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Sex-Dependent Impacts of Low-Level Lead Exposure and Prenatal Stress on Impulsive Choice Behavior and Associated Biochemical and Neurochemical Manifestations

Hiromi I. Weston, Douglas D. Weston, Joshua L. Allen, and Deborah A. Cory-Slechta¹

Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, 14642 USA

Abstract

A prior study demonstrated increased overall response rates on a Fixed Interval (FI) schedule of reward in female offspring that had been subjected to maternal lead (Pb) exposure, prenatal stress (PS) and offspring stress challenge relative to control, prenatal stress alone, lead alone and lead + prenatal stress alone (Virgolini et al., 2008). Response rates on FI schedules have been shown to directly relate to measures of self-control (impulsivity) in children and in infants (Darcheville et al., 1992; 1993). The current study sought to determine whether enhanced effects of Pb ± PS would therefore be seen in a more direct measure of impulsive choice behavior, i.e., a delay discounting paradigm. Offspring of dams exposed to 0 or 50 ppm Pb acetate from 2–3 months prior to breeding through lactation, with or without immobilization restraint stress (PS) on gestational days 16 and 17, were trained on a delay discounting paradigm that offered a choice between a large reward (three 45 mg food pellets) after a long delay or a small reward (one 45 mg food pellet) after a short delay, with the long delay value increased from 0 sec to 30 sec across sessions. Alterations in extinction of this performance, and its subsequent re-acquisition after reinforcement delivery was reinstated were also examined. Brains of littermates of behaviorally-trained offspring were utilized to examine corresponding changes in monoamines and in levels of brain derived neurotrophic factor (BDNF), the serotonin transporter (SERT) and the N-methyl-D-aspartate receptor (NMDAR) 2A in brain regions associated with impulsive choice behavior. Results showed that Pb ± PS-induced changes in delay discounting occurred almost exclusively in males. In addition to increasing percent long delay responding at the indifference point (i.e., reduced impulsive choice behavior), Pb ± PS slowed acquisition of delayed discounting performance, and increased numbers of both failures to and latencies to initiate trials. Overall, the profile of these alterations were more consistent with impaired learning/behavioral flexibility and/or with enhanced sensitivity to the downshift in reward opportunities imposed by the transition from delay discounting training conditions to delay discounting choice response contingencies. Consistent with these behavioral changes, Pb ± PS treated males also showed

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¹To whom reprint requests should be addressed: Box EHSC, Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, deborah_cory-slechta@urmc.rochester.edu; phone: 585-275-7060.

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reductions in brain serotonin function in all mesocorticolimbic regions, broad monoamine changes in nucleus accumbens, and reductions in both BDNF and NMDAR 2A levels and increases in SERT in frontal cortex, i.e., in regions and neurotransmitter systems known to mediate learning/behavioral flexibility, and which were of greater impact in males. The current findings do not fully support a generality of the enhancement of Pb effects by PS, as previously seen with FI performance in females (Virgolini et al., 2008), and suggest a dissociation of the behaviors controlled by FI and delay discounting paradigms, at least in response to Pb $\pm$ PS in rats. Collectively, however, the findings remain consistent with sex-dependent differences in the impacts of both Pb and PS and with the need to understand both the role of contingencies of reinforcement and underlying neurobiological effects in these sex differences.

Keywords

lead; prenatal stress; impulsivity; delay of reward; sex differences; serotonin

1. INTRODUCTION

Diagnosis of attention deficit hyperactivity disorder (ADHD), a dysfunction that is more prevalent in boys (Getahun et al., 2013), is based on the symptom domains of inattention, hyperactivity and impulsivity (American Psychiatric Association, 1994). Impulsivity, a construct defined as acting without appropriate deliberations and choosing short-term over long-term goals, has been considered to derive from mechanisms that include altered sensitivity to delay of reward (Luman et al., 2010). Learning disorders are a frequent comorbidity of ADHD (Cantwell and Baker, 1991; Jakobson and Kikas, 2007; Mayes et al., 2000), ADHD itself can be associated with deficits in executive function and academic achievement (Faraone et al., 1993; Seidman et al., 2001), and impulsivity has been linked to behavioral disorders that include drug abuse and addiction (de Wit, 2009) and gambling (Kertzman et al., 2011). Quantitative and validated measures of this construct have been extensively evaluated, specifically using self-control/delay of reward/delay discounting paradigms (Evenden and Ryan, 1996; Madden and Johnson, 2010).

Attention deficit, including impulsive-like behaviors, has been associated with low levels of lead (Pb) exposure in children (Boucher et al., 2012; Nigg et al., 2008; Nigg et al., 2010), and with analogous phenotypes in animal models (Brockel and Cory-Slechta, 1998; 1999; Morgan et al., 2001). Prenatal stress (PS) has also been tied to attention deficits (Li et al., 2010; Rodriguez and Bohlin, 2005; Wilson et al., 2012) that can include heightened impulsivity (Grizenko et al., 2008). This concurrence of outcomes is notable, given that the greatest proportion of children with elevated blood lead (PbB) levels reside in low socioeconomic status communities, where prenatal stress is also considered to be highly prevalent (Gavin et al., 2012; Lancaster et al., 2010; Miszkurka et al., 2012), consistent with the interpretation that these are co-occurring risk factors in the human environment. That elevated PbB levels and PS should lead to common adverse outcomes including cognitive and attention deficits, likely reflects the fact that both Pb exposure and prenatal stress also significantly modify the functions of the central nervous system's (CNS) fronto-striatal/mesocorticolimbic circuits, and its associated monoamine (dopamine and serotonin)-

glutamate balance (Barros et al., 2004; Berger et al., 2002; Cory-Slechta et al., 1998; 1999; Martinez-Tellez et al., 2009; Rossi-George et al., 2011; Virgolini et al., 2008), that have been shown to subserve these behavioral domains (Dalley et al., 2008; Dalley and Roiser, 2012; Kim and Lee, 2011; Luman et al., 2010; Pattij and Vanderschuren, 2008; Tripp and Wickens, 2009).

Based on this convergence of exposure, behavioral consequences and neurobiological substrates, and with the intent to develop animal models of Pb exposure that better simulate human environmental conditions, we hypothesized that prenatal stress could enhance or unmask neurotoxic consequences of Pb for those outcomes for which they share biological substrates. Consistent with this hypothesis, combined low level maternal Pb exposure and PS followed by offspring stress were found to significantly increase rates of responding of female rats on a fixed interval (FI) schedule of food reward, to levels consistent with a higher level of Pb exposure (Virgolini et al., 2008). Notably, increased rates of FI responding have been shown in both infants and children (Darcheville et al., 1992; 1993), to be a surrogate for impulsive choice behavior, defined operationally as preference for immediate small reward over delayed but larger reward.

The current study, therefore, sought to determine the impact of Pb on a delay of reward paradigm (delay discounting) widely employed in human and experimental studies and which provides a validated assessment of impulsive choice behavior in children diagnosed with ADHD (Marco et al., 2009), as well as the potential of PS to enhance any effects of Pb. Given the documented role of monoamine and glutamate circuitry in impulsive choice behavior, and our previous findings demonstrating the mitigating effects of behavioral testing on Pb $\pm$ PS-induced neurochemical changes (Cory-Slechta et al., 2013a; Cory-Slechta et al., 2013b), this study also examined cortico-mesolimbic neurotransmitter function at 2 months (mos) of age in littermates of behaviorally tested offspring, i.e., a time point immediately prior to initiation of behavioral testing to define changes in systems/regions that could contribute mechanistically to any observed adverse behavioral consequences.

2. METHODS

2.1 Dams and Pb Exposure

Four week old female Long Evans rats (Charles River, Germantown, NY) were randomly assigned to receive distilled deionized water drinking solutions containing 0 or 50 ppm Pb acetate and housed as same Pb-treatment condition pairs. Pb exposure was initiated 2–3 mos prior to breeding to ensure elevated bone Pb levels and Pb body burden at the time of conception and was continued throughout lactation, consistent with human environmental Pb exposure. Pb exposure ended at lactation to maintain consistency with the previous study of Pb $\pm$ PS effects on FI exposure (Virgolini et al., 2008) that specifically sought to evaluate the maternal contributions to pups. When female dams reached 3 mos of age, they were paired with 3 mos old male Long Evans rats for breeding. Animals were housed in a vivarium room maintained at 22 $\pm$ 2°C with a 12-h light-dark cycle (lights on at 0700h). Standard rat chow diet was provided *ad libitum*. All experiments were carried out according

to NIH Guidelines and were approved by the University of Rochester Medical School University Committee on Animal Resources.

2.2 Breeding and PS

Female rats were mated with males (2:1) across two estrous cycles. The presence of vaginal plugs or sperm was considered indicative of pregnancy and deemed gestational day 1 (GD1). Pregnant females in the 0 and 50 ppm Pb-treated groups were weighed and further randomly subdivided to a non-stress (NS) or prenatal stress (PS) condition. Beginning on GD1, all pregnant females were individually housed for the remainder of pregnancy and lactation.

On GD16 and 17, timed to correspond to the development of key brain regions (hypothalamic nuclei, hippocampus, striatum, frontal cortex) associated with the endpoints under study (Diaz et al., 1997; Yi et al., 1994), dams assigned to PS groups were weighed and subjected to a widely employed and extensively characterized restraint stress procedure consisting of three 45 min restraint sessions (1000, 1300, and 1600h) in plastic cylindrical devices (Ward and Weisz, 1984), a protocol we have previously verified to elevate corticosterone levels and alter catecholamine levels in frontal cortex and nucleus accumbens of dams (Cory-Slechta et al., 2004). NS dams were left undisturbed in their home cages. This resulted in four Pb-stress conditions with the following numbers of litters: 0-NS (n=20), 0-PS (n=15), 50-NS (n=19), and 50-PS (n=19).

2.3 Offspring Procedures

At delivery, designated as postnatal day 1 (PND1), litter size and body weight of the litter as a whole were recorded. On PND 5–6, litters were culled to no more than 8 pups, maintaining equal numbers of males and females wherever possible. Culled pups were used to extract trunk blood for Pb determinations. Remaining pups were weighed and weaned at PND21 and thereafter housed as same sex/treatment pairs.

From weaning, pups were provided with unrestricted access to the same standard rat chow and to water (0 ppm Pb) regardless of the prenatal Pb treatment. At 2 mos of age, brains were collected from a subset of pups (single pup/sex/litter) for determination of brain neurochemical and biochemical markers. For another subset, again using a single offspring/sex per litter ($n = 11$ /group), behavioral testing was initiated, and for another subset ($n=10$ /sex/group, each of which was also derived from independent litters) from these litters were maintained as non-behaviorally tested offspring for the same duration as behaviorally-tested littermates.

Prior to initiating behavioral testing, food intake was controlled for all offspring (behaviorally tested and non-tested) to achieve approximately 250g (females) or 300g (males) body weights. Caloric intake was regulated for the duration of the experiment to maintain this weight as required for food-motivated behavioral performance of the behaviorally tested subgroup. Because animals were pair-housed, individual feeding was accomplished by separating the residents through the introduction of a cage divider at the time of feeding, which remained in place for approximately 90–120 min.

2.4 Delay of Reward Paradigm

Behavioral test sessions were carried out Mon-Fri between 0900 and 1400h in rat operant chambers (ENV-008; Med Associates, St Albans, VT) housed in sound-attenuated enclosures ventilated by a fan, equipped with a grid floor, speaker, house light, and three standard (non-retractable) response levers (L, Left; C, Center; R, Right) configured horizontally on the front panel. Reinforcement consisted of the delivery of one or three 45-mg food pellet(s) (Bioserv, Frenchtown, NJ) depending on a rat's choice and behavioral contingencies, and data were controlled by Med-PC Version IV Research Control and Data Acquisition software. Lever press responding was first shaped using a variable time 20 sec fixed-ratio 1 schedule. The criterion for successful lever press training was the acquisition of 50 rewards in 30 min for each left, center and right levers.

Training on a delay discounting paradigm was subsequently initiated. In the training paradigm, a center lever press in the presence of the illumination of the houselight and the light above the center lever was required to start a trial. This response extinguished the center lever lights and illuminated the lights above the left or right lever ($p=0.5$). A response on the lever with illuminated lights then led to reward delivery (single 45 mg food pellet). After a 10 second (sec) post-reinforcement delay, the center lever was again operative and a new trial could be initiated. Failure to depress the correct lever or a repeated response on the center lever within 10 sec initiated a 2 sec time period out that was re-set with every additional incorrect center lever response. Sessions ended with the delivery of 50 rewards or after 30 min, whichever occurred first. A total of 4 training sessions were carried out.

Subsequently, the delay discounting paradigm was implemented. Each session consisted of the presentation of 3 blocks of trials, with the left lever designated as the long delay lever and the right lever as the short delay lever (Figure 1). Each block began with two forced choice trials, one forcing a long delay choice and one forcing a short delay choice, with a probability=0.5 of long or short on the first forced choice trial. The 2 forced choice trials within each block were thereafter followed by 10 choice trials. Forced trials operated as described above. In choice trials, a center lever response initiated the trial, shut off the center lever light, and illuminated the left and right lever lights. Responses on the right (short delay) lever extinguished both the right and left lever lights, and produced a flash of the right lever lights and the immediate delivery (0.1 sec, but hereafter referred to as 0 sec) of a single food pellet; this was followed by a 70 sec inter-trial interval before the center lever light was again illuminated providing the opportunity to initiate the next trial. Responses on the left (long delay) lever extinguished both the right and left lever lights, and after a specified delay period, produced a brief flash of the left lever lights and delivery of 3 food pellets. This was followed by an inter-trial interval of 70 sec minus the delay value, after which the center lever lights were re-illuminated again providing the opportunity to initiate the next trial. Center lever responses at any other point initiated a 2 sec timeout with all lights off that was re-set by any additional center lever responses. Failure to initiate a trial with a center lever response within 10 sec of the opportunity was recorded as either a forced or choice trial omission response and re-started the 10 sec opportunity clock. Sessions ended after completion of the 3rd block (36 total trials) or 45 min, whichever occurred first.

The initial delay value for the long delay lever was set at 0 sec, i.e., equal to that on the short delay lever, and was then increased across sessions to 10, 20 and then 30 sec, with 12 sessions at each delay value. The long delay value was subsequently again set at 0 sec for 18 sessions, after which an extinction stress probe challenge was carried out for 10 sessions in which all lights/sounds operated identically as described above, but no food pellets were actually delivered. The extinction probe was followed by 9 sessions at the 10 sec long delay to assess re-acquisition of performance.

The following outcomes measures were examined: percent choice of the long delay, large reward lever (number of long delay lever choices for larger reinforcement / total number of choices * 100), total trial omissions (number of 10 sec bins without a choice response in either a forced trial or choice trial, either summed across sessions (Figure 3) or as group mean values (Figures 5–7), latency to center lever responses (trial initiation), latency to the choice response (time to left or right lever response), premature left or right responses (number of left or right responses that were made when center lever trial initiation was required, either summed across sessions (Figure 3) or as group mean values (Figure 7)), time out responses (center lever responses made after trial initiation) and inter-trial interval responses (responses on left or right levers during the post-reward delay period either summed across sessions (Figure 3) or as group mean values (Figure 7)).

2.5 Blood and Brain Lead Determinations

Blood Pb measurements were determined from trunk blood of PND5–6 pups and of dams approximately 2–3 weeks after pup weaning by anodic stripping voltammetry using the Lead Care II system with a detection limit of 3.3 µg/dl. Cerebellar tissue from brain was analyzed for Pb levels in dams, collected at 2–3 weeks postweaning, was measured using atomic absorption spectrophotometry as previously described (Widzowski and Cory-Slechta, 1994).

2.6 Determination of Brain Monoamines

Brains of 2 mos old offspring were rapidly removed, dissected, and stored at –80°C until used for determinations of monoamines and western blot analysis. Levels of DA (dopamine), DOPAC (dihydroxyphenylacetic acid), HVA (homovanillic acid), NE (norepinephrine), 5-HT (serotonin) and 5-HIAA (5 hydroxyindoleacetic acid) from right hemisphere of frontal cortex, nucleus accumbens, striatum, hippocampus, hypothalamus, olfactory bulb and midbrain were analyzed using HPLC with electrochemical detection as described in detail previously (Cory-Slechta et al., 2013b). Concentrations of neurotransmitters were expressed as ng/mg protein. DA turnover was calculated as [DOPAC]/[DA]. Protein levels were determined using commercially available BCA assay kit according to the manufacture's instructions (Thermo Fisher Scientific Inc., Rockford, IL). Levels of detection (pg/ml), determined for each analyte using 20 blanks and 2 standard deviations were as follows: DA, 7.33; DOPAC, 7.42; HVA, 11.49; NE, 11.77; 5HT, 48.68 and 5-HIAA, 5.49.

2.7 Western Blot Analyses of BDNF, NMDAR 2A and SERT

2.7.1 Tissue preparation and protein extraction—For the determination of protein expression levels of interest, left hemispheres from frontal cortex, nucleus accumbens, hippocampus and hypothalamus were homogenized on ice in protein lysis buffer consisting of 50 mM Tris-HCl (pH 8.0), 150mM NaCl and 1 % Triton X-100. The homogenate was centrifuged at 14000 rpm for 20 at 4 °C. The resulting supernatant was collected, and protein levels were determined in an aliquot of each sample using the BCA assay kit (Thermo Fisher Scientific Inc., Rockford, IL).

2.7.2 SDS-page, blotting, probing and detection—Fifty µg of protein from each sample was denatured by mixing and boiling with 2× laemmli sample buffer (Bio-Rad, Hercules, CA) mixed with β-mercaptoethanol (5 %) at 1:1 ratio. Proteins were separated by electrophoresis on 12 % polyacrylamide gels and electroblotted onto nitrocellulose membranes (Bio-Rad, Hercules, CA) with sample loading counterbalanced across groups. Membranes were then blocked for 1 h at room temperature in 5 % non-fat milk (Bio-Rad, Hercules, CA) in a Tris-buffered saline (TBS) solution containing 0.05% tween-20 (Bio-Rad, Hercules, CA) (TBS-T). They were subsequently incubated overnight at 4°C with primary antibody diluted in 5 % milk in TBS-T as follows: rabbit anti-BDNF polyclonal antibody (AB1534, Millipore, Temecula, CA) diluted 1:700, rabbit anti-SERT polyclonal antibody (AB9726, Millipore, Temecula, CA) diluted 1:2000, mouse anti-NMDAR 2A monoclonal antibody (MAB5530, Millipore, Temecula, CA) diluted 1:800 and mouse anti-β-actin (A1978, Sigma-Aldrich, St. Louis, MO) diluted 1:20000. After washing with TBS-T 3 times for 10 min, membranes were incubated for 1 h at room temperature with horseradish peroxidase-linked secondary antibody (goat anti-rabbit IgG (172-1019, Bio-Rad, Hercules, CA) diluted 1: 5000 in 5 % milk in TBS-T for BDNF and SERT and goat anti-mouse IgG (172-1011, Bio-Rad, Hercules, CA) 1:5000 for NMDAR 2A and β-actin). Following washing of membranes with TBS-T 3 times for 10 min, proteins were visualized on X-ray film (X-OMAT AR Autoradiography film, Kodak, Rochester, NY) using Immun-Star HRP Chemiluminescent Substrate Kit (Bio-Rad, Hercules, CA). The target bands were compared with Precision Plus Protein Standards (Bio-Rad, Hercules, CA) to ensure that visualized bands corresponded to 18, 64, 165 and 42 KDa representing BDNF, SERT, NMDAR 2A and β-actin, respectively. Semi-quantitative densitometric analysis was carried out using Image J, an image processing program developed by NIH. Relative measurements of target protein levels were quantitated as area of the target bands minus the background intensity of the film and normalized to β-actin levels.

2.8 Statistical Analysis

Breeding outcomes, blood and brain Pb values, levels of neurotransmitters and BDNF, NMDAR 2A, and SERT were analyzed by 2-way ANOVAs with Pb and PS as between-group factors. Measures of delay discounting were analyzed using repeated measures analyses of variance with Pb and PS as between groups factors, and delay or sessions as a within group factor. Dependent upon ANOVA outcomes, subsequent Fishers least protected differences post-hoc tests were carried out. Our prior studies have repeatedly demonstrated clear sex differences in the consequences of Pb ± PS (Cory-Slechta et al., 2012a; Cory-Slechta et al., 2010; Cory-Slechta et al., 2012b; Cory-Slechta et al., 2008; Cory-Slechta et

al., 2009; Cory-Slechta et al., 2004; Cory-Slechta et al., 2013b; Rossi-George et al., 2009; Rossi-George et al., 2011; Virgolini et al., 2005; Virgolini et al., 2008); similar consequences were apparent in an initial overall analyses of percent long delay choice by session in the current study, as characterized by a significant interaction of Pb $\times$ sex ($F(47,3666)=2.16$, $p<0.0001$). Consequently, all analyses were carried out separately for each sex. Main effects as well as interactions are reported. In all cases, a p value of <0.05 was considered statistically significant, and where relevant, near significant effects ($p \leq 0.06$) are noted.

3. RESULTS

3.1 Breeding Outcomes

Multiple measures of breeding outcome were assessed (Table 1). Pb $\pm$ PS had no significant impact on numbers of litters or whole litter body weights, although a near significant PS-related decrement in weight per pup was found ($F(1,69)=3.93$, $p=0.051$). Of note, Pb significantly altered the male/female sex ratio, reducing the proportion of males by almost 20% ($F(1,70)=7.29$, $p=0.0087$).

3.2 Blood and Brain Pb Concentrations

Blood Pb (PbB) determinations were carried out approximately 2–3 weeks postweaning in dams and at PND5–6 in pups (Figure 2). At these time points, mean PbB values of dams exposed to 50 ppm ranged from 6–8 $\mu\text{g/dl}$, remained significantly higher than 0 ppm exposed groups ($F(1,52)=172.17$, $p<0.0001$), and did not differ in relation to PS; these values reflect steady-state PbBs under these exposure concentrations (Virgolini et al., 2008). PbBs of PND5–6 pups were also significantly increased by Pb ($F(1,38)=152.9$, $p<0.0001$), but to almost twice the levels of dams, i.e., to mean values ranging from 14–15 $\mu\text{g/dl}$ that did not differ in relation to sex or PS. Because these values were obtained at weaning, they reflect the significant increases in PbBs that occur in dams during gestation and lactation (Cory-Slechta et al., 2010).

Brain Pb values of dams, carried out using cerebellar tissue collected from a subset of 2–3 dams/treatment group at 2–3 weeks postweaning (remaining tissue were used for neurochemical analyses), likewise increased with Pb exposure level ($F(1,6)=19.56$, $p=0.0045$), with a suggestion that brain Pb values were further increased by PS; while this was not confirmed by a statistical interaction, plots of individual brain Pb values of dams (bottom right) show that brain values of all Pb+PS dams exceeded those of Pb only dams.

3.3 Delay of Reward Behavior

3.3.1 Baseline Behavior by Delay Interval

Males: As expected, when averaged across sessions, percent choice on the long delay lever declined with increasing delay value from 0 to 30 seconds (main effects of delay value: $F(3,120)=175.91$, $p<0.0001$), with a parallel reduction in total rewards obtained ($F(3,120)=153.32$, $p<0.0001$) and in numbers of premature left-right lever press responses ($F(3,120)=10.75$, $p<0.0001$) in males (Figure 3 **top**; Table 2). Latencies to respond on the center lever to initiate trials was also influenced by delay values ($F(3,120)=11.3$, $p<0.0001$),

with group mean values increasing from 0 to 10 and 20 seconds and declining at the 30 sec delay back towards 0 sec delay levels. A similar profile of effects was observed for choice response latencies ($F(3,120)=12.15$, $p<0.0001$) and for total trial omissions ($F(3,120)=20.89$, $p<0.0001$). Numbers of inter-trial interval responses declined as delay increased from 0 sec to 10 and 20 sec, and then tended to return at the 30 sec delay to levels consistent with the 0 sec delay values ($F(3,120)=6.52$, $p=0.0004$).

Effects of $Pb \pm PS$ on these baseline performance values as averaged across sessions were minimal, being restricted to a near significant effect of PS on percent of long delay choices ($F(1,120)=3.89$, $p=0.055$); the latter likely reflected the higher percentage long delay responses of the 50-PS group at the 10 sec delay, and similarly of the 0-PS and 50-PS groups at the 20 sec delay values. Despite trends towards increases in center and choice latencies, total omission responses and premature left right responses in $Pb \pm PS$ groups, none reached conventional levels of statistical significance.

Females: The profile of baseline performance of females across delay values was highly comparable to that of males (Figure 3 **bottom**; Table 2). Percent long delay responses (main effect of delay value: $F(3,120)=173.47$, $p<0.0001$), total rewards earned ($F(3,120)=223.41$, $p<0.0001$) and premature left-right lever responses ($F(3,120)=4.81$, $p=0.003$) all declined with increasing delay value, with center response latency ($F(3,120)=15.66$, $p<0.0001$), choice response latency ($F(3,120)=7.91$, $p<0.0001$) and choice trial omission responses ($F(3,120)=20.06$, $p<0.0001$) increasing from 0 sec delay values to 10 sec (all 3 measures) and 20 sec delay values (center and choice response latencies), before declining back towards 0 sec delay values or less at the 30 sec delay. As with males, numbers of inter-trial interval responses ($F(3,120)=11.99$, $p<0.0001$) tended to decline from the 0 sec delay values to 10 and 20 sec delay values with some suggestion of increases again in numbers at the 30 sec delay.

Only one effect of $Pb \pm PS$ on this baseline were seen across these averaged values, which consisted of a $Pb \times PS \times$ delay interaction for numbers of total rewards ($F(3,120)=3.47$, $p=0.018$). However, as can be seen, this interaction did not appear to reflect any consistent or systematic effects of $Pb \pm PS$.

3.3.2 Individual Plots for Percent Long Delay Response by Delay Interval—The suggestion of a more shallow delay function in response to $Pb \pm PS$ in males and the potential for individual differences was further examined in assessments of individual delay functions, as shown in Figure 4. As might be expected, group variability, as indicated by the range of scores, was most pronounced at the 10 sec delay interval, where mean values of the 0-NS groups of both males and females converged on indifference (50% choice). At the 10 sec delay, choice values of 6 of the 11 0-NS males and 5 of the 11 50-NS males exceeded 50%. In contrast, values of 7 of 11 0-PS males and all 11 of the 50-PS males exceeded 50%, findings found to be statistically significant using Chi-square methods (Chi-square=9.43(df=3), $p=0.024$). Similar, but non-significant trends were observed at the 20 sec delay value.

As with males, the greatest variability in females also occurred at the 10 sec delay value where group mean values tended to converge on indifference. No Pb $\pm$ PS-based differences in frequencies of subjects exceeding that value were found in females, however.

3.3.3 Pb $\pm$ PS-Induced Changes in Acquisition of Delay of Reward

Performance—While values averaged across sessions at each delay value reveal only minor Pb $\pm$ PS-related changes in delay of reward performance, effects across sessions on acquisition of performance were seen. In males, PS significantly increased the percent long delay choices at the 0 sec delay ($F(1,440)=4.61$, $p=0.0378$), a statistical outcome likely actually reflecting the lower values of the 50-NS group in collapsed comparisons of the PS vs. NS groups in statistical analyses (Figure 5, **top row**; Table 2). A corresponding slower increase in total number of rewards earned across sessions likewise occurred with Pb (Pb: $F(1,440)=6.53$, $p=0.015$; Pb $\times$ delay: $F(11,440)=3.17$, $p=0.0004$), effects that were particularly notable over the first 7 sessions. These were accompanied by a slower Pb related decline in center lever response latencies ($F(1,440)=8.06$, $p=0.007$) and by nearly significant Pb-related reductions in choice response latencies ($F(11,440)=1.76$, $p=0.059$).

At the 10 sec long delay, PS (generally 50-PS) nearly significantly ($F(1,440)=3.96$, $p=0.054$) flattened the slope of reduction of percent long delay responses across sessions (Figure 5, **second row**) in males, again with correspondingly slowed reductions in numbers of total rewards (Pb $\times$ delay: $F(11,440)=1.84$, $p=0.046$). Over these same sessions, PS (again, generally 50-PS) increased the numbers of total omission responses (PS $\times$ delay: $F(11,440)=1.95$, $p=0.032$).

At the 20 sec delay value (Figure 5, **third row**), near significant attenuation of the decline in long delay choices across sessions were associated with PS exposures in males ($F(1,440)=3.86$, $p=0.057$, and Pb-related increases in numbers of total omission responses remained evident ($F(1,440)=4.41$, $p=0.042$).

Pb $\pm$ PS-induced acquisition differences in females (Figure 5, **bottom row**) were seen only at the 0 sec delay value (Pb $\times$ PS $\times$ sessions: $F(11,429)=2.25$, $p=0.012$), where the 0-PS group in particular exhibited significantly lower percent of long delay choices than controls over the first 3 sessions. A corresponding profile of increases in total rewards across sessions at the 0 sec delay was seen ($F(11,429)=1.9$, $p=0.037$).

3.3.2 Re-instatement of a 0 sec Long Delay—Following the 30 sec long delay sessions, the long delay value was re-set to 0 sec for the next 18 sessions to re-assess acquisition under this condition (Figure 6; Table 2) and reproducibility of Pb $\pm$ PS effects initially seen in males (Figure 3) which included slower increases in both long delay choices and rewards, as well as increased center lever response latency and decreased choice latency. As Figure 6 shows, group mean levels of long delay choice and total rewards in the 50-NS group were lower but not statistically significantly reduced during reacquisition of long delay 0 sec performance. However, the increases in center lever response latency evoked by Pb did re-emerge ($F(1,680)=5.74$, $p<0.021$) and the Pb-related increase in total omissions previously seen at the 10 second delay was still noted but was no longer significant ($F(1,680)=3.48$, $p=0.069$).

3.3.3 Extinction of Delay of Reward Performance—The implementation of an extinction paradigm following the re-instatement of the 0 sec long delay value resulted in expected results (Figure 7; Table 2), which were fundamentally similar in both sexes. Specifically, extinction resulted in a reduction across sessions in long delay choice responses (main effect of sessions: males: $F(9,360)=31.75$, $p<0.0001$; females: $F(8,320)=13.86$, $p<0.0001$), premature left-right responses (males: $F(9,360)=6.23$, $p<0.0001$; females: $F(8,320)=3.76$, $p=0.0002$) and inter-trial interval responses (males: $F(9,360)=30.95$, $p<0.0001$; females: $F(8,320)=20.94$, $p<0.0001$), and increases in total response omissions (males: $F(9,360)=94.34$, $p<0.0001$; females: $F(8,320)=122.67$, $p<0.0001$), center response latencies (males: $F(9,360)=31.75$, $p<0.0001$; females: $F(8,320)=26.39$, $p<0.0001$) and choice response latencies (males: $F(9,360)=5.29$, $p<0.0001$; females: $F(8,320)=4.84$, $p<0.0001$). The only $Pb \pm PS$ effects seen during the extinction paradigm consisted of a near significant Pb -induced increase (primarily 50-PS-based) in choice response latencies in females ($F(1,333)=3.98$, $p=0.054$).

3.3.4 Reacquisition of 10 sec Long Delay of Reward Performance—Following extinction, the long delay value was set to 10 sec for a total of 9 sessions, during which $Pb \pm PS$ related changes were seen only in males (Figure 8; Table 2). These included a Pb -based delayed reduction in total omission responses, particularly in the initial sessions ($Pb \times$ sessions: $F(8,320)=2.02$, $p=0.044$) and PS -related increases in numbers of inter-trial interval responses, particularly in the final 3 sessions ($PS \times$ sessions $F(8,320)=2.92$, $p=0.0037$), while 50 ppm Pb tended to reduce numbers of inter-trial intervals during this period ($Pb \times PS \times$ sessions: $F(8,320)=3.75$, $p=0.0003$).

3.4 Regional Changes in BDNF, NMDAR 2A and SERT

Males: Alterations in BDNF, NMDAR 2A and SERT in males (Figure 9; Table 2) were seen in multiple brain regions. Specifically, Pb -related reductions in BDNF density were seen in both frontal cortex (approximately 40%, $F(1,34)=18.8$, $p=0.0001$) and hippocampus (approximately 25%, $F(1,36)=15.9$, $p=0.0003$), while PS increased BDNF density in both nucleus accumbens (41%, $F(1,35)=4.25$, $p=0.047$) and hippocampus (34%, $F(1,35)=4.57$, $p=0.04$). NMDAR 2A density was reduced by Pb in frontal cortex (56%, $F(1,34)=16.15$, $p=0.0003$) and hypothalamus (43%, $F(1,35)=22.98$, $p<0.0001$), while increased by PS in nucleus accumbens (19%, $F(1,35)=6.14$, $p=0.018$). Frontal cortex SERT levels were dramatically increased by Pb (248–479%, $F(1,34)=35.96$, $p<0.0001$) and by PS (up to 248%, $F(1,34)=7.35$, $p=0.01$), and included an interaction of Pb by PS ($Pb \times PS$: ($F(1,34)=5.72$, $p=0.023$), while PS alone increased SERT levels in nucleus accumbens (37%, $F(1,35)=4.24$, $p=0.047$). In hippocampus, a $Pb \times PS$ interaction ($F(1,35)=7.59$, $p=0.009$) resulted from the increased values of the $Pb+PS$ group relative to the 0- PS and 50- NS groups (post hoc comparisons: $p=0.007$ and 0.016 , respectively).

Females: Alterations in BDNF, NMDAR 2A and particularly SERT were also multi-regional in females (Figure 10; Table 2). Pb significantly reduced hippocampal BDNF (38%, $F(1,36)=15.9$, $p=0.0003$). A $Pb \times PS$ interaction in nucleus accumbens ($F(1,36)=5.69$, $p=0.022$) reflected a near significant reduction in the 50- NS group relative to 0- NS levels. NMDAR 2A levels were reduced by Pb in nucleus accumbens (34%, $F(1,36)=13.36$,

$p=0.0008$) and hypothalamus (44%, $F(1,36)=9.19$, $p=0.0045$). As with males, SERT levels were markedly increased by Pb in frontal cortex (up to 183%, $F(1,35)=8.47$, $p=0.0063$) and to a lesser extent in hypothalamus (25%, $F(1,36)=5.22$, $p=0.028$). SERT levels were reduced by Pb in hippocampus (30%, $F(1,36)=6.16$, $p=0.018$) and in nucleus accumbens, particularly in response to 50-NS (34%, Pb: $F(1,36)=10.43$, $p=0.0026$; Pb $\times$ PS: $F(1,36)=4.24$, $p=0.0467$).

3.5 Regional Changes in Brain Monoamines

Males: The most consistent treatment-related monoamine changes in males were in levels of 5-HT and its metabolite 5-HIAA (Figure 11; Table 2), which were altered in virtually all brain regions examined. Pb significantly reduced 5-HT levels in nucleus accumbens (37%, $F(1,35)=11.6$, $p=0.0017$) and striatum (38%, $F(1,35)=21.34$, $p<0.0001$), while PS reduced 5-HT levels in frontal cortex (26%, $F(1,34)=4.77$, $p=0.036$) and midbrain (25%, $F(1,35)=6.96$, $p=0.012$). 5-HIAA levels were reduced by Pb in frontal cortex (29%, $F(1,34)=11.62$, $p=0.0017$), nucleus accumbens (50%, $F(1,35)=22.93$, $p<0.0001$), and striatum (43%, $F(1,35)=21.91$, $p<0.0001$) and by PS in midbrain (23%, $F(1,35)=9.92$, $p=0.003$). In the hypothalamus, 5-HIAA was increased by Pb exposure (27%, $F(1, 35)=7.49$, $p=0.0097$).

From a regional perspective, the nucleus accumbens appeared to be the brain region most systematically affected by Pb $\pm$ PS, with Pb-induced reductions in both 5-HT and 5-HIAA in nucleus accumbens, as noted above, as well as Pb-related decrements in DA (19%, $F(1,35)=10.21$, $p=0.003$), HVA (12%, $F(1, 35)=5.76$, $p=0.022$) and NE (13%, $F(1, 35)=5.76$, $p=0.022$). Some changes in catecholamines were also found in other regions, including Pb-related reductions in HVA in frontal cortex (25%, $F(1,34)=4.37$, $p=0.044$) and in hypothalamus (39%, $F(1, 35)=17.46$, $p=0.0002$), in NE in striatum (25%, $F(1,35)=15.03$, $p=0.0004$), as well as PS-based near significant increases in HVA in midbrain (205%, $F(1,21)=4.01$, $p=0.058$).

Females: Monoaminergic changes in females in response to Pb $\pm$ PS were fewer in number and not systematic, as with serotonin in males (Figure 12; Table 2). The brain region most affected by Pb $\pm$ PS was hypothalamus, where Pb increased levels of DOPAC (19%, $F(1,36)=4.3$, $p=0.045$), NE (34%, $F(1,36)=11.15$, $p=0.002$) and 5-HIAA (36%, $F(1,36)=6.23$, $p=0.017$), while PS increased levels of 5-HT (27%, $F(1,36)=4.46$, $p=0.042$) and 5-HIAA (36%, $F(1,36)=5.06$, $p=0.03$), and Pb markedly reduced HVA (50%, $F(1,35)=8.23$, $p=0.007$). In addition, nucleus accumbens showed Pb-based reductions in DA (22%, $F(1,36)=4.7$, $p=0.037$), HVA (26%, $F(1,36)=14.33$, $p=0.0006$) and 5-HT (26%, $F(1,34)=5.91$, $p=0.021$), and reductions in frontal cortex levels of both NE (21%, $F(1,35)=5.64$, $p=0.023$) and 5-HIAA (22%, $F(1,35)=18.59$, $p=0.0001$), and of 5-HIAA in midbrain (16%, $F(1,36)=5.27$, $p=0.028$) were also noted. Frontal cortex 5-HIAA was also reduced by PS (45%, $F(1,35)=4.84$, $p=0.035$). In contrast, Pb-related increases were noted in striatal DOPAC (68%, $F(1,36)=11.06$, $p=0.002$) and DA TO (67%, $F(1,36)=14.77$, $p=0.0005$).

4. DISCUSSION

In a prior study, combined Pb +PS exposure followed by an offspring stress challenge increased rates of responding on a fixed interval (FI) schedule of food reward as compared to control, prenatal stress alone, lead alone and lead + prenatal stress alone in female rats (Virgolini et al., 2008). Since FI response rates have previously shown to be consistent with impulsive choice behavior in a delay discounting paradigm in both infants and children (Darcheville et al., 1992; 1993), the current study sought to further investigate the potential impacts of Pb $\pm$ PS on this behavioral domain using a more direct measure of impulsive choice behavior, i.e., a delay discounting paradigm that has been validated in children diagnosed with ADHD (Marco et al., 2009), and correspondingly, to determine whether PS-induced enhancements of Pb effects as seen on the FI schedule also generalized to delay discounting, another behavior that, like FI performance, is controlled by mesocorticolimbic DA/glutamate systems (Roesch and Bryden, 2011).

In the current study, Pb $\pm$ PS altered performance on the delay discounting paradigm primarily in male offspring, with effects that for some outcomes, appeared visually greater, in the 50-PS compared to the 50-NS group albeit not statistically confirmed. Collectively PS did not generally enhance Pb effects, nor, as hypothesized by increased FI rates, did Pb $\pm$ PS did enhance impulsive choice behavior, which would have been manifest as a more rapid decline in the percentage of long delay responses with increasing delay value. Instead, Pb $\pm$ PS-exposed males exhibited a slightly shallower delay function reflecting a greater preference for long delay choices at the indifference point (10 sec delay) as seen both in mean values collapsed across sessions and in individual delay functions. Further, Pb $\pm$ PS treatment also slowed acquisition of delay discounting performance, as shown by a slower increases at 0 sec and slower reductions at 10 and 20 sec delays in percent long delay responses across sessions. Pb $\pm$ PS also increased numbers of failures to and latencies to initiate trials (forced and choice trials). These latter performance disruptions tended to persisted across the duration of testing, such that even during the final condition (long delay of 10 seconds following the extinction paradigm), increased numbers of total trial omissions were again evident and ITI responses were increased by PS. Pb $\pm$ PS effects only emerged after the transition from the delay discounting training program to the more complex full delay discounting paradigm, indicating that these were not carry-over deficits from training.

Several putative explanations of the behavioral phenotype exhibited by males can be considered. One is the possibility of a generalized slowed overall responding produced by Pb $\pm$ PS. However, this seems unlikely as such an interpretation would also suggest that such deficits should also be seen during the delay discounting training paradigm that required a center lever response to initiate a trial followed by a response to the illuminated short or long delay lever, but no deficits were observed. Further, an explanation based on inability to inhibit responding would be seemingly inconsistent with increased total omission responses and increased center response latencies. A deficit in food motivation also seems unlikely given the absence of any body weight or growth differences with these Pb $\pm$ PS treatments and the ultimate acquisition of equivalent numbers of rewards and completed trials/session.

More likely explanations, accounts actually consistent with previously reported effects of Pb + PS, include impaired learning and/or behavioral inflexibility (Aleksandrov et al., 1999; Cohn et al., 1993; Newland et al., 1994; Pearson et al., 2010; Rice, 1993). The former would suggest that Pb $\pm$ PS impaired the ability to discriminate between the short and long delay conditions and with paradigm contingencies, and is consistent with the fact that the increased long delay choices occurred primarily during the initial phases of testing when long delay values increased from 0 to 20 sec, but not thereafter. Moreover, the accompanying alterations in numbers of and latencies to trial initiations could also be consistent with delayed acquisition of task contingencies. Further, if, as for monkeys, rats are insensitive to postreward delays, i.e., these are not associated with discounting unless cued (Blanchard et al., 2013; Pearson et al., 2010), meaning that performance is under the control of the initial delays but not post-reward delays, then short delay responses actually maximize reward, and Pb $\pm$ PS effects serve to delay maximization. The shallower delay function at long delay 10 sec could additionally reflect behavioral inflexibility/perseveration on the lever associated with greater reward magnitude at the 0 sec delay where >80% of responses had occurred under the 0 sec long delay condition. That it is not related to increased response omissions was indicated by the lack of any significant differences in group mean total trials completed at the 10 sec delay value (mean $\pm$ SE: 34.96 $\pm$.29 for 0-NS; 34.86 $\pm$.3 for 0-PS; 34.92 $\pm$.45 for 50-NS and 34.24 $\pm$.52 for 50-PS males).

Another mechanism that might be considered is that Pb $\pm$ PS produces an initially greater negative contrast effect with the downshift in reward magnitude (Pecoraro et al., 1999), or perhaps better framed here as reward opportunities, that occurred with the transition from the delay discounting training paradigm to the full paradigm. In the paradigm used here, choice of the short delay produced an immediate reward, but was then followed by a 70 sec ITI to the time of the next opportunity for trial initiation. In the case of a long delay choice, reward delivery was delayed by the specified long delay length, and thereafter followed by an ITI of 70 sec minus the delay value, so that the total trial length was not contingent upon the choice of long vs. short delay. Thus, either choice produced significant delays relative to training conditions where reward was effectively available every 10 seconds. Downshifts in reward magnitude or reward opportunities are associated with increased variability of behavior (Pecoraro et al., 1999) and could contribute to the increased failures to and latencies to initiate trials. The fact that the Pb $\pm$ PS-induced alterations were not seen during extinction may reflect the fact that significant effects of extinction were observed in all groups, control and treated groups increased across sessions during this period. It would also be of interest to test the possibility that the task itself was aversive for Pb $\pm$ PS males by offering an escape from the delay of reward paradigm. In the context of the current findings, studies using delay of reward that have offered a choice between a delay with an escape option, in which fast responses led to the avoidance of the delay, vs. delay with no escape option in which the delay followed the response regardless of latency, have found that individuals responded with a shorter latency in the delay escape option compared to the no escape delay, consistent with aversion to the delay (Broyd et al., 2012).

Changes in various neurotrophic/neurotransmitter markers at the time of initiation of behavioral testing were examined as potential mechanisms for the sex-related differences in the impact of Pb $\pm$ PS on delay of reward (Dalley et al., 2008; Pattij and Vanderschuren,

2008; Tripp and Wickens, 2009). Premised on the initial hypothesis related to impulsive choice behavior, the focus included monoamine/glutamate functions of mesocorticolimbic systems. Indeed, broad changes in serotonergic function across regions, in monoamine function in nucleus accumbens and reductions in BDNF, a molecule involved in regulation of dopamine mesocorticolimbic function and stress responsivity (Berton et al., 2006; Bustos et al., 2004; Guillin et al., 2004), and in NDMAR 2A density, indicative of glutamatergic dysfunction, accompanied by marked increases in SERT were found in frontal cortex in males at the time point at which behavioral testing was initiated. Many components of this broad profile of mesocorticolimbic neurotransmitter system alterations could contribute to alterations in performance as related to either deficits in acquisition of the contingencies of the paradigm and/or behavioral inflexibility/perseveration, behaviors clearly reflecting system-level control. In accord with the behavioral findings was the observation that these biochemical and neurochemical changes were less evident in females, consistent with the preferential impact of Pb $\pm$ PS on delay discounting seen here in males.

Serotonergic function is well known to be a critical mediator of the activity of many other neurotransmitters, including dopamine (Hensler et al., 2013) and glutamate (Delille et al., 2013), in regions that include ventral tegmental area and substantia nigra, nucleus accumbens, hippocampus, amygdala and prefrontal cortex (Dalley and Roiser, 2012). Serotonergic function has also been associated with impulsive choice behavior, with depletion increasing impulsive choice behavior in delay of reward paradigms (Bizot et al., 1999; Mobini et al., 2000; Wogar et al., 1993) and selective serotonin reuptake inhibitors reducing impulsive choice behavior (Baarendse and Vanderschuren, 2012; Wolff and Leander, 2002). Notably, such effects can be sex-dependent. For example, in humans, 5-HT depletion achieved via acute tryptophan depletion increased impulsive choice behavior assessed by a Continuous Performance Test in males, while reducing it in females (Walderhaug et al., 2007). Despite the marked changes in serotonergic function seen here, the role of serotonin in the observed behavioral deficits of males is not clear, given that increase impulsive choice behavior, interpreted as a greater propensity to short delay choices, was not observed. Further, while increases in frontal cortex SERT were seen and could be consistent with reduced impulsive choice behavior, they also occurred in females.

Pb $\pm$ PS-induced effects in males could also be considered in relation to a 'flexible approach' hypothesis recently proposed, which asserts that nucleus accumbens dopamine function is required for initiation of actions related to reward (Nicola, 2010), including return to the operant levers for reward availability. Indeed, Pb $\pm$ PS was associated with reductions in nucleus accumbens DA and HVA in male littermates at the time of behavioral testing, and could therefore potentially contribute to longer choice trial latencies and increased trial omissions. The fact that long periods of reward unavailability could occur either in the long delay period itself, or in the post-reward delay that preceded the opportunity to initiate a new trial provides a significant number of such opportunities. However, reductions in nucleus accumbens DA and HVA were also seen in female littermates at this time point, and yet females did not show corresponding increases in response omissions and latencies. Nor were deficits seen during the training phase of delay of reward in males when these neurochemical changes were also present.

The possibility that negative contrast effects contribute to the deficits in Pb $\pm$ PS males in delay discounting performance, given their emergence after the transition from the training program to the full delay discounting paradigm, could suggest other possible neurochemical mechanisms, particularly related to amygdala function. Numerous studies have demonstrated a role for amygdala function in mediation of negative contrast (Salinas and McGaugh, 1996; Salinas et al., 1993; Young and Williams, 2010), whereas control is not conferred by regions such as hippocampus and nucleus accumbens (Gilbert and Kesner, 2002; Leszczuk and Flaherty, 2000; Liao and Chuang, 2003). Further, it appears that amygdalar control is hemispheric, with preferential involvement of the right hemisphere (Coleman-Meschies et al., 1996; Young and Williams, 2010). Clearly assessment of amygdala changes should be a future component of these efforts, and, interestingly, we have previously demonstrated brain hemispheric differences in the trajectory of effects of Pb $\pm$ PS (Cory-Slechta et al., 2013b), although unfortunately amygdala was not examined in that study.

One goal of the current study was to determine the extent to which PS might enhance Pb-related effects, as previously seen with FI performance of females (Virgolini et al., 2008) in another mesocorticolimbic-mediated behavior. While Pb clearly altered delay of reward performance in males in this study, statistically significant evidence for enhancement of such effects by PS was not consistently observed. While suggestions of such trends in males were evident for trial omission responses, and for frontal cortex 5-HT and 5-HIAA, and for midbrain HVA, for example, they were not of sufficient magnitude to produce statistically significant Pb by PS interactions. Hence, the degree of PS-related enhancement observed here appears to be of lesser magnitude than that seen for FI performance, and occurred in males rather than females. These comparative findings underscore the dissociation of FI performance and impulsive choice behavior (Darcheville et al., 1992; 1993), at least in rats, although the two performances were not measured in the same subjects in this study as they were by Darcheville et al. The current results, coupled with our prior FI study (Virgolini et al., 2008), however, again demonstrate clear sex-related differences in response to Pb $\pm$ PS and indicate the need to further understand how differences in behavioral contingencies and neurobiology imposed by these factors converge to generate sex differences, findings of import to the development of behavioral interventions.

Finally, there was evidence in the current study that PS increased brain Pb levels of dams to values greater than that produced by Pb alone, a potential mechanism, in addition to others, by which enhanced effects of Pb +PS could occur. This is a new observation not seen in our prior studies that have relied primarily on blood Pb as a measure. Future studies should include measures of the trajectory of brain Pb levels in offspring over time as well.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Pb $\pm$ PS reduced impulsive choice behavior of male offspring at the indifference point in a delay discounting task
- Pb $\pm$ PS slowed acquisition of impulsive choice behavior across sessions preferentially in males
- These effects may relate to impaired learning/behavioral flexibility and/or increased sensitivity to changes in reward opportunities.

Figure 1.

Schematic of the behavioral contingencies of a choice trial in the delay discounting paradigm. In choice trials, in the presence of the houselight and center lever lights, a response on the center lever was required to initiate a trial. Failure to press the center lever within 10 sec was recorded as an omission and initiated a 2 sec time out period in which all lights were off; responses during the time out re-set the 2 sec period. Responses on either the right or left lever instead of the center lever within the designated 10 sec window had no consequence and were recorded as premature right/left responses. A correct center lever response turned off the center lever lights and turned on the lights above both the left and right levers. An additional center lever response after this resulted again in a 2 sec time out that was re-set with any additional center lever responses. At the end of the time-out period the lights above the right and left levers are once again illuminated for a choice response. A response on the left lever turned off the right lever lights and initiated the long delay

interval. At the end of the interval, the left lever lights and the houselight flashed and 3 rewards were delivered with 0.5 sec between each delivery. This was followed by an inter-trial interval (ITI) of 70 sec minus the long delay time at which point. A choice response on the right lever results in flashing of the right lever and houselights and delivery of a single reward and initiates the 70 sec ITI. At the end of the ITI, the houselight and center lever lights are illuminated signaling the opportunity for trial initiation.

Figure 2.

Top row: mean $\pm$ SE blood Pb values ($\mu\text{g/dl}$) of dams after 7 weeks of Pb exposure ($n=8-19/\text{group}$) and of male and female, as indicated, offspring at PND5-6 (left and right, respectively; $n=3-10/\text{group}$). Bottom row: mean $\pm$ SE cerebellar Pb values (ng/mg wet weight; $n=2/3$ per group) of dams (left) at 2-3 weeks postweaning, with individual values plotted on the right. Pb signifies main effect of Pb in the repeated measures ANOVA.

Figure 3.

Group mean $\pm$ SE values for performance measures of males on the delay discounting paradigm in relation to long delay values for the treatment groups as indicated ($n=11$ /group). Repeated measures ANOVA: Delay signifies main effect of delay value; Pb signifies main effect of Pb; ~PS signifies marginally significant effect of PS. $N=11$ /group.

Figure 4.

Individual plots for long delay choice by delay interval for each treatment group for males and females, as indicated; each line in each plot depicts an individual subject.

Figure 5.

Group mean $\pm$ SE values for treatment groups across sessions for various measures of performance for males for the 0 sec long delay sessions (top row), for the 10 second long delay sessions (second row), and for the 20 sec long delay sessions (third row) and for females for the 0 sec long delay value sessions (bottom row). Statistical outcomes from repeated measures ANOVAs: Sessions= main effect of sessions, Pb= main effect of Pb; PS=main effect of PS, ~PS=marginally significant effect of PS, PS $\times$ session= interaction of Pb by session, Pb $\times$ sessions=interaction of Pb by sessions, Pb $\times$ PS $\times$ sessions=interaction of Pb by PS by session.

Figure 6.

Group mean $\pm$ SE values for performance measures of treatment groups of males during reinstatement of the 0 sec long delay value. Statistical outcomes from repeated measures ANOVAs: Sessions= main effect of sessions, Pb= main effect of Pb.

Figure 7.

Group mean $\pm$ SE values for performance measures of treatment groups of males (top row) and females (bottom row) across sessions of the extinction paradigm. Statistical outcomes from repeated measures ANOVAs: Sessions= main effect of sessions, Pb= main effect of Pb.

Figure 8.

Group mean $\pm$ SE values for performance measures of treatment groups of males during the reinstatement of the 10 sec long delay value. Statistical outcomes from repeated measures ANOVAs: Sessions= main effect of sessions, PS $\times$ session= interaction of Pb by session, Pb $\times$ sessions=interaction of Pb by sessions, Pb $\times$ PS $\times$ sessions=interaction of Pb by PS by session.

Figure 9.

Group mean $\pm$ SE relative densities of BDNF (left column) NMDAR 2A (middle column) and SERT (right column) in frontal cortex (top row), nucleus accumbens (second row), hippocampus (third row) and hypothalamus (bottom row) of males for indicated treatment groups. ANOVAs: Pb = main effect of Pb; PS = significant effect of PS; Pb $\times$ PS = significant interaction of Pb by PS.

Figure 10.

Group mean $\pm$ SE relative densities of BDNF (left column), NMDAR 2A (middle column) and SERT (right column) in frontal cortex (top row), nucleus accumbens (second row), hippocampus (third row) and hypothalamus (bottom row) of females for indicated treatment groups. ANOVAs: Pb = main effect of Pb; PS = significant effect of PS; Pb $\times$ PS indicates significant interaction of Pb by PS.

Figure 11.

Group mean $\pm$ SE levels (ng/mg protein) of DA, DOPAC, HVA, DA TO, NE, 5-HT and 5-HIAA from left to right columns in frontal cortex, nucleus accumbens, striatum, midbrain and hypothalamus (from top to bottom row) of males by treatment group as indicated (n=9–11/group). ANOVAs: Pb = main effect of Pb; PS = significant effect of PS; ~Pb = nearly significant effect of Pb; Pb $\times$ PS indicates significant interaction of Pb by PS.

Figure 12.

Group mean $\pm$ SE levels (ng/mg protein) of DA, DOPAC, HVA, DA TO, NE, 5-HT and 5-HIAA from left to right columns in frontal cortex, nucleus accumbens, striatum, midbrain and hypothalamus (from top to bottom row) of females by treatment group as indicated (n=9–11/group). ANOVAs: Pb = main effect of Pb; PS = significant effect of PS.

Table 1Group Mean $\pm$ S.E. Breeding Outcomes by Treatment Group

	0-NS	0-PS	50-NS	50-PS
Numbers of Litters	20	15	19	19
Number of pups per litter	12.5 $\pm$ 1.0	14.5 $\pm$ 0.8	13.9 $\pm$ 0.6	14.2 $\pm$ 1.0
Number of males per litter	7.0 $\pm$ 0.5	7.7 $\pm$ 0.6	6.3 $\pm$ 0.6	6.9 $\pm$ 0.7
Number of females per litter	5.4 $\pm$ 0.7	6.5 $\pm$ 0.6	6.8 $\pm$ 0.6	7.1 $\pm$ 0.6
Sex Ratio (male/female) [*]	1.5 $\pm$ 0.2	1.4 $\pm$ 0.2	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1
Body weight/pup [#]	6.2 $\pm$ 0.1	5.8 $\pm$ 0.1	6.0 $\pm$ 0.1	5.9 $\pm$ 0.1

^{*} Main effect of Pb (F(1,70)=7.29, p=0.0087)[#] Main effect of PS (F(1,69)=3.93, p=0.051)

TABLE 2

Summary of Pb ± PS-Based Behavioral and Neurochemical Alterations by Sex

Behavioral Alterations ¹						Neurochemical Alterations ²										
	Long Delay Choice	Total Omissions	Center Resp Latency	Choice Resp Latency	Inter-Trial Interval Response		BDNF	NMDAR2	SERT	DA	DOPAC	HVA	DA TO	NE	5HT	SHIAA
♂ 0	↓ Pb		↑ Pb	↓ Pb		♂ FC	↓ Pb	↓ Pb	↓ Pb	↓ Pb		↓ Pb		↓ Pb	↓ PS	↓ Pb
10	↑ Pb+PS	↑ Pb+PS				NAC	↑ PS	↑ PS	↑ PS	↓ Pb,PS		↓ Pb		↓ Pb	↓ Pb	
20	~↑ PS	↑ Pb				STR			↑ Pb+PS					↓ Pb	↓ Pb	↓ Pb
0 Reacq			↑ Pb			MB						↑ Pb			↓ PS	↓ PS
Ext						HYP0		↓ Pb			↓ PS	↓ Pb				↑ Pb
10 Reacq		↑ Pb			↑ PS ↓ 50-NS											
♀ 0	↓ PS					♀ FC			↑ Pb					↓ Pb		↓ Pb,PS
10						NAC	↑ Pb, PS	↓ Pb	↓ Pb	↓ Pb		↓ Pb			↓ Pb	
20						STR	↓ Pb	↑ PS	↓ Pb		↓ Pb		↑ Pb			
0 Reacq						MB										↓ Pb
Ext				↑ Pb+PS		HYP0		↓ Pb	↑ Pb		↑ Pb	↓ Pb		↑ Pb	↑ PS	↑ Pb,PS
10 Reacq																

¹ 0, 10, 20 refer to long delay values; 0 Reacq to the reinstatement of the 0 sec delay; ext to the extinction probe sessions and 10 Reacq to the reinstatement of the 10 sec delay; arrows indicate direction of effect and associated effects of Pb ± PS; ~ signifies marginally significant effect

² FC = frontal cortex, NAC = nucleus accumbens, STR = striatum; MB = midbrain; HYP0 = hypothalamus. Arrows indicate direction of effect and associated effects of Pb ± PS