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Sex- and brain region- specific effects of prenatal stress and lead exposure on permissive and repressive post-translational histone modifications from embryonic development through adulthood

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Abstract

Developmental exposure to prenatal stress (PS) and lead (Pb) can affect brain development and adversely influence behavior and cognition. Epigenetic-based gene regulation is crucial for normal brain development and mis-regulation, in any form, can result in neurodevelopmental disorders. Post-translational histone modifications (PTHMs) are an integral and dynamic component of the epigenetic machinery involved in gene regulation. Exposures to Pb and/or PS may alter PTHM profiles, promoting lifelong alterations in brain function observed following Pb $\pm$ PS exposure. Here we examined the effects of Pb $\pm$ PS on global levels of activating marks H3K9Ac and H3K4Me3 and repressive marks H3K9Me2 and H3K27Me3 at different developmental stages: E18, PND0, PND6 and PND60. Dams were exposed to 0 or 100 ppm Pb beginning 2 months prior to breeding followed by no PS (NS) or PS resulting in 4 offspring treatment groups per sex: 0-NS (control), 0-PS, 100-NS and 100-PS. Global levels of PTHMs varied from E18 through adulthood even in control mice, and were influenced by sex and brain-region. The developmental trajectory of these PTHM levels was further modified by Pb $\pm$ PS in a sex-, brain region-and age-dependent manner. Females showed a preferential response to Pb alone in frontal cortex (FC) and differentially to PS alone and combined Pb + PS in hippocampus (HIPP). In males, PS-induced increases in PTHM levels in FC, whereas PS produced reductions in HIPP. Pb $\pm$ PS-based changes in PTHM levels continued to be observed in adulthood (PND60), demonstrating the lasting effect of these early life environmental events on these histone marks. These results indicate that epigenetic consequences of Pb $\pm$ PS and their contribution to mechanisms of toxicity are sex dependent. Additional studies will assist in understanding the functional significance of these changes in PTHM levels on expression of individual genes, functional pathways, and ultimately, their behavioral consequences.

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Keywords

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1. Introduction

The modification of histones at their amino-terminal tails by acetylation, methylation, sumoylation, phosphorylation, and/or ubiquitylation plays a fundamental role in gene regulation (Fischle et al., 2003) throughout development and is closely linked to transcriptional regulation required for many biological processes necessary for the proper development and maturation of the nervous system. Of these histone modifications, histone methylation and acetylation have been extensively studied in regard to their roles in gene expression and genome stability. Histone methylation has different roles in various biological processes, depending on the site and type of histone modified. For example, histone H3K4 methylation is associated with actively transcribed genes whereas histone H3K9 methylation is linked to heterochromatin formation and is associated with transcriptional repression. Like histone H3K9 methylation, histone H3K27 methylation is also involved in transcriptional repression. Conversely, epigenetic marks such as histone H3K9 acetylation and H3K4 methylation are associated with transcriptional activation (Kouzarides, 2007). Thus, there are various histone modifications and effector proteins that may play important roles in dictating correct spatial and temporal gene expression patterns during development (Jenuwein and Allis, 2001). These modifications can be read by transcriptional machinery and give rise to various patterns of expression that are dynamic, complex, and specific to particular cell types (Strahl and Allis, 2000), and in the brain, to specific cells located in specific brain regions. For example, epigenetic regulation has been suggested to play roles in neural, astrocytic, and oligodendrocytic differentiation (Ballas et al., 2005; Takizawa et al., 2001; Ye et al., 2009), neurogenesis (Kim et al., 2009; Mandel et al., 2011), synaptic plasticity (Pittenger and Kandel, 2003; Whitlock et al., 2006), and maintenance of neuronal identity (Kim et al., 2008).

In addition to their important and complex roles during the development of the brain, histone modifications also play a major role in modulating various cognitive processes in developing and mature brains. The epigenetic landscape remains 'plastic' throughout brain development and aging, with ongoing dynamic regulation occurring in neurons and non-neuronal cells throughout the lifespan. Alterations in post-translational histone modifications have been assessed in relation to cognitive and behavioral functioning including the regulation of impulsive behaviors, aggressiveness, and attention (Archer et al., 2012; Mill and Petronis, 2008) and linked to a variety of neurodevelopmental disorders (Parkel et al., 2013). Dynamic regulation of post-translational histone modifications have also been shown to play an important role in at least some forms of learning and the stabilization of long-term memories (Gupta et al., 2010), suggesting an important role of dynamic changes in gene expression and repression in normal cognitive functioning (Gupta et al., 2010).

Brain development, maturation, and functioning are vulnerable to lead (Pb) toxicity and clinical evidence indicates that the adverse consequences of developmental Pb exposure on

cognition, behavior, and brain function persist into adulthood (Yuan et al., 2006; Cecil et al., 2008). In humans, Pb exposure does not occur in isolation but often occurs in conjunction with other risk factors for adverse neurodevelopmental outcomes. One other such risk factor is prenatal stress (PS). Lead exposure and PS can be co-occurring and/or sequentially occurring risk factors, particularly in lower-socioeconomic-status communities (Keenan et al., 2007; Thayer and Kuzawa, 2014). Additionally, Pb exposure and PS also share biological substrates and can produce common adverse outcomes. For example, both Pb and PS adversely impact the hypothalamic–pituitary–adrenal (HPA) axis and associated glucocorticoid receptors, as well as target brain mesocorticolimbic dopamine–glutamate systems (Cory-Slechta et al., 1998, 1999; Berger et al., 2002; Barros et al., 2004; Virgolini et al., 2008; Martinez-Tellez et al., 2009; Rossi-George et al., 2011). Both Pb and PS also have effects on the epigenome (Oberlander et al., 2008; Senut et al., 2012; Faulk et al., 2013; Matrisciano et al., 2013) yet little is known about how these effects might be manifested from early development through adulthood. We previously reported differences in levels of histone modifications H3K9/14Ac and H3K9Me3 and the developmental trajectory of changes in these histone levels from day of birth (PND0) to postnatal day 6 (PND6) in hippocampus (HIPPO) and frontal cortex (FC) and that these parameters were differentially affected by Pb, PS, and the combination, in a sex and brain-region-dependent manner (Schneider et al., 2016). The current study was performed to extend these findings by examining the extent to which Pb, PS and Pb + PS affects levels of other post-translational histone modifications (PTHMs) H3K9Ac, H3K4Me3 (activating marks) and H3K9Me2 and H3K27Me3 (repressive marks) in FC and HIPPO during fetal development (E18), early postnatal life (PND0, PND6) and in adulthood (PND60) in males and females. These PTHMs were chosen for study as they have been associated with cognitive functioning (Peixoto and Abel, 2013; Parkel et al., 2013; Jarome et al., 2014), environment exposures (Hou et al., 2012; Eid et al., 2016), postnatal stress (Weaver et al., 2004) and may be differentially regulated in males and females (McCarthy et al., 2009; Shen et al., 2015; McCarthy and Nugent, 2015). These specific PTHMs have not been previously studied in the context of Pb exposure and PS.

2. Materials and methods

2.1. Animals, lead exposure, and prenatal stress

The use of animals was in compliance with NIH Guidelines for the Care and Use of Laboratory Animals and the study was approved by the institutional animal care and use committee at the University of Rochester School of Medicine. Two-month-old female C57/Bl6 mice (Jackson Laboratories, Bar Harbor ME) were randomly assigned to receive distilled deionized water drinking containing 0 or 100 ppm Pb acetate for 2 months prior to breeding and throughout lactation. This Pb exposure concentration have been used in our prior studies and produce blood lead values in offspring at postnatal days 5–6 of approximately 10 µg/dL (Schneider et al., 2016). Standard rat chow diet (Lab Diet, Laboratory Rodent Diet) was provided ad libitum. Female mice were mated with males (1:1) for 4 to 5 days to cover the duration of an estrous cycle. Gestational day 1 (GD1) was designated as the second day after pairing. Pregnant females in the 0 and 100 ppm Pb-treated groups were weighed and randomly assigned to a non-stress (NS) or prenatal stress (PS)

condition. Half of the dams in each Pb group were exposed to restraint stress (PS) 3×/day at for 45 min/day (1000, 1300, and 1600 h) from gestational day 15–18 or no stress (NS). This yielded 4 treatment groups/sex: 0-NS (no Pb, no PS), 0-PS (no Pb, PS), 100-NS (Pb, no PS) and 100-PS (Pb and PS) with no more than 1 pup/sex/dam to preclude litter specific effects. The stress procedure consisted of three 30-min restraint sessions in plastic cylindrical devices, a protocol previously verified to elevate corticosterone levels (Cory-Slechta et al., 2004). No stress dams were left undisturbed in their home cages. Frontal cortex and HIPP were removed from male and female mouse embryos at the developmental day E18 and rapidly frozen for later analysis. Tail snips were taken from pups and genomic DNA was extracted from tail snips and the sex of the embryos was confirmed by Jarid 1C (X-chromosome-specific gene)/Jarid 1D (Y-chromosome-specific gene) PCR (Clapcote and Roder, 2005). For the postnatal groups, the dams were treated as described above and at delivery (PND0), postnatal day 6 (PND6), or postnatal day 60 (PND60) animals were euthanized and FC and HIPP were removed and frozen for later analysis. The sex of PND0 and PND6 pups was verified as described above.

2.2. Measurement of post-translational histone modification levels

Histones were purified using the Active Motif Histone Purification mini kit (Cat. No. 40026) using manufacturer instructions with some modifications. Briefly, the FC and HIPP from E18, PND0, PND6 and PND60 mice (five biological replicates per treatment condition) were homogenized in 0.25 ml extraction buffer and incubated overnight at 4 °C on a rotating platform. Before purifying the histone proteins, the crude histone extract was neutralized by adding 5× neutralization buffer. The histone proteins were then purified using spin columns provided with the kit and washed 3 times with 0.5 ml histone wash buffer and eluted in 0.05 ml elution buffer. The histone proteins were precipitated overnight by adding perchloric acid to a final concentration of 4% and then pelleted by spinning at 14,000 rpm for 1 h at 4 °C. The high levels of salts were removed by washing twice with 0.5 ml 4% perchloric acid, followed by acetone containing 0.2% HCl and again with acetone. After air drying, the pellet was dissolved in 0.03 ml sterile water, the histone protein concentration was measured using a Qubit Protein Assay Kit (Invitrogen, Cat no. Q33212) (according to manufacturer instructions), and the protein extract was then snap frozen and stored at –80C.

The Active Motif Histone H3 PTM Multiplex Kit (Cat No. 33115) was used to examine global changes in PTHM levels. The protocol was followed as described by the manufacturer and 150 ng of purified histone was used for assay. For normalization of histone H3 levels between different samples, the histone H3 Total Ab-conjugated beads (Active Motif, Cat. No. 33116) were multiplexed with the histone PTM Ab-conjugated beads (H3K9Ac: Cat. No. 33117, H3K4Me3: Cat. No. 33121, H3K9Me2: Cat. No. 33119 and H3K27Me3: Cat. No. 33125). Five biological replicates per treatment condition were assayed. Data were collected using FLEXMAP3D instrument and analyzed using xPONENT software (Luminex Corp.).

2.3. Statistical analyses

To analyze PTHM levels in the normal (no Pb, no PS: 0-NS) condition one-way analysis of variance (ANOVA) was conducted with developmental time point (Day) as a between

groups factors. For analysis of PTHM level differences by developmental time point (Day) in relation to developmental exposures to Pb, PS or the combination, three factor ANOVAs with Pb (100 ppm, 0 ppm), PS or NS, and developmental time point (Day-E18, P0, P6 and PND60) as between group factors were carried out. Given that majority of analyses identified three-way interactions between Pb, PS and Day, two-factor ANOVAs were conducted, separated by Day, with Pb and PS as between group factors, with subsequent post-hoc tests as appropriate dependent upon main effect or interaction outcomes. All analyses were conducted separately by sex, given the known sex-specific effects of Pb on the brain. Statistical outliers were assessed and removed following a Grubb's test. All analyses were conducted using JMP (version 12). P values ≤ 0.05 were considered statistically significant. Data are presented as group mean $\pm$ S.E.

3. Results

3.1. Developmental trajectory of global PTHM levels in normal mouse frontal cortex

The developmental trajectory of PTHMs H3K9Ac, H3K4Me3, H3K9Me2 and H3K27Me3 at E18, PND0, PND6 and PND60 in normal (0-NS) FC of male and female mice is shown in Fig. 1. For females, one-way ANOVA revealed time (Day) as a significant modifier of each PTHM (H3K9Ac: $F(3,15) = 65.7$, $p < 0.0001$; H3K4Me3: $F(3,15) = 9.2$, $p = 0.001$; H3K9Me2: $F(3,15) = 23.0$, $p < 0.0001$; H3K27Me3: $F(3,15) = 13.4$, $p = 0.0002$). Subsequent post-hoc tests confirmed dramatic increases in H3K9Ac, H3K9Me2, H3K27Me3 levels at PND6 relative to E18, followed by significant reductions relative to E18 at PND60. The one exception was H3K4Me3 where an increase was noted relative to E18 only for PND0. In male FC, a main effect of day was also found for all 4 PTHMs (H3K9Ac: $F(3,15) = 76.8$, $p < 0.0001$; H3K4Me3: $F(3,15) = 12.1$, $p = 0.0003$; H3K9Me2: $F(3,15) = 39.6$, $p < 0.0001$ and H3K27Me3: $F(3,15) = 32.6$, $p < 0.0001$). In males, levels of all PTHMs increased at PND0, and then markedly further increased at PND6 followed by a decrease at PND60 back to levels observed at E18.

3.2. Effects of Pb, PS, and the combination on global PTHM levels in frontal cortex

Changes in PTHM levels in female FC by Day for each of the 4 treatment groups is shown in Fig. 2. Statistical analysis using three factor ANOVA with Pb, PS and Day as between group factors confirmed significant three-way interactions for each of the 4 PTHMs (Pb $\times$ PS $\times$ Day: $F(15,60) = 21.08, 15.0, 16.1, 15.4$, respectively, $p < 0.0001$ each). Consequently, post-hoc *t*-tests (all p values ≤ 0.05) were carried comparing treatment groups at each time period and significant differences from the 0-NS control group were observed at each time period. Increases in levels of the activating mark H3K9Ac at day E18 were observed in the 0-PS, 100-NS and 100-PS groups, and in the 0-PS and 100-NS groups at PND0 and at PND60. Increases in H3K4Me3 levels were observed in the 100-NS group at PND0 and in the 100-PS group at PND6. Similar patterns were observed for the two repressive marks, H3K9Me2 and H3K27Me3. The most marked effects observed were increases in levels of all PTHMs in the 100-NS group at PND0, which ranged in magnitude from 220 to 252% of 0-NS control.

In addition, post hoc *t*-tests were carried out across time periods relative to E18 for each treatment group, with the results shown in Table 1. For the most affected PTHM, H3K9Ac, differences from E18 occurred in all 4 treatment groups at each time period, except PND6 for the 0-PS group. H3K27Me3 was the least affected PTHM across time and treatment groups. The direction of the changes in PTHM levels differed by the specific PTHM and by Day.

Changes in PTHM levels in male FC by Day for each of the 4 treatment groups are shown in Fig. 3. The pattern of change observed in male FC differed from that observed in female FC. Statistical analyses confirmed main effects of Day for all 4 PTHMs (H3K9Ac: $F(15,60) = 456.0$, $p < 0.0001$; H3K4Me3: $F(15,60) = 45.12$, $p < 0.0001$; H3K9Me2: $F(15,60) = 142.67$, $p < 0.0001$, and H3K27Me3: $F(15,60) = 107.98$, $p = 0.0001$). PTHM levels increased from E18 to PND0 and further increased at PND6 before declining back to levels seen at E18. The magnitude of increases at PND0 ranged from 150 to 302%, 272 to 401%, 218 to 262% and 147 to 205 for H3K9Ac, H3K4Me3, H3K9Me2 and H3K27Me3, respectively. The further increases observed between PND0 and PND6 ranged from 150 to 233%, 203 to 240%, 164 to 253% and 165 to 226%, respectively. Main effects of PS in statistical analyses for H3K9Ac, H3K9Me2 and H3K27Me3 ($F(60) = 10.95$, $p = 0.0016$, $F = 4.77$, $p = 0.033$, and $F = 5.66$, $p = 0.021$ respectively) resulted from their slightly higher levels, particularly in the 0-PS group at PND0 and PND6.

Results of post hoc *t*-tests carried out within each treatment group across days relative to E18 are shown in Table 1 and show the significant increases at PND0 relative to E18, particularly for H3K9Ac and H3K9Me2, and the marked increases at PND6 that were significant in all 4 treatment groups relative to their corresponding E18 levels.

3.3. Developmental trajectory of global PTHMs levels in hippocampus in normal mice

The PTHM levels in HIPP for 0-NS females and males across different ages are shown in Fig. 4. Significant main effects of Day were found for all 4 PTHMs for females (H3K9Ac: $F(3,15) = 28.0$, $p < 0.000$; H3K4Me3: $F(3,15) = 4.9$, $p = 0.01$; H3K9Me2: $F(3,15) = 14.6$, $p = 0.000$; and H3K27Me3: $F(3,15) = 18.7$, $p < 0.0001$). Post hoc *t*-tests (all p values < 0.05) showed this to be the result of increases in all 4 PTHMs at PND6 relative to their treatment group level at E18. These increases persisted for PTHMs H3K9Ac, H3K9Me2 and H3K27Me3 at PND60.

Significant main effects of Day were also confirmed in ANOVA for all 4 PTHMs in male HIPP (H3K9Ac: $F(3,15) = 262.0$, $p < 0.0001$; H3K4Me3: $F(3,15) = 42.0$, $p < 0.0001$; H3K9Me2: $F(3,15) = 73.7$, $p < 0.0001$; and H3K27Me3: $F(3,15) = 143.6$, $p < 0.0001$). Post-hoc *t*-tests (all p values < 0.05) showed these changes to be due to significant increases in all 4 PTHMs both at PND6. The increases observed in male HIPP at PND6 did not persist out to PND60.

3.4. Effects of Pb, PS, and the combination on global PTHM levels in hippocampus

The changes in PTHM levels in female HIPP by Day for each of the 4 treatment groups are shown in Fig. 5. A three way interaction of Day $\times$ Pb $\times$ PS was observed for H3K4Me3, H3K9Me2 and H3K27Me3 ($F(15,60) = 3.4$, $p = 0.02$; $F(15,60) = 6.02$, $p = 0.0012$; and

$F(15,60) = 4.5$, $p = 0.006$, respectively). For H3K9Ac, multiple interactions were found, including $Pb \times PS$ ($F(1,60) = 10.1$, $p = 0.002$), $Day \times Pb$ ($F(3,60) = 6.3$, $p = 0.0008$) and $Day \times PS$ ($F(3,60) = 6.1$, $p = 0.001$). Overall, increases in PTHM levels were seen across Days for all treatment groups for all PTHMs, but differed for individual PTHMs by age of the animal and by treatment group. Significant differences from 0-NS controls, as determined by post hoc t -tests (all p values < 0.05), showed increases across time in the 0-PS group and remained significantly higher than control at PND60 for H3K4Me3, H3K9Me2 and H3K27Me3 with a similar trend for H3K9Ac. In contrast, the 100-PS group showed a reduction relative to control by PND60 that was significant for H3K9Ac and H3K27Me3.

Table 2 summarizes the results of post hoc t -tests carried out within each treatment group across different ages relative to E18. As it indicates for females, increases in PTHM levels at PND6 were significant in all treatment groups for all 4 PTHMs. In the case of H3K4Me3, increases were also seen in the 0-PS, 100-NS and 100-PS groups at PND0.

Changes in PTHM levels in male HIPP by Day for each of the 4 treatment groups are shown in Fig. 6. A three-way interaction of $Day \times Pb \times PS$ was confirmed by ANOVA for all 4 PTHMs (H3K9Ac: $F(15,60) = 11.8$, $p < 0.0001$; H3K4Me3: $F(15,60) = 3.1$, $p = 0.03$; H3K9Me2: $F(15,60) = 6.0$, $p = 0.0012$; and H3K27Me3: $F(15,60) = 5.8$, $p = 0.001$). Subsequent post hoc t -tests (all p values < 0.05) confirmed significant differences from 0-NS controls in the 100-NS group at E18 and at PND0 for H3K9Ac and H3K9Me2. Further, greater increases occurred at PND6 in the 100-PS group relative to 0-NS controls for both H3K9Ac and H3K4Me3 levels.

The results of post hoc t -tests carried out within each treatment group in males across Days, relative to E18, are summarized in Table 2. For males, virtually all 4 treatment groups displayed significant increases in all 4 PTHMs at PND6, and for H3K27Me3 at PND0 as well. No changes were seen in the 0-NS group at PND0 in levels of H3K9Ac, H3K4Me3 or H3K9Me2, relative to those observed at E18, although such changes were observed in the 0-PS and 100-NS groups and in the 100-PS group for H3K9Ac and H3K9Me2.

4. Discussion

The epigenome is an important target for environmental exposures occurring during development. The current study examined the extent to which developmental exposure to Pb, PS and Pb + PS affects levels of PTHMs H3K9Ac, H3K4Me3 (activating marks) and H3K9Me2 and H3K27Me3 (repressive marks) in FC and HIPP during fetal development (E18), early postnatal life (PND0, PND6) and in adulthood (PND60). Results showed sex-dependent differences in the developmental trajectory of the PTHMs examined within each brain structure (i.e., FC and HIPP) in normal animals (no Pb/no PS) as well as sex- and brain region-dependent differences in PTHM levels in response to Pb, PS, and Pb + PS. Our findings thus demonstrate that time course data differentiated by brain region and sex are needed to understand the dynamics of epigenetic changes across brain development and their relation to specific physiological processes.

In normal (no Pb/no PS) animals, the different enrichment levels of PTHMs at different developmental time points suggest that during the development of FC and HIP, different regulatory events are occurring that are shaping the structural and functional status of these brain regions and that are likely associated with expression of unique gene regulatory networks that are modulated at least in part by the levels of PTHMs presently examined. In general, males showed dramatic increases in PTHM levels between E18 to PND6 in both FC and HIP and females showed less marked increases over time that generally, but not uniformly, also peaked at PND6. Considering the dynamic nature of the epigenome during early development, the findings of Pb, PS and Pb + PS-induced changes in global PTHM levels detected during early embryonic development (i.e., E18) could have far-reaching effects on the structural and functional development of the brain. Rapid changes are also occurring in the rodent brain at PND0 (Bockhorst et al., 2008), including processes of myelination, synaptogenesis, and axonal pruning (Semple et al., 2013), which occur differently in FC and HIP. At PND6 in the rodent, a highly orchestrated series of neurodevelopmental processes continue to take place, with gliogenesis peaking along with increasing axonal and dendritic growth and complexity of neuronal connections (Semple et al., 2013). At PND6, males had higher levels of acetylated, dimethylated and trimethylated H3 in FC and HIP than did females and these differences may be hormonally dependent. While the differences in H3 acetylation may be related to testosterone levels, H3 methylation does not appear to be testosterone related and it has been suggested that this early postnatal sex difference in H3 levels may not be organized by early hormonal exposures but instead may be the result of other genomic influences (Nugent and McCarthy, 2011).

Adverse neurodevelopmental effects of Pb, PS and the combination, as demonstrated in human studies and paralleled in animal models, can be persistent (Anderson et al., 2016; Brubaker et al., 2010; Canfield et al., 2003; Cecil et al., 2008; Cohn et al., 1993; Jett et al., 1997; Lanphear et al., 2005; Li et al., 2016). Findings from this study confirm long-lasting changes in PTHM levels in response to these exposures, as evidenced in FC and HIP at PND60 with Pb exposure having been discontinued at PND21, with effects differing by sex and by brain region. In PND60 male and female FC, PTHM levels were decreased from those observed at PND6. A similar trend was observed in male HIP but in females, PTHM levels mostly remained high at PND60. In PND60 female FC, there were significant interactive effects of Day $\times$ Pb $\times$ PS on all PTHMs while such effects were not observed in males. In PND60 male HIP, interactive effects of Day $\times$ Pb $\times$ PS were seen on all PTHMs whereas this was only the case for H3K4Me3 and H3K27Me3 in female HIP. By PND60, adult levels of synaptic density, myelination, and neurotransmitter levels are achieved (Semple et al., 2013) and thus it is important that effects from perinatal Pb exposure and PS on PTHM levels persist into adulthood and can potentially continue to influence gene transcriptional profiles.

Sex differences in the response to Pb have been repeatedly described and include changes at the molecular and behavioral levels (Anderson et al., 2012; Cory-Slechta et al., 2010; Virgolini et al., 2008). In a study using similar Pb exposures as used here, female but not male mice exhibited hypermethylation; however, no significant changes in whole-genome DNA methylation were found in cortex in that study (Sanchez-Martin et al., 2015).

Similarly, sex differences in response to PS have been described. For example, male offspring were reported to show greater impacts of PS on hippocampal neuronal and glial markers than females (Barbie-Shoshani et al., 2016), whereas in other cases, females tended to show enhanced consequences (Benoit et al., 2015; Huang et al., 2013). In some studies, males exhibited more adverse behavioral consequences of PS, while females showed a greater number of changes in gene expression (Van den Hove et al., 2013). Our present results are consistent with evidence for a sex differential in vulnerability to perinatal risks (DiPietro and Voegtline, 2017) and raise the possibility that epigenetic alterations resulting from Pb, PS and the combination during embryogenesis and early brain development could have far-reaching effects on brain development. The mechanisms through which sex imparts vulnerability or protection to the developing nervous system are not clear, however, variations in placental functioning and the neuroendocrine milieu have been suggested as potential contributors (DiPietro and Voegtline, 2017).

5. Conclusions

At least for the PTHMs examined here, changes were observed over the course of brain development in a brain region- and sex-dependent manner. The developmental trajectories of these PTHMs were modified by Pb $\pm$ PS, in a sex-, brain region- and age-dependent manner. Females appeared to show a preferential response to Pb alone in FC and differentially to PS alone and combined Pb + PS in HIPP. In males, PS-induced increases were seen in FC, whereas PS produced reductions in HIPP. The fact that Pb $\pm$ PS-induced changes continue to be observed even in adulthood (PND60), with PS present only during gestation and Pb exposure ending at weaning, emphasizes the lasting imprint of these early life environmental events on these histone marks. As we have observed here and reported previously (Schneider et al., 2016; Rossi-George et al., 2011), effects of Pb and/or PS alone can be observed on a variety of measures whereas in some cases, combined Pb + PS appears to restore outcome measures back to control levels. It is unlikely that the combination of two such adverse impacts would be less detrimental than each single impact alone, and instead suggests a potential situation in which physiological compensation pathways may be triggered in response to combined exposures. Further studies are needed to better understand the physiological basis of such responses as well as to discern the influence of the currently observed changes in global PTHM levels on expression of individual genes and functional pathways in order to better understand the functional relevance of the present findings.

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References

- Anderson DW, Pothakos K, Schneider JS. Sex and rearing condition modify the effects of perinatal lead exposure on learning and memory. *Neurotoxicology*. 2012; 33(5):985–995. [PubMed: 22542453]
- Anderson DW, Mettil W, Schneider JS. Effects of low level lead exposure on associative learning and memory in the rat: influences of sex and developmental timing of exposure. *Toxicol Lett*. 2016; 246:57–64. [PubMed: 26812500]

- Archer T, Oscar-Berman M, Blum K, Gold M. Neurogenetics and epigenetics in impulsive behaviour: impact on reward circuitry. *J Genet Syndr Gene Ther.* 2012; 3(3):1000115. [PubMed: 23264884]
- Ballas N, Grunseich C, Lu DD, Speh JC, Mandel G. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell.* 2005; 121(4):645–657. [PubMed: 15907476]
- Barbie-Shoshani Y, Shoham S, Bejar C, Weinstock M. Sex-specific effects of prenatal stress on memory and markers of neuronal activity in juvenile rats. *Dev Neurosci.* 2016; 38(3):206–219. [PubMed: 27372837]
- Barros VG, Berger MA, Martijena ID, Sarchi MI, Perez AA, Molina VA, Tarazi FI, Antonelli MC. Early adoption modifies the effects of prenatal stress on dopamine and glutamate receptors in adult rat brain. *J Neurosci Res.* 2004; 76(4):488–496. [PubMed: 15114621]
- Benoit JD, Rakic P, Frick KM. Prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression. *Behav Brain Res.* 2015; 281:1–8. [PubMed: 25496779]
- Berger MA, Barros VG, Sarchi MI, Tarazi FI, Antonelli MC. Long-term effects of prenatal stress on dopamine and glutamate receptors in adult rat brain. *Neurochem Res.* 2002; 27(11):1525–1533. [PubMed: 12512957]
- Bockhorst KH, Narayana PA, Liu R, Ahobila-Vijjula P, Ramu J, Kamel M, Wosik J, Bockhorst T, Hahn K, Hasan KM, Perez-Polo JR. Early postnatal development of rat brain: in vivo diffusion tensor imaging. *J Neurosci Res.* 2008; 86(7):1520–1528. [PubMed: 18189320]
- Brubaker CJ, Dietrich KN, Lanphear BP, Cecil KM. The influence of age of lead exposure on adult gray matter volume. *Neurotoxicology.* 2010; 31(3):259–266. [PubMed: 20226811]
- Canfield RL, Henderson CR Jr, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. *N Engl J Med.* 2003; 348(16):1517–1526. [PubMed: 12700371]
- Cecil KM, Brubaker CJ, Adler CM, Dietrich KN, Altaye M, Egelhoff JC, Wessel S, Elangovan I, Hornung R, Jarvis K, Lanphear BP. Decreased brain volume in adults with childhood lead exposure. *PLoS Med.* 2008; 5(5):e112. [PubMed: 18507499]
- Clapcote SJ, Roder JC. Simplex PCR assay for sex determination in mice. *Biotechniques.* 2005; 704:706.
- Cohn J, Cox C, Cory-Slechta DA. The effects of lead exposure on learning in a multiple repeated acquisition and performance schedule. *Neurotoxicology.* 1993; 14:329–346. [PubMed: 8247407]
- Cory-Slechta DA, O'Mara DJ, Brockel BJ. Nucleus accumbens dopaminergic mediation of fixed interval schedule-controlled behavior and its modulation by low-level lead exposure. *J Pharmacol Exp Ther.* 1998; 286(3):794–805. [PubMed: 9694936]
- Cory-Slechta DA, O'Mara DJ, Brockel BJ. Learning versus performance impairments following regional administration of MK-801 into nucleus accumbens and dorsomedial striatum. *Behav Brain Res.* 1999; 102:181–194. [PubMed: 10403026]
- Cory-Slechta DA, Virgolini MB, Thiruchelvam M, Weston DD, Bauter MR. Maternal stress modulates the effects of developmental lead exposure. *Environ Health Perspect.* 2004; 112(6):717–730. [PubMed: 15121516]
- Cory-Slechta DA, Stern S, Weston D, Allen JL, Liu S. Enhanced learning deficits in female rats following lifetime Pb exposure combined with prenatal stress. *Toxicol Sci.* 2010; 117(2):427–438. [PubMed: 20639260]
- DiPietro JA, Voegtline KM. The gestational foundation of sex differences in development and vulnerability. *Neuroscience.* 2017; 342:4–20. [PubMed: 26232714]
- Eid A, Bihaqi SW, Renehan WE, Zawia NH. Developmental lead exposure and lifespan alterations in epigenetic regulators and their correspondence to biomarkers of Alzheimer's disease. *Alzheimers Dement (Amst).* 2016; 2:123–131. [PubMed: 27239543]
- Faulk C, Barks A, Liu K, Goodrich JM, Dolinoy DC. Early-life lead exposure results in dose- and sex-specific effects on weight and epigenetic gene regulation in weanling mice. *Epigenomics.* 2013; 5(5):487–500. [PubMed: 24059796]
- Fischle W, Wang Y, Allis CD. Histone and chromatin cross-talk. *Curr Opin Cell Biol.* 2003; 15(2):172–183. [PubMed: 12648673]

- Gupta S, Kim SY, Artis S, Molfese DL, Schumacher A, Sweatt JD, Paylor RE, Lubin FD. Histone methylation regulates memory formation. *J Neurosci*. 2010; 30(10):3589–3599. [PubMed: 20219993]
- Hou L, Zhang X, Wang D, Baccarelli A. Environmental chemical exposures and human epigenetics. *Int J Epidemiol*. 2012; 41(1):79–105. [PubMed: 22253299]
- Huang Y, Chen S, Xu H, Yu X, Lai H, Ho G, Huang Q, Shi X. Pre-gestational stress alters stress-response of pubertal offspring rat in sexually dimorphic and hemispherically asymmetric manner. *BMC Neurosci*. 2013; 14:67. [PubMed: 23829597]
- Jarome TJ, Thomas JS, Lubin FD. The epigenetic basis of memory formation and storage. *Prog Mol Biol Transl Sci*. 2014; 128:1–27. [PubMed: 25410539]
- Jenuwein T, Allis CD. Translating the histone code. *Science*. 2001; 293(5532):1074–1080. [PubMed: 11498575]
- Jett DA, Kuhlmann AC, Farmer SJ, Guilarte TR. Age-dependent effects of developmental lead exposure on performance in the morris water maze. *Pharmacol Biochem Behav*. 1997; 57:271–279. [PubMed: 9164582]
- Keenan K, Gunthorpe D, Grace D. Parsing the relations between SES and stress reactivity: examining individual differences in neonatal stress response. *Infant Behav Dev*. 2007; 30(1):134–145. [PubMed: 17292786]
- Kim D, Frank CL, Dobbin MM, Tsunemoto RK, Tu W, Peng PL, Guan JS, Lee BH, Moy LY, Giusti P, Broodie N, Mazitschek R, Delalle I, Haggarty SJ, Neve RL, Lu Y, Tsai LH. Deregulation of HDAC1 by p25/Cdk5 in neurotoxicity. *Neuron*. 2008; 60(5):803–817. [PubMed: 19081376]
- Kim HJ, Leeds P, Chuang DM. The HDAC inhibitor, sodium butyrate, stimulates neurogenesis in the ischemic brain. *J Neurochem*. 2009; 110(4):1226–1240. [PubMed: 19549282]
- Kouzarides T. Chromatin modifications and their function. *Cell*. 2007; 128(4):693–705. [PubMed: 17320507]
- Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J, Roberts R. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect*. 2005; 113(7):894–899. [PubMed: 16002379]
- Li Y, Xie C, Murphy SK, Skaar D, Nye M, Vidal AC, Cecil KM, Dietrich KN, Puga A, Jirtle RL, Hoyo C. Lead exposure during early human development and DNA methylation of imprinted gene regulatory elements in adulthood. *Environ Health Perspect*. 2016; 124(5):666–673. [PubMed: 26115033]
- Mandel G, Fiondella CG, Covey MV, Lu DD, Loturco JJ, Ballas N. Repressor element 1 silencing transcription factor (REST) controls radial migration and temporal neuronal specification during neocortical development. *Proc Natl Acad Sci U S A*. 2011; 108(40):16789–16794. [PubMed: 21921234]
- Martinez-Tellez RI, Hernandez-Torres E, Gamboa C, Flores G. Prenatal stress alters spine density and dendritic length of nucleus accumbens and hippocampus neurons in rat offspring. *Synapse*. 2009; 63(9):794–804. [PubMed: 19489049]
- Matrisciano F, Tueting P, Dalal I, Kadriu B, Grayson DR, Davis JM, Nicoletti F, Guidotti A. Epigenetic modifications of GABAergic interneurons are associated with the schizophrenia-like phenotype induced by prenatal stress in mice. *Neuropharmacology*. 2013; 68:184–194. [PubMed: 22564440]
- McCarthy, MM., Nugent, BM. At the frontier of epigenetics of brain sex differences; *Front Behav Neurosci*. 2015. p. 9doi:<http://dx.doi.org/10.3389/fnbeh.2015.00221>
- McCarthy MM, Auger AP, Bale TL, De Vries GJ, Dunn GA, et al. The epigenetics of sex differences in the brain. *J Neurosci*. 2009; 29(41):12815–12823. [PubMed: 19828794]
- Mill J, Petronis A. Pre- and peri-natal environmental risks for attention-deficit hyperactivity disorder (ADHD): the potential role of epigenetic processes in mediating susceptibility. *J Child Psychol Psychiatry*. 2008; 49(10):1020–1030. [PubMed: 18492038]
- Nugent BM, McCarthy MM. Epigenetic underpinnings of developmental sex differences in the brain. *Neuroendocrinology*. 2011; 93(3):150–158. [PubMed: 21411982]

- Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*. 2008; 3(2):97–106. [PubMed: 18536531]
- Parkel S, Lopez-Atalaya JP, Barco A. Histone H3 lysine methylation in cognition and intellectual disability disorders. *Learn Mem*. 2013; 20(10):570–579. [PubMed: 24045506]
- Peixoto L, Abel T. The role of histone acetylation in memory formation and cognitive impairments. *Neuropsychopharmacology*. 2013; 38(1):62–76. [PubMed: 22669172]
- Pittenger C, Kandel ER. In search of general mechanisms for long-lasting plasticity: aplysia and the hippocampus. *Philos Trans R Soc Lond B Biol Sci*. 2003; 358(1432):757–763. [PubMed: 12740123]
- Rossi-George A, Virgolini MB, Weston D, Thiruchelvam M, Cory-Slechta DA. Interactions of lifetime lead exposure and stress: behavioral; neurochemical and HPA axis effects. *Neurotoxicology*. 2011; 32(1):83–99. [PubMed: 20875452]
- Sanchez-Martin FJ, Lindquist DM, Landero-Figueroa J, Zhang X, Chen J, Cecil KM, Medvedovic M, Puga A. Sex- and tissue-specific methylome changes in brains of mice perinatally exposed to lead. *Neurotoxicology*. 2015; 46:92–100. [PubMed: 25530354]
- Schneider JS, Anderson DW, Kidd SK, Sobolewski M, Cory-Slechta DA. Sex-dependent effects of lead and prenatal stress on post-translational histone modifications in frontal cortex and hippocampus in the early postnatal brain. *Neurotoxicology*. 2016; 54:65–71. [PubMed: 27018513]
- Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. Brain development in rodents and humans: identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol*. 2013; 106–107:1–16.
- Senut MC, Cingolani P, Sen A, Kruger A, Shaik A, Hirsch H, Suhr ST, Ruden D. Epigenetics of early-life lead exposure and effects on brain development. *Epigenomics*. 2012; 4(6):665–674. [PubMed: 23244311]
- Shen EY, Ahern TH, Cheung I, Straubhaar J, Dincer A, Houston I, de Vries GJ, Akbarian S, Forger NG. Epigenetics and sex differences in the brain: a genome-wide comparison of histone-3 lysine-4 trimethylation (H3K4me3) in male and female mice. *Exp Neurol*. 2015; 268:21–29. [PubMed: 25131640]
- Strahl BD, Allis CD. The language of covalent histone modifications. *Nature*. 2000; 403(6765):41–45. [PubMed: 10638745]
- Takizawa T, Nakashima K, Namihira M, Ochiai W, Uemura A, Yanagisawa M, Fujita N, Nakao M, Taga T. DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. *Dev Cell*. 2001; 1(6):749–758. [PubMed: 11740937]
- Thayer ZM, Kuzawa CW. Early origins of health disparities: material deprivation predicts maternal evening cortisol in pregnancy and offspring cortisol reactivity in the first few weeks of life. *Am J Hum Biol*. 2014; 26(6):723–730. [PubMed: 24599586]
- Van den Hove DL, Kenis G, Brass A, Opstelten R, Rutten BP, Bruschettini M, Blanco CE, Lesch KP, Steinbusch HW, Prickaerts J. Vulnerability versus resilience to prenatal stress in male and female rats; implications from gene expression profiles in the hippocampus and frontal cortex. *Eur Neuropsychopharmacol*. 2013; 23(10):1226–1246. [PubMed: 23199416]
- Virgolini MB, Rossi-George A, Lisek R, Weston DD, Thiruchelvam M, Cory-Slechta DA. CNS effects of developmental Pb exposure are enhanced by combined maternal and offspring stress. *Neurotoxicology*. 2008; 29(5):812–827. [PubMed: 18440644]
- Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci*. 2004; 7(8):847–854. [PubMed: 15220929]
- Whitlock JR, Heynen AJ, Shuler MG, Bear MF. Learning induces long-term potentiation in the hippocampus. *Science*. 2006; 313(5790):1093–1097. [PubMed: 16931756]
- Ye F, Chen Y, Hoang T, Montgomery RL, Zhao XH, Bu H, Hu T, Taketo MM, van Es JH, Clevers H, Hsieh J, Bassel-Duby R, Olson EN, Lu QR. HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the beta-catenin-TCF interaction. *Nat Neurosci*. 2009; 12(7):829–838. [PubMed: 19503085]

Yuan W, Holland SK, Cecil KM, et al. The impact of early childhood lead exposure on brain organization: a functional magnetic resonance imaging study of language function. *Pediatrics*. 2006; 118(3):971–977. [PubMed: 16950987]

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Fig. 1.

Group mean $\pm$ SEM normalized PTHM levels (H3K9Ac, H3K4Me3, H3K9Me2 and H3K27Me3) from 4 to 5 biological replicates in frontal cortex examined in female and male 0-NS control animals at each developmental time point. Results of statistical analysis: Day = significant main effect of day. * = significantly different from corresponding E18 treatment group value.

Fig. 2.

Group mean $\pm$ SEM normalized PTHM levels (H3K9Ac, H3K4Me3, H3K9Me2 and H3K27Me3) from 4 to 5 biological replicates examined in female frontal cortex for indicated treatment groups (0-NS = no Pb, no stress; 0-PS = no Pb + prenatal stress; 100-NS = 100 ppm Pb + no stress; 100-PS = 100 ppm Pb + prenatal stress). Results of statistical analysis: Day = significant main effect of Day, PS = Significant main effect of PS, $\times$ Day $\times$ PS = Significant interaction of Day by PS, Day $\times$ Pb $\times$ PS = Significant interaction of Day by Pb by PS. * = significantly different from 0-NS control at indicated day.

Fig. 3. Group mean $\pm$ SEM normalized PTHM levels (H3K9Ac, H3K4Me3, H3K9Me2 and H3K27Me3) from 4 to 5 biological replicates examined in male frontal cortex for indicated treatment groups (0-NS = no Pb, no stress; 0-PS = no Pb + prenatal stress; 100-NS = 100 ppm Pb+ no stress; 100-PS = 100 ppm Pb + prenatal stress). Results of statistical analysis: Day = significant main effect of Day, PS = Significant main effect of PS, Day $\times$ PS = Significant interaction of Day by PS, Day $\times$ Pb $\times$ PS = Significant interaction of Day by Pb by PS. * = significantly different from 0-NS control at indicated day.

Fig. 4.

Group mean $\pm$ SEM normalized PTHM levels (H3K9Ac, H3K4Me3, H3K9Me2 and H3K27Me3) in hippocampus from 4 to 5 biological replicates examined in female and male 0-NS control animals at each developmental time point. Results of statistical analysis: Day = significant main effect of day. * = significantly different from corresponding E18 treatment group value.

Fig. 5. Group mean $\pm$ SEM normalized PTHM levels (H3K9Ac, H3K4Me3, H3K9Me2 and H3K27Me3) from 4 to 5 biological replicates examined in female hippocampus for indicated treatment groups (0-NS = no Pb, no stress; 0-PS = no Pb + prenatal stress; 100-NS = 100 ppm Pb+ no stress; 100-PS = 100 ppm Pb + prenatal stress). Results of statistical analysis: Day = significant main effect of Day, PS = Significant main effect of PS, Day $\times$ PS = Significant interaction of Day by PS, Day $\times$ Pb $\times$ PS = Significant interaction of Day by Pb by PS. * = significantly different from 0-NS control at indicated day.

Fig. 6.

Group mean $\pm$ SEM normalized PTHM levels (H3K9Ac, H3K4Me3, H3K9Me2 and H3K27Me3) from 4 to 5 biological replicates examined in male hippocampus for indicated treatment groups (0-NS = no Pb, no stress; 0-PS = no Pb + prenatal stress; 100-NS = 100 ppm Pb+ no stress; 100-PS = 100 ppm Pb + prenatal stress). Results of statistical analysis: Day = significant main effect of Day, PS = Significant main effect of PS, Day $\times$ PS = Significant interaction of Day by PS, Day $\times$ Pb $\times$ PS = Significant interaction of Day by Pb by PS. * = significantly different from 0-NS control at indicated day.

Table 1

Changes in FC PTHM levels by day for each of the treatment groups relative to E18.

	FEMALE FRONTAL CORTEX											
	H3K9Ac			H3K4Me3			H3K9Me2			H3K27Me3		
	PND0	PND6	PND60	PND0	PND6	PND60	PND0	PND6	PND60	PND0	PND6	PND60
0-NS	↓	↑	↓	↑				↑	↓			↓
0-PS	↓		↓	↑				↑	↓			
100-NS	↑	↓	↓	↑	↑		↑		↓	↑		
100-PS	↓	↑	↓	↑	↑		↑		↓	↑		↓
	MALE FRONTAL CORTEX											
	H3K9Ac			H3K4Me3			H3K9Me2			H3K27Me3		
	PND0	PND6	PND60	PND0	PND6	PND60	PND0	PND6	PND60	PND0	PND6	PND60
0-NS	↑	↑	↓		↑		↑	↑		↑	↑	
0-PS	↑	↑		↑	↑		↑	↑			↑	
100-NS	↑	↑	↓		↑		↑	↑			↑	
100-PS	↑	↑			↑		↑	↑		↑	↑	

↑ indicates significant increase and ↓ indicates significant decrease in PTHMs levels relative to E18.

Table 2

Changes in HIPP PTHM levels by day for each of the treatment groups relative to E18.

	FEMALE HIPPOCAMPUS											
	H3K9Ac			H3K4Me3			H3K9Me2			H3K27Me3		
	PND0	PND6	PND60	PND0	PND6	PND60	PND0	PND6	PND60	PND0	PND6	PND60
0-NS	↓	↑	↑		↑			↑			↑	↑
0-PS		↑	↑	↑	↑	↑	↑	↑	↑	↑		↑
100-NS		↑		↑	↑	↑		↑			↑	↑
100-PS		↑		↑	↑		↑	↑		↑	↑	
	MALE HIPPOCAMPUS											
	H3K9Ac			H3K4Me3			H3K9Me2			H3K27Me3		
	PND0	PND6	PND60	PND0	PND6	PND60	PND0	PND6	PND60	PND0	PND6	PND60
0-NS		↑			↑			↑		↑	↑	↑
0-PS	↑	↑		↑	↑		↑	↑		↑	↑	
100-NS	↑	↑		↑	↑		↑	↑	↑	↑	↑	↑
100-PS	↑	↑		↑	↑		↑	↑		↑	↑	

↑ indicates significant increase and ↓ indicates significant decrease in PTHM levels relative to E18.