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Developmental stress and lead (Pb): Effects of maternal separation and/or Pb on corticosterone, monoamines, and blood Pb in rats

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ABSTRACT

The level of lead (Pb) exposure in children has decreased dramatically since restrictions on its use were implemented. However, even with restrictions, children are exposed to Pb and still present with cognitive and behavioral deficits. One prominent aspect of the exposome of these children is that many come from low social economic status (SES) conditions, and low SES is associated with stress. In order to compare the combined effects of early stress and Pb, Sprague-Dawley rats were exposed to vehicle or Pb either alone or in combination with maternal separation stress during brain development (i.e., postnatal day (P)4–P11, P19, or P28). Maternally separated/isolated pups had lower body and thymus weights during exposure and had increased levels of blood Pb compared with vehicle controls. Isolation, but not Pb, affected the response to an acute stressor (standing in shallow water) when assessed on P19 and P29, but not earlier on P11. Interactions of Pb and isolation were found on monoamines in the neostriatum, hippocampus, and hypothalamus on turnover but not on levels, and most changes were on dopamine turnover. Isolation had greater short-term effects than Pb. Interactions were dependent on age, sex, and acute stress.

1. Introduction

Lead (Pb) continues to be an environmental health risk despite restrictions on its use. Children are especially susceptible to Pb exposure (Lidsky and Schneider, 2003), and this exposure is associated with altered cardiovascular function (Gump et al., 2005), encephalopathy (Patel and Athawale, 2009), genotoxicity (Mendez-Gomez et al., 2008), and cognitive and behavioral deficits, the latter including but not limited to decreased IQ (Chiodo et al., 2007; Needleman et al., 1979; Surkan et al., 2007; Wang et al., 1989; Winneke et al., 1985), altered language function (Yuan et al., 2006), social problems (Chiodo et al., 2007; Burns et al., 1999), and increased juvenile adjudication (Dietrich et al., 2001). Even levels of Pb below the Centers for Disease Control's 10 μg/dL level of concern (CDC, 2005) result in neuropsychological and IQ reductions (Neal and Guilarte, 2010; Bellinger, 2011). Because of the persistence of Pb in the environment, it continues to be found in children, albeit at low levels (Bellinger, 2008; Lanphear et al., 2000). However, these levels have not been modeled often in animals and represent a gap in understanding how such levels affect brain development.

Pb exposure also has effects on stress mechanisms (Gump et al., 2005, 2008). Interactions between Pb and stress have been reported in children (Tong et al., 2000) and in animals (Weston et al., 2014; Cory-Slechta et al., 2004, 2009, 2010, 2012, 2013a,b; Rossi-George et al., 2009, 2011; Virgolini et al., 2006, 2008a,b). The latter are from a laboratory primarily using a paradigm in which female Long-Evans rats are given Pb (50 or 150 ppm in drinking water) for 2 months prior to mating, throughout gestation, and up to weaning or through adulthood. Restraint stress in this model is to the dam and consists of three 45 min episodes given on two days of gestation: embryonic (E) days 15 and 16 (where evidence of mating is counted as E0) and may be characterized as an acute stress paradigm. This model, and its variations, show a number of

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interactions in which the effects of Pb are increased by the prenatal stress exposure. However, this model does not address the effects of chronic developmental stress.

Chronic stress can cause neurotoxicity (McEwen and Stellar, 1993; McEwen, 1998) and hypothalamic–pituitary–adrenal (HPA) axis dysfunction (Lupien et al., 1999; McEwen et al., 1992). In young children and rodents, the HPA axis passes through a stage of reduced responsiveness to stress (stress hyporesponsive period; SHRP). The SHRP is hypothesized to be a neuroprotective mechanism to prevent excitotoxicity when glucocorticoid receptors are developing (De Kloet et al., 1988; Sapolsky and Meaney, 1986). Stressors that exceed the buffering capacity of the SHRP result in adverse consequences (Anisman et al., 1998; Gos et al., 2008; Gruss et al., 2008).

A number of stressors may interfere with child developmental markers, one of which is low socioeconomic status (SES). Children in such settings face a variety of difficulties that may include impoverishment, family disruption, overcrowding, poor diet, threats, violence, and other challenges that result in elevated cortisol levels and other markers of stress (Gump et al., 2005; Lupien et al., 2000, 2001). These conditions can co-occur when children live in older housing that tend to have higher levels of Pb paint, dust, and or soil contamination (Goyer, 1996; Jacobs et al., 2002; Lanphear et al., 1998; Levin et al., 2008; Meyer et al., 2003; Muntner et al., 2005; Schnaas et al., 2004).

In the present study, we tested the interaction of Pb exposure in combination with the chronic stressor of maternal separation (pup isolation; ISO). We hypothesized that the combination would be more detrimental than either factor alone. ISO has been used in many studies. It consists of isolating pups from the dam and littermates during a period that spans the SHRP (Kosten and Kehoe, 2005). The ISO model alters stress markers (McCormick et al., 1998), brain monoamines (Kehoe et al., 1998; Kosten et al., 2004), fear conditioning, and DNA methylation (Kao et al., 2012). Here, offspring were separated on P4 for 4 h/day until the day prior to assessment on P11, P19, or P29. These days were chosen to match a previous experiment of similar design but using barren-cage stress instead of maternal separation (Graham et al., 2011). The P4–28 period of brain development in rats is approximately analogous to late gestation to early childhood in humans (Bayer et al., 1993; Clancy et al., 2007a,b). In combination with ISO, pups were treated every other day with 1 or 10 mg/kg of Pb acetate by gavage from P4–10, P4–18, or P4–28. The day after the last treatment, pups were challenged with an acute stressor or left undisturbed. Pb doses used here were designed to produce blood Pb (BPb) concentrations similar to those observed in humans (Lanphear et al., 2000; Bellinger, 2008). The effects of chronic stress and Pb were assessed on brain monoamines, organ weights associated with stress and immunity, and corticosterone. The ultimate purpose was to develop a combination model suitable for studies on long-term behavioral effects and test whether maternal separation induces effects similar to or different from barren cage rearing as used in our previous experiment (Graham et al., 2011).

2. Materials and methods

2.1. Animals

Male and nulliparous female Sprague-Dawley Crl:CD (IGS) rats (strain 001, Charles River Laboratories, Raleigh, NC) were acclimated for at least 1–4 weeks in the vivarium prior to breeding. The vivarium is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, and care was provided in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Animals were maintained on a 14:10 h light:dark cycle (lights on at 600 h) with controlled temperature ($19 \pm 1\,^{\circ}C$) and humidity ($50\% \pm 10\%$). To control exposure to other metals in the diet, animals were maintained on an NIH-07 diet throughout the experiment in which metal content was certified by assay to be below preset values (PMI Corp., Lab Diet Auto 5018). Food and water were given ad libitum. For breeding, a male and female were cohabitated until the presence of a sperm plug was detected (E0). On E1, females were transferred to polycarbonate cages ($46 \times 24 \times 20$ cm) containing woodchip bedding and housed singly. Day of birth was designated P0, and on P1 litters were culled to 12 pups (6 males and 6 females) using a random number table. If litters had less than 12 pups on P1, 1 or 2 pups from another litter born on the same day were added to attain a uniform litter size of 12; this occurred in 17 litters (17.7%) of the 96 litters in the experiment. Of the 17 litters that had pups added, 13 received 1 pup (13.5%) and 4 received 2 pups (4.2%). The experiment was approved by the Institutional Animal Care and Use Committee. Personnel conducting the study were blind to the group membership of the pups.

2.2. Pb exposure and rearing conditions

The experiment had five independent variables: 2 rearing conditions, 3 exposure groups, 3 test ages, 4 levels of the acute stressor, and 2 sexes. The rearing conditions were maternal separation or no maternal separation. The three exposure groups were 0, 1, or 10 mg/kg Pb acetate. The three ages were P11, 19, or 29. The four levels of the acute stressor were no Shallow Water Stress (SWS) and 0, 30, and 60 min after SWS. The two sexes were males and females. Hence, the study design was a $2 \times 3 \times 3 \times 4 \times 2$ design, the experiment had 144 cells, hence 96 litters $\times$ 12 offspring per litter = 1152 pups; 1152 pups divided by 144 cells resulted in 8 males and 8 females in each cell of the study stratified by litter. The dependent variables were body and organ weights, monoamines, and corticosterone.

Pb administration and ISO began on P4. Pb was given in a vehicle of 0.01 M anhydrous sodium acetate; VEH. Two male and female pairs per litter were gavaged with 0, 1, or 10 mg/kg Pb acetate (1Pb and 10Pb, respectively) in a volume of 3 mL/kg VEH as in our earlier experiment, and in that experiment we also showed that gavaging per se did not increase plasma corticosterone (Graham et al., 2011). Rats were gavaged every other day from P4 until P10, 18, or 28. Offspring assessed on P29 had their dams removed from the cage on P28 at which time the weanlings were housed by sex, 3 rats/cage (1 from each exposure group).

From P4 until assessment, ISO offspring were removed from their dam and isolated individually in a quiet room for 4 h in a small clean paper container with lid (14 cm height $\times$ 13 cm diameter at the bottom and 16 cm diameter at the top). Control animals remained with their dam throughout this period (Standard rearing: STD).

On P11, P19, or P29 separate subsets within each group were exposed to an acute stressor (shallow water stressor, SWS) or left undisturbed. The SWS animals were placed for 30 min in clean cages ($28 \times 16 \times 12$ cm polycarbonate cages) filled with room temperature water to a depth of 2 cm for P11, 3 cm for P19, and 4 cm for P29 animals. During the middle of the light cycle (approximately 1000–1400 h), one pup from each condition at each age was decapitated 0, 30, or 60 min after removal from SWS or directly upon removal from their home cage (baseline). Trunk blood was collected in 12×75 mm polyethylene tubes containing 0.05 mL of 2% EDTA for corticosterone assay. An additional blood sample was taken from animals on P29 for BPb analysis. Monoamine determinations were from rats not subjected to SWS. The neostriatum, hypothalamus, and hippocampus were dissected over ice using a brain block (Zivic-Miller, Pittsburgh, PA) as described (Grace et al., 2010). Spleen, thymus, and adrenals were