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DEVELOPMENTAL LEAD EXPOSURE CAUSES STARTLE RESPONSE DEFICITS IN ZEBRAFISH

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Abstract

Lead (Pb^{2+}) exposure continues to be an important concern for fish populations. Research is required to assess the long-term behavioral effects of low-level concentrations of Pb^{2+} and the physiological mechanisms that control those behaviors. Newly fertilized zebrafish embryos (<2 hours post fertilization; hpf) were exposed to one of three concentrations of lead (as $PbCl_2$): 0, 10, or 30 nM until 24 hpf. 1) *Response to a mechanosensory stimulus*. Individual larvae (168 hpf) were tested for response to a directional, mechanical stimulus. The tap frequency was adjusted to either 1 or 4 taps/sec. Startle response was recorded at 1000 fps. Larvae responded in a concentration-dependent pattern for latency to reaction, maximum turn velocity, time to reach V_{max} and escape time. With increasing exposure concentrations, a larger number of larvae failed to respond to even the initial tap and, for those that did respond, ceased responding earlier than control larvae. These differences were more pronounced at a frequency of 4 taps/sec. 2) *Response to a visual stimulus*. Fish, exposed as embryos (2–24 hpf) to Pb^{2+} (0–10 μM) were tested as adults under low light conditions ($\sim 60 \mu W/m^2$) for visual responses to a rotating black bar. Visual responses were significantly degraded at Pb^{2+} concentrations of 30 nM. These data suggest that zebrafish are viable models for short- and long-term sensorimotor deficits induced by acute, low-level developmental Pb^{2+} exposures.

Keywords

behavior; lead; mechanosensory; startle response; visual; zebrafish

1. INTRODUCTION

Lead (Pb^{2+}) exposure continues to be an important concern for fish populations (Mueller et al. 2011; Ebrahimi and Taherianfard 2010; Buekers et al. 2009; Hinck et al. 2006; Schmitt et

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al. 2006). While Pb^{2+} has been shown to be an endocrine disruptor (Iavicoli et al. 2009; Levesque et al. 2003; Weber 1993), it is most known for its effects on neuronal function and neurodevelopment (Neal et al. 2011; Reddy et al. 2007; Costa et al. 2004; Oberto 1996). Numerous studies have identified mechanisms of Pb^{2+} neurotoxicity, especially the long-term outcomes due to gestational exposures in mammals (Basha and Reddy 2010; Dearth et al. 2004, 2002; Yang et al. 2003; Devoto et al. 2001). Less research has been conducted to identify critical periods of development that are particularly sensitive to Pb^{2+} and whose toxic effects are observed at later time points in life (Bunn et al. 2001). Among the long-term changes identified to date are learning deficits (Sun et al. 2005; Carvan et al. 2004) and altered responses to environmental stimuli (Moreira et al. 2001; Morgan et al. 2001). This study was designed to focus on the effects on locomotor function due to low-level, developmental Pb^{2+} exposure.

Significant work has focused on Pb^{2+} -induced alterations in learning and memory in a variety of vertebrate species (e.g., Carvan et al. 2004 [zebrafish]; Kuhlmann et al. 1997; Cory-Slechta 1995a, b [rats]; Stickler-Shaw and Taylor 1991 [frogs]). Pb^{2+} toxicity in fish also manifests itself through changes in locomotor activity patterns (Weber and Spieler 1994) and sensorimotor responses (Weber et al. 1997). A subset of such studies exists for studies on vertebrate species, specifically rats, analyzing the effects of low-level developmental Pb^{2+} on these particular behaviors (Leasure et al. 2008; Dietrich et al. 1985; Reiter et al. 1975); no studies examine the effect of very low-level Pb^{2+} exposure when isolated only to the ontogenetic periods in which neural network development occurs.

To fill the gap in our knowledge about the effects of low-level, developmental Pb^{2+} exposures in fishes, this study used larval zebrafish to assess changes in specific components of the startle response, the reaction to a directional stimulus, a behavior that is one of the first to be observed in newly hatched fishes. Fish embryos are particularly sensitive to environmental contaminants, especially within the first 24 hours post fertilization (Lahnsteiner 2008). In that regard, the following questions were asked: Do acute, very low-level developmental Pb^{2+} exposures cause sensorimotor deficits? If so, are the resulting behavioral phenotypes concentration-dependent even at these low exposure levels? If that is also true, do those effects extend to later life-history stages? Are the observed effects more pronounced in specific aspects of the startle response and escape swim? Answers to these questions will provide a foundation for examinations into how Pb^{2+} differentially alters neurodevelopment and behavioral outcomes.

2. MATERIALS AND METHODS

2.1 Treatment of Glassware and Plasticware

All laboratory materials made of plastic were washed thoroughly in a 10% solution of a nontoxic, biodegradable detergent (Simple Green™; Sunshine Makers, Inc., Huntington Harbour, CA), rinsed repeatedly in ultra-pure Milli-Q™ water (Millipore Corp., Medford, MA), and immersed in a 30mM Na_4EDTA (Fisher Scientific, Hanover Park, IL) solution overnight to remove all surface adsorbed metal ions; glassware was washed and rinsed similarly but immersed in a 10% HNO_3 (Fisher Scientific, Hanover Park, IL) solution overnight. Glass and plasticware were then rinsed in ultra-pure Milli-Q™ water.

2.2 Breeding and Egg Collection

Adult zebrafish from a wild-type laboratory strain originally acquired as aquarium fish from Ekkwill Waterlife Resources (Gibson, FL) were used. Fish were maintained at 26–28°C on a 14-hour light and 10-hour dark cycle in a flow-through, 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 hours post fertilization (hpf), counted, and placed into metal-free, glass culture dishes (100 mm diameter $\times$ 50 mm depth; N = 100 eggs/dish) containing an embryo development medium (each liter contains 0.875 g NaCl, 0.038 g KCl, 0.120 g MgSO₄, 0.021 g KH₂PO₄, and 0.006 g Na₂HPO₄)

2.3 Exposure Regimen

A stock solution of 1 mM PbCl₂ was prepared at pH 2 until fully dissolved. Using 1.0 M KOH, pH was adjusted to 6.5. Eggs were rinsed twice in Pb²⁺-free medium (as determined by ICP-MS analysis) and transferred (N = 100 eggs/dish; <2 hpf) to a metal-free glass dish (100 mm diameter $\times$ 50 mm depth) containing 100 ml of medium with Pb²⁺ at 0, 10 or 30 nM. At 24 hpf, eggs were rinsed three times with Pb²⁺-free medium and placed in clean, metal-free glass dishes with Pb²⁺-free medium until tested. Visual response experiments used concentrations that extended the range of exposure levels to include 3 and 100 nM Pb²⁺.

2.4 Mechanosensory Response

Responses to directional stimuli are observed in fish larvae and, therefore, are a useful tool to assess neurotoxicity. Details for this behavioral assay are described in Weber (2006). Briefly, larvae (n = 10/exposure regimen/tap frequency; 7 days post fertilization [dpf]) were placed individually into a white plastic chamber (15 mm diam., 3 mm deep). The chamber was lit using a fiber optic cable (Solarc, Model LB-24; Welch Allyn, Inc., Skaneateles Falls, NY, USA) and was the only source of light in the room. A small fan forced air over the experimental apparatus to prevent temperature changes that would alter responses. After a 3-minute acclimation period, a solenoid was remotely activated to control for intensity and tap frequency (either 1 or 4 taps/sec, i.e., one stimulus every 1000 or 250 msec, respectively, with each tap lasting approximately 4 msec). The tap intensity used for these experiments was the lowest at which $>90\%$ of all control larvae responded. Larval motion was recorded at 1000 fps using a PCI1024 high-speed CCD video camera (Motion Engineering, Inc., Indianapolis, IN, USA). The following four measures were recorded: latency of reaction (elapsed time interval in msec between moment solenoid arm taps the chamber pin and the initiation of the startle response), $V_{\max}$ (maximum turn velocity in body lengths [BL]/sec), time to reach $V_{\max}$ (msec) and duration of escape swim (total time elapsed in msec from beginning of the startle response to the end of the escape swim, i.e., cessation of caudal tail movement).

Using FLOTE version 2.0 (Burgess et al. 2009), traces of the larval swim pattern were created for the 4 tap/sec trials to assess potential changes in locomotor activity as a function of developmental Pb²⁺ exposure in addition to calculating latency of reaction, $V_{\max}$, and duration of escape swim.

2.5 Visual Response

The behavioral apparatus follows previously published accounts (Weber et al. 2008; Li and Dowling 1997) as modified for testing adults and to allow for different drum speeds and directions. Briefly, individual fish grouped by exposure concentration (n = 12; 4 months old, 1.5–2.0 cm standard length) were placed into one of four opaque compartments (gray PVC bottom and white plastic walls) within a glass container. This design allowed each fish to view and react to a rotating black bar (see below) without behavioral influence from a neighboring fish. The glass container (10 cm diam., 5 cm depth) was suspended by brackets over a rotating drum of white PVC plastic (diam. 11 cm, depth 4 cm). On the drum, black

electrical tape (1 cm width $\times$ 5 cm height) was used to create a mark for eliciting a startle response when it comes into the fish's field of vision. Zebrafish adults typically react to the rotating black bar by either eliciting a C-start escape response or an avoidance maneuver. After a 5-minute low light acclimation time, the number of C-start reactions to the leading edge was recorded over a 5-minute period. Since there is a circadian rhythm to light sensitivity (Li and Dowling 1998), all experiments were performed from 1300–1500 h each day.

Zebrafish are able to see well under low light conditions. To assess potential visual deficits, very low light conditions ($\sim 60 \mu\text{W}/\text{m}^2$) were created using a white light diode attached to a 500 μm optical fiber distribution bundle inserted through a PVC collar. This assured even distribution of equal spectral integrity throughout the chamber. A visible wavelength photodiode suspended in the observation chamber was connected to a digital meter (Micronta® 22, Radio Shack, Ft. Worth, TX) which also manipulated light intensity. To allow the observer to monitor fish behaviors under these low light levels, a ring of infrared (IR) LEDs (960 nm) was also inserted into the same PVC collar. Fish do not see in IR wavelengths (Nawrocki et al. 1985). The IR intensity could also be manipulated remotely. To allow for observer monitoring, an IR-sensitive CCD camera (Sony Hyper HAD B&W Video Camera) recorded images that were stored on a remote computer to facilitate observations without disturbing the test fish. The drum speed was set at 10 rev/min. Data were analyzed for total number of reactions/5 min using Pinnacle Studio Quick Start version 9.1 (Brothersoft.com) software.

2.6 Statistical Analyses

After video images from larval responses to the mechanosensory stimuli were analyzed using FLOTE v2.0 (Burgess et al. 2009), data were subjected to repeated measures ANOVA to compare responses at each stimulus within each trial (within subject factor = stimulus time; measures = latency of reaction, V_{max} , time to reach V_{max} and duration of escape swim; between subject factor = Pb^{2+} concentration). Repeated measures tests required no missing cells in the data spreadsheet. Therefore, non-responses were treated in the following manner: since the solenoid arm retracted at approx. 25 msec and occasionally induced a larval response, non-responders were given a value of 25 msec for both latency and time to reach V_{max} . Capping these variables at a specific value effectively raised the threshold for showing statistical significance. Since a non-responder did not move, those cells in the data spreadsheet for V_{max} (BL/sec) and duration of escape swim (msec) were given the value of "0", i.e., no movement. Bonferroni intervals were used for multiple comparison tests over each stimulus time for the effect of exposure concentrations. Fisher's Exact Test (2×3 contingency table; responders vs. non-responders $\times$ treatment group) was used to determine significance of exposure regimen effects at each stimulus time per trial. Level of significance for all statistical analyses was set at $\alpha = 0.05$.

Data for the visual response data and ICP-MS analyses were subjected to a one-way ANOVA for effect of Pb^{2+} concentration. Level of significance was set at $\alpha = 0.05$.

All statistical analyses and data presentations were accomplished using SPSS 13 software (IBM Corporation, Somers, NY).

3. RESULTS

3.1 Pb^{2+} Uptake

The uptake of Pb^{2+} by zebrafish embryos during the first 24 hpf was directly dependent upon the exposure concentrations, i.e., higher concentrations of Pb^{2+} in the exposure medium corresponded to greater Pb^{2+} in the 24 h-old embryo (Fig. 1; ANOVA, $P < 0.01$,

$F_{9,97}$, $df = 2$; Pearson's coefficient of correlation, $r^2 = 0.836$). Each exposure concentration was significantly different in the level of Pb^{2+} in the embryos ($P < 0.05$). There was no detectable Pb^{2+} in the larvae used for these tests (data not shown).

3.2 Mechanosensory Stimulus Response

3.2.1 Stimulus Frequency of 1 tap/sec—All control larvae ($n = 10$) responded to the stimulus at both the initial tap and the second tap 1000 msec later (Fig. 2). While fewer larvae developmentally exposed to 10 nM Pb^{2+} responded to the initial stimulus ($n = 9$), that number did not change over time. However, larvae from the 30 nM Pb^{2+} group demonstrated a large drop in the number responding to the second stimulus ($n = 8$ vs. 2, respectively; Fisher's Exact Test, $P < 0.0001$).

Mean response latency: Analyses over time using repeated measures ANOVA to account for multiple stimuli/trial resulted in significant differences as a function of Pb^{2+} concentration ($P < 0.05$; Fig. 3A) with longer reaction times to the second stimulus in larvae developmentally exposed to 30 nM Pb^{2+} vs. either of the other two exposure groups.

Mean time to reach V_{max} : Analyses over time using repeated measures ANOVA to account for changes over recurring stimuli, however, resulted in highly significant differences as a function of Pb^{2+} concentration ($P < 0.001$; Fig. 3B); time to reach V_{max} increased at a larger rate with the second stimulus in larvae developmentally exposed to 30 nM Pb^{2+} than larvae from either the control or 10 nM Pb^{2+} groups.

Mean V_{max} : Analyses over time using repeated measures ANOVA to account for changes with each stimulus in the trial resulted in significant differences as a function of Pb^{2+} concentration ($P < 0.01$; Fig. 3C) with V_{max} decreasing more with the second stimulus in larvae developmentally exposed to 30 nM Pb^{2+} vs. either of the other two exposure groups. In fact, the other two groups demonstrated no significant change between the first and second stimulus.

Mean duration of the startle and escape response: Differences at specific times of stimulation over the course of the 2-second trial were apparent (repeated measures ANOVA, $P < 0.05$; Fig. 3D) with total response time decreasing at a larger rate with the second stimulus in larvae developmentally exposed to 30 nM Pb^{2+} vs. either of the other two exposure groups.

3.2.2 Stimulus Frequency of 4 taps/sec—At all but one time points during each trial, $\geq 60\%$ of control larvae responded to each stimulus (Fig. 4A). Decreased responsiveness relative to control larvae with the initial stimulus followed by further depressed responses was observed in larvae developmentally exposed to either concentration of Pb^{2+} (Figs. 4B, C; Fisher's Exact Test, $P < 0.05$). Whereas control larvae tend to swim around the periphery of the startle test chamber, there was a progressive, concentration-dependent disruption of that pattern with Pb^{2+} exposure (Fig. 5). While larvae usually began each session at or near the chamber edge, there was less activity at the edge and a larger amount of movement along the chamber's diameter, especially for the larvae exposed to 30 nM Pb^{2+} .

Mean response latency: Analyses over time using repeated measures ANOVA demonstrate that while 10 and 30 nM Pb^{2+} are not significantly different, they were significantly slower to respond to stimuli than control larvae at some time points (Bonferroni multiple comparisons test, $P < 0.005$; Fig. 6A).

Mean time to reach V_{max} : Using repeated measures ANOVA resulted in significant differences at some time points only between the 0 and 30 nM Pb^{2+} groups (Bonferroni multiple comparisons test, $P < 0.05$; Fig. 6B), i.e., there no significant differences between 0 and 10 nM Pb^{2+} groups.

Mean V_{max} : Analyses over time using repeated measures ANOVA resulted in significant differences between control and either Pb^{2+} exposure group at specific time points (Bonferroni multiple comparisons test, $P < 0.05$; Fig. 6C).

Mean duration of the startle and escape response: Differences at specific times of stimulation over the course of the 2-second trial were apparent (repeated measures ANOVA, $P < 0.05$) There were no significant differences between either Pb^{2+} exposure concentration at any time point (Bonferroni multiple comparisons test, $P < 0.05$; Fig. 6D).

3.3 Visual Startle Response

At very low levels of developmental Pb^{2+} (3 nM) exposure, there was a significant reduction in reaction to the rotating black bar (one-way ANOVA, $P < 0.001$; Student-Newman-Keuls multiple comparison test, $P < 0.05$; Fig. 7).

4. DISCUSSION

Developmental Pb^{2+} exposures caused changes in larval responses to a tapping stimulus. While the time required to initiate a response increased, maximum head turn velocity and duration of the escape swim decreased with increasing Pb^{2+} concentration. In addition, the number of larvae who failed to respond to a stimulus increased with Pb^{2+} concentration. These changes in behavioral response became more pronounced when the stimulus frequency increased from 1 to 4 taps/sec. Decreases in adult visual startle responses also demonstrated a concentration-dependent change.

The simple neural network of the startle response followed by an escape swim allows for a first-step examination of the relationship between Pb^{2+} neurotoxicity and behavioral outcomes. This study used two sensorimotor networks in a zebrafish, mechanosensory and visual, to assess the effect of very low-level, developmental Pb^{2+} exposure on larval startle responses. Exposure regimens were within the period in which embryonic motor circuits form (Thomas et al. 2009).

The Pb^{2+} levels used in this study are three orders of magnitude lower than that reported in very low-level Pb^{2+} exposure mammalian studies that, in some studies, extended beyond gestation into early postnatal stages (e.g., Fortune and Lurie 2009). Metal cation uptake in fish eggs often becomes bound in the perivitelline fluid of the zona radiata once the egg has undergone water hardening after 24 hpf (Rombough 1985). Since only 50% of the Pb^{2+} during waterborne exposures actually enters the embryo due to the binding capacity of the perivitelline fluid (Zhang et al. in press), we actually observed behavioral effects at embryo Pb^{2+} concentrations that were at or below detection by ICP-MS.

Several research groups have tracked the swim pattern of fish larvae exposed to a range of organic chemicals (Irons et al. 2010 [neuroactive drugs]; Jin et al. 2010 [bifenthrin]; Powers et al. 2010 [Ag^+]; Winter et al. 2008 [pharmaceuticals]; Levin et al. 2004 [chlorpyrifos]). These experiments, however, used video recording speeds that were sufficient only for tracking general locomotor activity without external physical stressors that would initiate a startle response. This paper describes results based on high-speed videography to view the series of fast movements that constitute the startle and escape behaviors in response to repeated directional stimuli. This study examined the effects of a chemical stressor, Pb^{2+} , on

two forms of sensory perception (mechanosensory [lateral line system] and visual [retinal neurons]) and the startle response they trigger. The startle, or C-start, response represents the behavioral outcome of the integration of sensory neurons (sensory perception), Mauthner cells (information integration), interneurons and neuromuscular junctions (neuromuscular activation) in which there is a rapid movement away from the source of the stimulus, resulting in the body flexing in a C-shape before it straightens out to escape.

Our results cannot be specifically associated with any one aspect of the startle response neural pathway because these data do not differentiate between Pb^{2+} -induced impaired detection (sensory), altered integration (M-cell), impaired response (neuromuscular) or increased fatigue (respiratory/muscular). Still, these data are consistent with several possible mechanisms and it is likely that multiple sites of Pb^{2+} neurotoxicity exist. First, the lateral line system in fishes and the neuromasts that constitute the sensory cells of that system are highly sensitive to metal exposure (Froehlicher et al. 2009). One explanation for the delayed response evident in the Pb^{2+} -exposed larvae (Fig. 3A) is an increase in the sensory threshold of the lateral line neuromasts, as observed in frog and rat retinal cells exposed to Pb^{2+} (Fox et al. 2008; Fox et al. 1991; Fox and Sillman 1979). Second, depressed neuronal function also may have occurred in the descending interneurons that connect the M-cell to the lateral trunk muscles. At a stimulus frequency of 1 tap/sec, both the maximum head turn velocity (V_{max}) and time to reach V_{max} (Figs. 3B, C) was significantly different at the higher concentration level of 30 nM Pb^{2+} . These differences became more pronounced for all exposure concentrations at the higher stimulus frequency of 4 taps/sec (Figs. 6B, C). At this higher stimulus frequency, a progressive degeneration in escape swim activity was observed (Fig. 5). Rather than a majority of the swimming activity occurring at the chamber periphery, there was progressive, concentration-dependent switch to activity centrally located, if the larvae responded at all to the stimulus. Third, there are four major neurotransmitter and neurotransmitter receptor sites in the piscine M-cell: glutamate, glycine, γ -amino butyric acid (GABA) and serotonin (5-HT). These can be found in both the developing embryo and the adult fish (Patten and Ali 2007; Rigo and Legendre 2006; McLean and Fetcho 2004; Hatta et al. 2001; Triller et al. 1997; Diamond 1968) and all are affected by Pb^{2+} (Fortune and Lurie 2009; Xing et al. 2009; Fitsanakis and Aschner 2005; Spieler et al. 1995; Weber et al. 1991). Fourth, while no data exist for the effect of developmental Pb^{2+} on depolarization in fish neurons, it has been shown that post hatch exposures inhibit contraction of single muscle fibers and suppress fast and slow Ca^{2+} currents in crayfish (Zacharová et al. 1993). These observations correspond to the decrease in escape swim time duration (Figs. 3D, 6D) and the compressed track patterns in zebrafish larvae developmentally exposed to Pb^{2+} .

Visual stimuli, as used in this study, elicit a Mauthner cell-initiated startle response similar to that observed with mechanosensory stimulation (Eaton et al. 1977). In the experiments reported in this study, the ability to detect contrast (a black bar against a white background) is an important first step to initiate the visual startle response. Changes in the sensitivity to such contrasts and the resultant alterations in behavioral responses are a useful method to assess the effects of neurotoxicant exposures. A change of response suggests that the functionality of either the ON- or OFF-center receptive fields has been compromised. The optomotor responses to visual stimuli used in these experiments depend upon the sum of electrical signals transmitted from the circular receptive fields of the bipolar and amacrine cells to the ganglion and then through the optic nerve to the optic tectum. The formation of these retinal neurons occurs during the period of developmental Pb^{2+} exposure (0–24 hpf) used in this study (Schmitt and Dowling 1999, 1994) which is responsible for raising the threshold of activation in frog rod cells (Fox et al. 1991). Similarly, a change in sensory threshold may have occurred in the visual startle response test in adult zebrafish developmentally exposed to Pb^{2+} (Fig. 7) due, in part, to changes in sensory perception of

borders. Additional effects on information integration may be occurring at higher brain centers, since Pb^{2+} reduces dopamine levels in the rainbow trout optic tectum (Rademacher et al. 2001).

5. CONCLUSIONS

Low-level developmental Pb^{2+} exposures induce behavioral disruptions in mechanosensory startle responses of larval zebrafish. These altered behaviors manifest themselves as disruptions in ability to respond to a stimulus, as well as the time required for responding, maximum head turn velocity and duration of escape swim. Furthermore, the pattern of the escape swim is changed in larvae from Pb^{2+} -exposed embryos. Rather than swimming along the test chamber periphery, those larvae that do respond to the stimuli, spend most of their escape swim time along the diameter of the test chamber. In addition, these same exposure regimens have the capacity to cause long-term effects in startle behaviors, e.g., visual responses in adult zebrafish, even at very low developmental Pb^{2+} concentrations.

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Highlights

Low-level developmental Pb^{2+} exposures induce behavioral disruptions in mechanosensory startle responses of larval zebrafish. These altered behaviors manifest themselves as disruptions in ability to respond to a stimulus, as well as the time required for responding, maximum head turn velocity and duration of escape swim. Furthermore, the pattern of the escape swim is changed in larvae from Pb^{2+} -exposed embryos. Rather than swimming along the test chamber periphery, those larvae that do respond to the stimuli, spend most of their escape swim time along the diameter of the test chamber. In addition, these same exposure regimens have the capacity to cause long-term effects in startle behaviors, e.g., visual responses in adult zebrafish, even at very low developmental Pb^{2+} concentrations.

Figure 1.

Concentration-dependent uptake of Pb^{2+} in whole embryo during 2–24 hours post fertilization ($n = 100$ embryos/sample; 3 samples/concentration). Values = mean $\pm$ SE. * = $P < 0.05$; ** = $P < 0.01$

Figure 2.

Time- and concentration-dependent larval response to repeated stimuli at a frequency of 1 tap/sec. ■ = 0 nM Pb²⁺; □ = 10 nM Pb²⁺; ▒ = 30 nM Pb²⁺. Values = proportional number of larvae responding at each stimulus ± SD. Interval between each stimulus = 1000 msec. n = 10/exposure concentration. * = P < 0.0001 relative to control value at equivalent stimulus time.

Figure 3A:

Figure 3B:

Figure 3C:

Figure 3D:

Figure 3.

Responses to each stimulus at a frequency of 1 tap/sec. ■ = 0 nM Pb²⁺; □ = 10 nM Pb²⁺; ▒ = 30 nM Pb²⁺. **A.** Time (msec) to initial response (reaction latency); **B.** Time (msec) to reach maximum head turning velocity ($V_{\max}$); **C.** $V_{\max}$ (body lengths/sec); **D.** Duration (msec) of response (startle response + escape swim). Values = mean $\pm$ SE. Interval between each stimulus = 1000 msec. n = 10/exposure concentration. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ relative to control value at equivalent stimulus time.

Figure 4A:

Figure 4B:

Figure 4C:

Figure 4.

Time- and concentration-dependent larval response to repeated stimuli at a frequency of 4 taps/sec. **A.** ■ = 0 nM Pb²⁺; **B.** □ = 10 nM Pb²⁺; **C.** ▒ = 30 nM Pb²⁺. Values = proportional number of larvae responding at each tap stimulus ± SD. Interval between each stimulus = 250 msec. n = 10/exposure concentration. * = P < 0.05; ** = P < 0.01; *** = P < 0.005 relative to control value at equivalent stimulus time.

Figure 5.

Representative traces of movement of larvae developmentally exposed to 0, 10 or 30 nM Pb^{2+} in response to a directional stimulus at a frequency of 4 taps/sec for 2.25 seconds. Interval between each stimulus = 250 msec.

Figure 6A:

Figure 6B:

Figure 6C:

Figure 6D:

Figure 6.

Responses to each stimulus at a frequency of 4 taps/sec. ■ = 0 nM Pb^{2+} ; □ = 10 nM Pb^{2+} ; ▒ = 30 nM Pb^{2+} . **A.** Time (msec) to initial response (reaction latency); **B.** Time (msec) to reach maximum head turning velocity (V_{max}); **C.** V_{max} (body lengths/sec); **D.** Duration (msec) of response (startle response + escape swim). Values = mean $\pm$ SE. Interval between each stimulus = 250 msec. $n = 10$ /exposure concentration. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$ relative to control value at equivalent stimulus time.

Figure 7.

Effect of developmental Pb^{2+} exposure on the adult expression of visual responses to a rotating black bar (10 revolutions/min) under low light conditions ($N = 12$ /exposure concentration). Bars (mean $\pm$ SE) with different letters are statistically different from mean control response (ANOVA, $P < 0.001$, Student-Newman-Keuls post hoc test, $P < 0.05$). Means with the same letter are not statistically different.