necessary for learning to occur. That is, MeHg exposure might impair the perception of the CS of the light or the US of the body shock or might impair the motor coordination of swimming cross the divider as opposed to the impairment of learning process specifically. Both older and younger zebrafish exposed to MeHg showed the levels of escape responses during training similar to those of their control groups during training, indicating that zebrafish exposed to MeHg were able to perceive the body shock and the opening under the divider, and were able to swim cross the divider to the dark side to escape the shock. Even the 0.1 μM MeHg group of older zebrafish that showed more general and profound behavioral deficits was able to perceive the body shock and the opening under the divider, and was able to swim through the opening under the divider to escape the body shock as indicated by their higher level of escape responses during training. Furthermore, visual deficits produced by MeHg exposure tend to occur following chronic mercury exposure, and include color vision impairments and diminished visual acuity (Barboni et al., 2009; Feitosa-Santana et al., 2010; Neghab et al., 2012). In the present study, the 2-week MeHg exposure was short-term and a bright light was used as the CS. It is unlikely that the MeHg exposure in the present study produced visual deficits that caused zebrafish unable to see the CS of the bright light as MeHg zebrafish were able to perceive the opening under the divider to swim through to escape the body shock. Thus, MeHg exposures in the current study did not impair the sensory or motor processes necessary for learning to occur. Therefore, MeHg exposure in the present study was most likely to impair learning process specifically.

While numerous papers have been published identifying adult effects on learning after developmental exposures to MeHg, others have focused on adult-only exposures either through diet or occupation (e.g., Bourdineaud et al., 2008; Carvalho et al., 2007; Yokoo et al., 2003; Dolbec et al., 2000; Satoh, 2000; Lebel et al., 1998). Most of these studies have, however, differentiated between learning outcomes due to adult-only exposures and adult effects of gestational, lactational, or lifetime exposures. The majority of those studies that did make such a distinction involved occupational exposures to elemental mercury and not MeHg, e.g., dental workers, chemical production workers or gold miners (Li et al., 2011; Hilt et al., 2009; Powell, 2000; Ritchie et al., 1995; Smith et al., 1983). None, however, of those reports compared learning outcomes due to MeHg exposures at either early-mid or late adult stages. The importance of such comparisons revolves around findings that as one ages, neurodegeneration increases and the ability to protect against neural damage decreases, which results in greater potential sensitivity to environmental contaminants (Spencer et al., 2000). The present experiments, therefore, were designed to provide insights into the interaction between the adult aging process and the intensity of age-specific learning effects of short-term, adult-only MeHg exposure; it is to the best of our knowledge, the first such study. While these data did not directly compare age-specific differences in brain structure as a result of short-term, adult exposures to MeHg, they do suggest that further research into the interaction between the cellular and organismal changes that occur during the aging process and the increased susceptibility to environmental contaminants in human and wildlife populations needs to be investigated.

The present study used zebrafish because of its short generation times, high number of eggs per female, and ease of breeding. Our previous studies utilized zebrafish in studying the neurobehavioral effects of embryonic MeHg exposure (Smith et al., 2010; Xu et al., 2012). The present study used zebrafish to investigate the age-dependent neurobehavioral effects of adult MeHg exposure. Our ongoing studies have utilized zebrafish to explore the transgenerational heritability of the neurobehavioral effects of embryonic MeHg exposure. Zebrafish are also useful in examining whether short-term MeHg exposure during juvenile stage or early-adult stage or middle age produces a life-long effect. Thus, due to its short generation times, high number of eggs per female, and ease of breeding, zebrafish has become a useful organism for studying the neurobehavioral effects of environmental contaminants.

5. Conclusion

MeHg exposure produced learning impairments at a much lower concentration of MeHg exposure and more severely in old adult compared to young adult zebrafish.

Conflicts of interest

None.

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