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Developmental Lead and/or Prenatal Stress Exposures Followed by Different Types of Behavioral Experience Result in the Divergence of Brain Epigenetic Profiles in a Sex, Brain Region, and Time-Dependent Manner: Implications for Neurotoxicology.

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Developmental Lead and/or Prenatal Stress Exposures Followed by Different Types of Behavioral Experience Result in the Divergence of Brain Epigenetic Profiles in a Sex, Brain Region, and Time-Dependent Manner: Implications for Neurotoxicology

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Abstract

Over a lifetime, early developmental exposures to neurocognitive risk factors, such as lead (Pb) exposures and prenatal stress (PS), will be followed by multiple varied behavioral experiences. Pb, PS and behavioral experience can each influence brain epigenetic profiles. Our recent studies show a greater level of complexity, however, as all three factors interact within each sex to generate differential adult variation in global post-translational histone modifications (PTHMs), which may result in fundamentally different consequences for life-long learning and behavioral function. We have reported that PTHM profiles differ by sex, brain region and time point of measurement following developmental exposures to Pb±PS, resulting in different profiles for each unique combination of these parameters. Imposing differing behavioral experience following developmental Pb±PS results in additional divergence of PTHM profiles, again in a sex, brain region and time-dependent manner, further increasing complexity. Such findings underscore the need to link highly localized and variable epigenetic changes along single genes to the highly-integrated brain functional connectome that is ultimately responsible for governing behavioral function. Here we advance the idea that increased understanding may be achieved through iterative reductionist and holistic approaches. Implications for experimental design of animal studies of developmental exposures to neurotoxicants include the necessity of a ‘no behavioral experience’ group, given that epigenetic changes in response to behavioral testing can confound effects of the neurotoxicant itself. They also suggest the potential utility of the inclusion of salient behavioral experiences as a potential effect modifier in epidemiological studies.

Keywords

behavioral experience; lead; stress; epigenetics; frontal cortex; hippocampus; connectome

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INTRODUCTION

1. Deleterious Effects of Developmental Exposures to Lead and Prenatal Stress on the Central Nervous System (CNS): Potential Contributions of Epigenetic Alterations

Environmental agents that impact children's cognitive development and function include chemical stressors, e.g., exposures to lead (Pb), as well as non-chemical stressors, such as prenatal stress (PS). Both Pb and PS have been reported to produce cognitive deficits and reductions in IQ, attention deficits, and later behavioral problems in children, findings that are paralleled in animal models [1–12]. These common adverse consequences of Pb and PS likely arise through their shared biological targets, specifically the HPA axis [13–15] and the brain mesocorticolimbic system that includes prefrontal cortex and hippocampus [16–24]; additionally, the HPA axis and brain mesocorticolimbic systems are highly interactive [25].

There is increasing interest in the role of epigenetic impacts in brain of such exposures and their potential relationships to associated behavioral toxicology and neurotoxicity. Epigenetic alterations have been described in response to Pb and to PS in both frontal cortex (FC) and in hippocampus (HIPP), components of the mesocorticolimbic system that are critical to the mediation of cognitive function [26, 27], suggesting such modifications could relate to associated cognitive deficits. For example, developmental Pb alters the expression of DNA methyltransferases and DNA-binding proteins (DNMT1, DNMT3a, and MeCP2) in rat HIPP, with effects expressed differently by sex [28]. Analysis of genome wide DNA methylation in the cortex (region(s) not specified) and whole HIPP of 2 month old C57/Bl6 mice from dams with gestational and lactational blood Pb levels of 0.9–1.3 μg/dL (levels in offspring not reported) showed sex-dependent Pb effects on DNA methylation [29]. Studies of developmental Pb on Alzheimer's-like disease report lifelong changes in global PTHM levels in cortex after exposures to 0 or 0.2% Pb acetate in drinking water via the dam from birth to weaning. Initial Pb-induced increases in the activating mark H3K9Ac were seen in 20 day old males followed by subsequent reductions out to 700 days of age, while reductions in the repressive mark H3K4Me2 were lifelong; levels of the repressive mark H3K27Me3 were increased over this total time period [30].

Epigenetic changes in CNS also occur in response to PS. Among such changes are increased methylation of BDNF exon IV critical to synaptic plasticity [31] and altered expression levels of the gene *Gpm6a* which plays an important role in neuronal differentiation and plasticity via changes in DNA methylation status and posttranscriptional regulation by microRNAs [32]. Numerous genes involved in epigenetic regulation were differentially expressed after PS in rats, including HDAC4, methyltransferase-like 2 and MBD [33]. PS at different times during gestation in mice produced changes in corticotropin-releasing hormone and glucocorticoid receptor gene methylation profiles, with consequences that were sex-specific [34]. A number of changes in expression levels of DNA methyltransferases (DNMT1, DNMT3a) in FC and HIPP, particularly in GABAergic interneurons, were reported in mice exposed to PS [35].

As alluded to above, such changes are often sex-specific [34, 36–38]. Our recent studies, however, which have focused to date primarily on global post-translational histone

modifications (PTHMs), suggest far greater complexity in CNS epigenetic profiles that will need to be considered in any elaboration of epigenetic mechanisms underlying CNS behavioral function and dysfunction. In addition to these consistent sex differences [38] are both brain region- and time point of measurement-dependent differences in brain epigenetic responses to Pb and PS. For example, offspring of C57Bl6 mouse dams treated from 2 mos prior to breeding through lactation with 0 or 100 ppm Pb drinking solutions combined with either no prenatal restraint stress or PS administered 3x/day for 30 min each time from gestational days 11–19 showed brain region differences as indicated by differing PTHM profiles in FC as compared to HIPP [37]. Each treatment group (0-NS: no Pb, no PS; 0-PS: no Pb, PS; Pb-NS: Pb, no PS; Pb-PS: Pb and PS) showed unique transitions in PTHMs from P0 to P6 and these treatment-related differences varied between the HIPP and FC. Specifically, in HIPP, reductions in expression levels of both marks were seen between PND0 and PND6 in females, with no impacts of Pb±PS. In contrast, expression levels of these marks increased across time in male HIPP, and were influenced by Pb±PS: at PND6, Pb reduced expression levels of H3K9/14Ac by 50%, and Pb+PS reduced levels of H3K9Me3. In FC, expression levels of both marks again decreased significantly between PND0 and PND6 in females, but here Pb±PS did influence outcomes: Pb alone reduced expression levels of H3K9/14Ac at PND0 and PS alone reduced levels of H3K9Me3. In male FC, expression levels of both marks also declined between PND0 and PND6, while PS alone significantly increased H3K9/14Ac at PND0 [37].

Subsequent extension of these studies to a panel of 4 PTHMs (global levels of H3K9ac, H3K4Me3, H3K9Me2 and H3K27Me3) examined at embryonic day 18 and PND0, 6 and 60 in FC and HIPP further underscored these complexities [39]. Sex, brain region and time-dependent changes occurred in controls even in the absence of Pb±PS. Influences of Pb±PS were likewise sex, brain region and time-dependent. For example, developmental Pb exposure increased expression levels of all PTHMs in FC at PND0 in female, but not male, offspring, whereas males showed a prominent increase in expression levels of all 4 PTHM at PND6 that was further increased by Pb+PS. In HIPP, increases in expression levels in response to PS only were seen in females at PND60, as were significant reductions in H3K9Ac and H3K27Me3 in response to Pb+PS, whereas prominent increases at PND6 were again seen in males, but tended to be significantly reduced by prior PS exposures.

Results from these studies indicate that at *any given time point, global PTHM profiles differ by context, i.e., developmental exposure conditions* (control, Pb, PS or Pb+PS), *sex and brain region*, as summarized in Table 1 adapted from data taken at PND6 from a recently reported study from our laboratories [39]. It presents changes in the expression levels of the 4 PTHMs (H3K9Ac, H3K4Me3, HeK9Me2 and H3K27Me3) as a percent of control group expression levels (100%) for all treatment groups (rows) [39], with arrows indicating direction of change. Table 1 shows that Pb alone produced almost no changes in FC or HIPP PTHM expression levels at this time point in males, whereas PS alone increased FC expression levels, but reduced levels in HIPP of all 4 PTHMs, while Pb+PS increased levels of all marks in both FC and HIPP. Patterns in females differed from those in males, with PS alone decreasing HIPP global expression levels of all 4 marks, and reducing H3K27Me3 in FC, whereas Pb alone reduced global expression levels relative to controls of the PTHMs H3K9Ac, H3K9Me2 and H3K27Me3 and generated minor increases in all 4 PTHMs in

HIPP. Pb+PS females exhibited robust increases in all 4 PTHMs in FC, but reduced expression levels of H3K9Ac in HIPP. Based on the known critical interactions of FC and HIPP in mediating cognitive function [40, 41], interactions between these two regions would now also likely differ from control. Consequently, near the time of birth, PTHM profiles of all groups of both sexes differ within and across brain regions, as summarized in Table 1, likely influencing interactions between FC and HIPP.

In sum, brain epigenetic responses to such CNS stressors as Pb exposure and PS are not only sex-dependent, but also brain region dependent [37, 39]. Furthermore, these profiles change over time, meaning that conclusions or hypotheses generated from a single point in time, particularly during development, may not reflect trends though development or final adult epigenetic profiles, indicating the significant impact of environmental contexts in determination of these PTHM profiles. Of course, our assessments to date, in beginning these studies, have focused on global PTHM expression levels in these brain regions, and thus they represent a type of averaging which may obscure more localized gene and regional changes still to be elaborated. In addition, the brain is composed of multiple cell types and multiple areas within each region, which studies are showing likewise differ in their epigenetic response to environmental events [42, 43]. Extension to these additional factors will provide additional details, that, in concert with global patterns, may help facilitate understanding of how Pb, PS and behavioral experience influence epigenetic profiles, CNS protein production and regional function; all of which are needed for appropriate behavioral output.

2. Contrasting Behavioral Experiences Following Developmental Exposures to Pb and/or PS Result in Further Divergence of Brain Epigenetic Profiles

Of course, birth is inevitably followed by exposures to other environmental events that will encompass a wide range of different conditions and evoke behavioral responses from an individual (i.e., they will undergo behavioral experience). Such conditions can include events that are adverse/negative (e.g., early behavioral adversity, such as parental divorce, child abuse or neglect) or positive (parental affection and attention, reward, accomplishment, stability, etc.) in nature and, importantly, these experiences further impact future CNS function [44–47]. Such behavioral experiences have been shown to alter brain epigenetic profiles. For example, in rodents, differences in levels of early maternal care produce functional and persistent changes in DNA methylation of the glucocorticoid receptor [48]. Early caregiver maltreatment altered mRNA levels of epigenetic regulators Dnmt1, Dnmt3a, MeCP2, Gadd45b and HDAC 1 in adult male rats and significantly decreased Gadd45b in females [49]. Conversely, early environmental enrichment in mice increased histone acetylation of the BDNF gene in adulthood, but BDNF gene expression levels increased only after stimulation, suggesting that early enrichment permitted rapid increases in BDNF in response to a challenge [50]. Similarly, numerous human studies of traumatic stress during the prenatal period are associated with changes in DNA methylation [44, 51, 52].

As illustrated in Figure 1 (shown for one sex only)**,** environmental events early after birth would act upon epigenetic profiles that already differ in response to developmental Pb, PS and Pb+PS exposure for each sex, i.e., these environmental events act upon already

differentiated epigenetic profiles [77], and thus could result in further divergence of the trajectories of these profiles, based on the unique combination of early insults and types of environmental experience. Even under no exposure conditions (control), different environmental experiences could produce divergence of the consequent brain epigenetic profile.

To further simulate the human environmental trajectory of in utero exposures followed by other environmental events, offspring from each of the 4 treatment groups per sex (0-NS, 0-PS, Pb-NS, Pb-PS) were subsequently exposed to either no behavioral experience, positive behavioral experience or negative behavioral experience, with no more than 1 pup/sex used from each dam to preclude any litter specific effects within behavioral experience groups. 'Positive' behavioral experience consisted of exposure to 30 behavioral test sessions in which food reward was available on a fixed interval (FI) 60 second schedule of reinforcement [53–55] in operant chambers (Med Associates, Model ENV-307W) housed in sound-attenuating cabinets equipped with fans for ventilation and white noise generation. Three levers were located horizontally across the back wall of the chamber, with a liquid dipper and dual pellet dispenser for reinforcer (20 mg food pellet; PJ Noyes) delivery on the front (opposite) wall. On the FI schedule, the first lever press response occurring on the designated active lever after the 60 second interval elapsed, resulted in food delivery and initiated the next 60 second interval. Responses during the 60 second interval itself had no programmed consequences. Sessions lasted 30 min and a total of 30 sessions were carried out on a M-F schedule. 'Negative' behavioral experience consisted of exposure to a single 5 min session of forced swim followed one week later by a single 30 min episode of restraint stress. These were specifically limited to one experience each to preclude habituation effects. For restraint stress, mice were placed in a restrictive Plexiglas tube where they remained for a period of 30 minutes. In the forced swim paradigm, mice were placed in a pail of water from which escape was not possible, and which was too deep to allow mice to use their tail to balance. For the 'no behavioral experience' condition, mice simply remained in their home cages (paired by sex and treatment) during the entire period of behavioral experience afforded to the other groups. Since the positive behavioral experience, i.e., FI schedule of reward, required mice to be food-motivated, mice in all groups were food restricted to maintain 90% of their ad-lib weights beginning at approximately 50 days of age for the duration of the experiment.

As suggested by **a** growing literature describing the impacts of early behavioral adversity on brain epigenetic profiles [56–58], Figure 1 hypothesizes that these different behavioral experiences will produce further divergence of trajectories of epigenetic profiles and do so in a sex and brain region-dependent manner, as the different patterns/shapes and arrows under all different conditions are intended to convey. Indeed, Table 2 illustrates examples of some observed differences in global PTHM levels for the Pb+PS group relative to the control group for H3K4Me3 in males and H3K27Me3 for females calculated as a percent of the non-behavioral control group expression levels (Cory-Slechta et al., unpublished data). As before, the different shapes/patterns for each condition convey the different epigenetic profiles already associated with each condition. Subsequently, variations in behavioral experiences can differentially influence epigenetic profiles even in the absence of

developmental exposures to Pb±PS. In control males for example, negative behavioral experience reduced H3K4Me3 levels in FC by 27%, whereas positive behavioral experience reduced expression levels of this mark by 47% in HIPP. In control females, little impact of either positive or negative experience relative to no behavioral experience was seen on levels of H3K27Me3 expression in FC, but both experiences reduced expression of this mark by >30% in HIPP. As can also be seen, these different types of behavioral experiences produced divergent profiles in groups exposed to Pb+PS, which again differed by sex and brain region as well as from profiles in the control group. In the male Pb+PS group, positive experience further increased FC H3K4Me3 expression levels by 52%, but negative experience further decreased this mark by 48% in HIPP. In the female Pb+PS group, both types of behavioral experience reduced H3K27Me3 expression levels in FC by 20–30% as did positive behavioral experience in HIPP by 54%.

While these data derive from animal models of developmental exposures to Pb, PS or Pb+PS and to specific behavioral experiences, the resulting complexities are not likely to be unique to these particular developmental exposures or behavioral experiences, nor to FC and HIPP. Epigenetic changes occur throughout the brain in response to environmental events. In fact, a recent study measuring *in vivo* epigenetic imaging of HDACs in human subjects showed them to be simultaneously highly expressed in multiple brain regions, with differences between gray and white matter as well as between cortical and subcortical gray matter [59]. Further, histones work in ‘codes’ via multiple post-translational modifications occurring at their N-terminal tails which exhibit significant cross talk at a local level, producing dynamic and flexible chromatin marking that ultimately determines the pattern of gene expression to a given environmental stimulus; these changes in histone codes have been shown to vary in a region-specific manner following similar cognitive/behavioral stimuli, suggesting that multiple changes are occurring at single genes culminating in global changes that vary across multiple brain regions following the integrated behavioral response [60].

How such widespread and yet highly localized epigenetic impacts within the CNS ultimately relate to brain function and behavior is a critical question, as the brain is a highly-networked interactive system of systems [61, 62], with an extensive body of fMRI studies demonstrating existence of overlapping functional (behavioral) networks [63]. Indeed, CNS responses represent the integrated output of a network of systems of the brain, and not the modular response of a specific brain region or site or cell type [64–66]. *Thus, the networked brain must somehow be linked to patterns of localized epigenetic changes.* Correspondingly, studies in both human and mouse/rat brain demonstrate that the functional connectivity of brain regions can actually be predicted by clustered gene expression data, particularly of synaptic activity-related genes [67, 68]. Data sciences could begin to assist in determining whether this linkage relies on patterns of specific epigenetic changes or corresponding emergent global epigenetic consequences. Further, human behavior is highly dynamic meaning that linkages must be extremely rapid; removing your hand from a flame is far faster than the time required to produce changes in epigenetic profiles, gene transcription and subsequent protein production.

Most behavioral epigenetic studies to date have focused on very specific and single behavioral functions, such as learning or drug abuse, and the associated studies have

typically used a very limited set of behavioral paradigms to measure that behavioral function. Assessments of the epigenetic underpinnings of learning and memory, for example, have primarily relied on the use of fear conditioning, a shock-based Pavlovian conditioning paradigm, although a few studies have examined object and spatial memory and taste memory [69] and thus comparative changes in resulting epigenetic profiles in relation to differential characteristics of behavioral experience are as yet unknown.

However, the differential epigenetic impact of specifically divergent behavioral experiences, as observed in our studies, is not without precedent, although such comparisons have been extremely limited. The effects of histone deacetylase inhibitors, compounds that increase levels of acetylation at histone tails and thus open up chromatin structure and facilitate gene expression [70], were examined in honeybees. Changes in rewarding vs. aversive olfactory conditioning (memories) were compared: bees were trained to discriminate between rewarding (positive conditioned stimulus; limonene) vs. aversive (negative conditioned stimulus; natural vanilla) odors that were the conditioned stimuli for sucrose vs. saturated NaCl solutions, respectively. As it showed, histone deacetylase inhibitors systematically impaired discrimination memory of aversive stimuli, but had no impact on reward-based conditioning, despite the fact that both were measures of learning. These effects were shown not to reflect differences in sucrose sensitivity or locomotor activity [70].

A study by Bousiges et al [71] suggests controllability vs. uncontrollability in the environmental experience as a modulator of PTHM changes as well. Specifically, they compared global dorsal hippocampal histone acetylation in rats that underwent water maze learning with a constant location visible platform from which escape was possible (hidden platform; controllable/predictable stressor) vs. one in which the platform was moved on each trial (variable platform; uncontrollable/unpredictable stressor). Under those conditions, significant increases were seen in H2B and H4 acetylation levels in the hidden platform group relative to the variable platform group. In another experiment, H3 acetylation expression was markedly increased in the variable platform experienced animals relative to levels obtained from rats that simply remained in their home cage. Such findings are consistent with our results described above and with the fact that differences in the nature of the environmental experience can markedly influence the epigenetic outcome.

That differences in environmental experience lead to divergence of epigenetic profiles is also suggested by the findings of Cheung et al. [43] in human post-mortem tissue. This study showed that while there existed highly significant correlations of neuron-enriched H3K4me3 peaks across 11 human subjects, there were also significant individual differences in human prefrontal cortex neuronal H3K4me3 epigenomes, likely reflecting the different environmental experiences of these individuals.

3. Challenges to Understanding the Epigenetic Profiles Arising from the Convergence of Adverse Neurodevelopmental Exposures and Subsequent Behavioral Experience

The primary intention of this article is to highlight the interactive effects upon the brain epigenome of developmental behavioral and toxicant exposures with subsequent environmental events that may ultimately result in behavioral toxicity. However, it also underscores the significant need to link the complexity of regionally-specific changes in

epigenetic profiles to differences in inter-region brain function and ultimately to behavior, a linkage likely not achievable based on a totally reductionist strategy [72]. These findings from our studies summarized above indicate that depictions of an effect of Pb or PS on brain histone marks are highly context-dependent. In that spirit, the complexity demonstrated to date in our studies generates many exciting new questions and experimental design implications for environmental neurotoxicology.

A. Environmental Experiences per se Can Modify Brain Epigenetic Profiles, but the Nature of the Experience May Significantly Influence the Resulting Epigenetic Profile: What Characteristics of the Experience Underlie Those Differences?—As the above question implies, it is not presently clear what the critical characteristics are that differentiate the epigenetic impacts of the various behavioral experiences used here, or more broadly in the literature. Is it possible, for example to classify categories of, e.g., positive vs. negative behavioral experiences in which members within each category produce similar epigenetic patterns that would be translatable to human experiences? With respect to operant learning per se, a recent study using money gain vs. money loss in humans suggests that stimulus-presentation based positive reinforcement is actually functionally different from stimulus elimination-based negative reinforcement [73], even though both lead to the same behavioral outcome. What are the characteristics of behavior that would allow such classification and consistent epigenetic pattern induction?

Our choices of positive vs. negative experience were based on our assessment of the controllability/predictability of outcome of these behavioral paradigms, as has been used to distinguish stress-inducing from resilience-producing paradigms [74]. Specifically, food-rewarded responding is both controllable and predictable, whereas the single exposures to forced swim and restraint stress were both uncontrollable and unpredictable and are supported by the findings of Bousiges et al [71] cited above. However, additional systematic research will be required to address other potential differences, e.g., in our studies, the positive behavioral experience paradigm involved food delivery, whereas the negative behavioral experience did not, the negative behavioral experience also required more intense motor behavior. By further operationalizing these behaviors and including additional controls, we will also be able to separate epigenetic alterations associated with metabolism demands or digestion.

As current studies have tended to focus on a single or perhaps two brain regions in assessment of epigenetic profiles, a more holistic networked-based epigenetic characterization has not yet been approached and may indefinitely remain cost prohibitive, even though a sizeable literature documents the fact that brain function is characterized by overlapping large scale functional networks (the connectome, [61, 62]), and that network connectivity can actually be predicted by local clustered gene expression, corresponding to the interactive nature of genetic underpinnings of brain function [67, 75].

B. What are the Implications for Experimental Design of Neurotoxicology Studies?—The fact that different epigenetic profiles emerge in response to different environmental experiences has critical implications for the design of experimental studies of epigenetic effects of neurodevelopmental environmental stressors, such as Pb and/or PS. Our

previous studies [76, 77] confirm that these different behavioral experiences likewise differentiate the effects of Pb ±PS on brain neurochemistry and corticosterone levels and on subsequent cognitive function. If environmental/behavioral experience has the capacity to change brain neurochemistry and epigenetic marks as well as even peripheral hormone levels, then assessment of 'mechanisms' of e.g., Pb at a molecular/biochemical/cellular level in animals that have undergone environmental/behavioral experience might actually reflect an interaction of that behavioral experience with Pb, rather than the impact of Pb alone, thus confounding interpretation. This makes inclusion of a 'no behavior' control group critical in studies aimed at ascertaining epigenetic mechanisms of environmental stressors per se. It is important to note that a 'no behavioral experience' control group is actually an environmental impossibility, as some, albeit deprived, stimulation and behavioral experience occurs even in pair-housed mice. However, the 'no behavior' control group nevertheless provides a requisite alternative group indicating what changes occur based on daily vivarium and social experiences at baseline. These interactions of behavioral experience with in utero events also suggest the importance of understanding environmental experience in human epidemiological studies as a potential covariate or confounder, or, more informatively, as an effect modifier of subsequent epigenetic readouts to assess its interaction with a chemical exposure.

C. The Brain Functions as a Highly-Networked System of Systems: Is There Epigenetic Profile Patterning Across Brain Regions/Networks? Are There Patterns of Signatures of Epigenetic Changes within Brain Systems?—An understanding of the modulation of epigenetic profiles in brain in response to environmental events will ultimately require elaboration of a complex interactive profile of changes, not only locally, but also across the brain [78, 79]. Although many studies have examined brain epigenetic consequences of various chemical exposures or environmental events, it has been more typical to do so in a specific brain region or gene or site rather than across brain regions or at potential correlations across regions [80].

Even then, given the functional connectome of the brain, can a strictly reductionist approach focused on a single brain region, ever yield a full mechanistic understanding of behavioral variation? At another scale, given the highly context-dependent nature of epigenetic changes needed for functional protein transcription, can a single epigenetic change alone ever yield a full mechanistic understanding of the gene-protein relationship? It seems more likely that an iterative reductionist-holistic approach will prove more fruitful, in searching for patterns of epigenetic changes that link to functional brain networks [72]. Such an understanding is critical because it could ideally be utilized to devise specific behavioral interventions to reverse earlier adverse environmental impacts [81].

D. The Human Environment is Dynamic and Environmental Experiences Occur Across the Life Time: The Course and Trajectory of Such Experiences are Unique to Each Individual—The rightmost side of Figure 1 is included to remind us of the fact that the trajectory of environmental events that can influence brain epigenetic marks continues over the course of a human's lifetime, with the sum and time course of such events unique to each individual. This raises several critical questions. How dynamic are epigenetic

changes in brain? Do sequential salient environmental events or exposures simply modify epigenetic profiles that have already been altered by former exposures or events? Are some epigenetic changes produced by specific experiences more persistent than others or even intractable? If so, which experiences result in such responses? That the latter can be the case is suggested by studies reporting alterations in promoter methylation of the glucocorticoid receptor gene in leukocyte DNA from healthy adults following early childhood maltreatment [82]. But not all children end up dysfunctional, some are resilient to these adversities [83]. As noted above, such questions may be best approached via an iterative shifting between holism and reductionism [72].

Conclusions

Clearly, there are significant hurdles to advancing the understanding of the epigenetic consequences of environmental stressors for brain and its control over behavior. Understanding the characteristics by which behaviors can be classified or grouped with respect to brain epigenetic impact may provide one strategy to facilitate this understanding. For example, it is important to determine whether common epigenetic profiles exist for any single environmental experience or behavioral domain of interest, i.e., those epigenetic networks/consequences that generalize across different episodes of an environmental event or across behavioral paradigms that measure a specific behavioral function. In the case of behavioral functions, for example, what are the epigenetic profiles that occur across operant learning paradigms; and do they differ from Pavlovian learning? What about positive vs. negative reinforcement operant learning paradigms? Both increase response frequency, but the procedures differ. Are these patterns observable at specific epigenetic targets, global regional patterns or observed in dysfunction of regional connectivity? Presumably, overlapping marks would relate to the learning itself. Comparative studies addressing such questions would help to identify epigenetic changes that relate to the behavioral function and not to the specific paradigm or to specific features of the paradigm or stimuli used, such as shock delivery or food delivery. In addition, it would be useful to compare experiences that differ in specific types of controlling characteristics, e.g., controllable/predictable vs. uncontrollable/unpredictable, extinction (removal of reward) vs. punishment, etc. Such studies should collectively begin to define groupings of events/behaviors and permit more generalizability in terms of consequent epigenetic marks that are important.

Albeit potentially limited by current costs, it will ultimately be important to assess epigenetic profiles more broadly and correlatively across brain regions, recognizing that modularity and site specific changes are only a component of integrated brain function [67, 75]. Looking with both breadth and depth at integration as well as modularity may assist in finding more generalized markers important to specific environmental events or behavioral domains.

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HIGHLIGHTS

- Developmental exposures to Pb, PS and Pb+PS result in sex, brain region and time-dependent changes in expression levels of global PTHMs in FC and HIPP.
- Subsequently imposed behavioral experiences (i.e., interactions of an individual with its environment), differentially influence these PTHMs, resulting in a further divergence of brain epigenetic profiles by type of behavioral experience within each sex and brain region.
- Linking regional and cell-type specific epigenetic changes to systems and patterns of network organization comprising the brain connectome (whole brain structural and functional (behavioral) networks) to ultimately explain behavior is likely to require an iterative reductionist/holistic approach.
- Defining the features of behavioral experiences that produce common brain epigenetic changes could facilitate linkages of regional and cell-type specific epigenetic changes to the functional brain connectome.
- Understanding the epigenetic influences in brain of Pb or PS alone may be confounded by behavioral experience that must be considered in the design of experimental animal studies and could serve as an informative epigenetic effect modifier in human epidemiological studies.

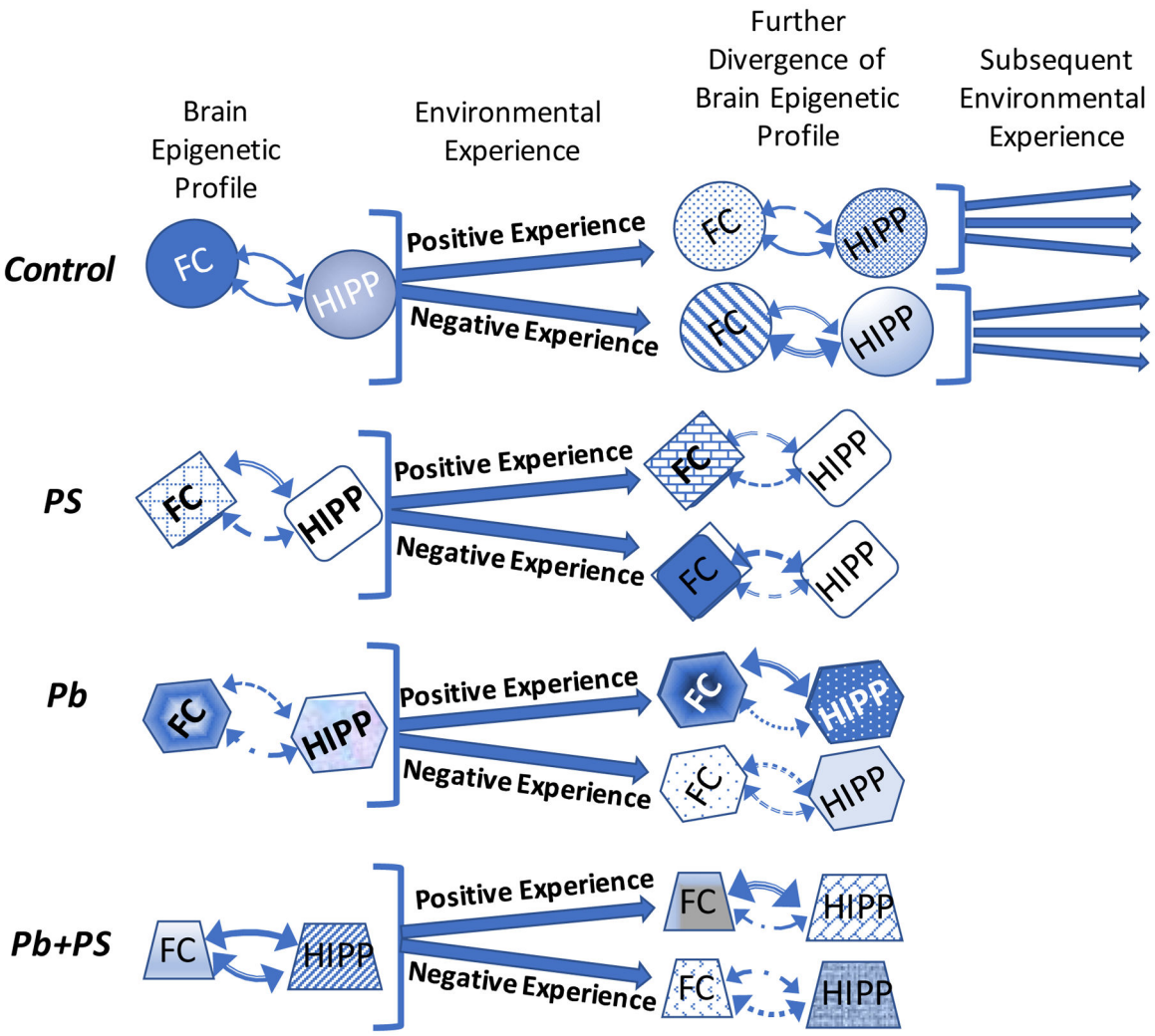

Figure 1.

Schematic depicting the divergence of brain PTHM epigenetic profiles with developmental exposures followed by subsequent behavioral experiences. During the course of brain development, exposures may occur to a variety of insults, here illustrated by Pb, PS or Pb +PS relative to no such exposures. Brain epigenetic profiles are thus altered early in life after such exposures by sex and brain-region, such that the corresponding epigenetic profiles in FC and HIPP differ in each such exposure group (row), as would the interactive functioning of FC and HIPP which is critical to cognitive functions; this is indicated by the different shapes/patterns for each region/developmental treatment condition prior to behavioral experience. Birth is followed by various environmental experiences that themselves can alter epigenetic marks, but these experiences will be acting on an epigenetic base that already differs by group due to prior in utero exposures. This results in further divergence of FC and of HIPP PTHM epigenetic profiles and their interactions as indicated schematically by the further changes in shapes/patterns for each brain region/developmental exposure/behavioral experience conditions. This is illustrated for just one sex, and a completely different set of patterns would occur with females. Over the life course, further environmental experience

will occur over time (far right), providing yet additional such changes. In essence, each individual may end with a unique profile of changes over the course of this trajectory.

Table 1 a

Changes in group mean ± S.E. expression levels of 4 post-translational histone modifications as a percent of the 0-NS control group (baseline) for the indicated treatment groups as measured at postnatal day 6 in frontal cortex and hippocampus

	Frontal Cortex				***Hippocampus***			
Males	**H3K9ac**	**H3K4Me3**	**H3K9Me2**	**H3K27Me3**	**H3K9ac**	**H3K4Me3**	**H3K9Me2**	**H3K27Me3**
0-PS	113±2.3↑	136±30.3↑	106±19.5↑	111±18.2↑	88±4.7↓	77±4.4↓	86±7.2↓	88±5.8↓
100-NS	102±6.6↑	100±13.6-	102±15.8↑	102±6.4↑	100±4.6-	103±10.2↑	99±5.0↓	103±4.2↑
100-PS	120±3.3↑	127±13.9↑	121±6.8↑	122±8.8↑	109±2.0↑	117±4.0↑	112±7.0↑	109±5.8↑
Females	**H3K9ac**	**H3K4Me3**	**H3K9Me2**	**H3K27Me3**	**H3K9ac**	**H3K4Me3**	**H3K9Me2**	**H3K27Me3**
0-PS	93±14.2↓	108±30.0↑	93±16.2↓	80±28.9↓	85±7.3↓	65±2.7↓	81±9.0↓	78±10.8↓
100-NS	73±15.0↓	110±22.4↑	72±17.6↓	79±17.5↓	106±6.5↑	103±11.3↑	104±7.4↑	107±8.3↑
100-PS	118±7.0↑	167±19.8↑	125±13.6↑	118±13.7↑	83±4.9↓	101±14.3↑	95±8.5↓	94±7.3↓

[a]Up arrows indicate increased mean values relative to control, down arrows represent decreased mean values relative to control, while dashes indicate no change. Red shaded boxes represent statistically significant increases relative to control in post-hoc tests following overall ANOVAs with Pb and PS and time (E18, PND0, PND6 and PND60) as factors, while green shaded boxes represent statistically significant decreases. Patterns of arrows differ for each post-translational histone modification by region and sex. Male 0-NS FC control S.E. values were 5.7, 19.6, 10.7 and 9.8. respectively for H3K9ac, H3K4Me3, H3K9Me2 and H3K27Me3, respectively; corresponding values for male HIPP were 2.4, 12.0, 8.4 and 5.2; for female FC 5.5, 9.7, 9.9 and 12.2; for female HIPP 5.9, 14.5, 2.8 and 4.3.

Table 2 a

Group mean ± S.E. changes in expression level of the indicated post-translational histone modification in male and female mice from the control or Pb+PS group along with positive or negative behavioral experience. Values are percent of corresponding no behavioral testing controls (adapted from Cory-Slechta et al., unpublished data).

	Male		Female	
	Frontal Cortex	***Hippocampus***	***Frontal Cortex***	***Hippocampus***
Control	**H3K4Me3**		**H3K27Me3**	
Positive	112±10.3↑	53±14.6↓	97±17.6↓	66±4.4↓
Negative	73±17.4↓	106±6.9↑	91±17.6↓	68±13.9↓
Pb+PS				
Positive	152±11.8↑	53±11.4↓	73±12.4↓	46±19.5↓
Negative	73±16.3↓	52±11.2↓	80±19.5↓	75±14.2↓

[a]Up arrows indicate increased mean values relative to control, down arrows represent decreased mean values relative to control, while dashes indicate no change. Red shaded boxes represent statistically significant increases relative to control in post-hoc tests following overall ANOVAs with behavior, Pb and PS as factors, while green shaded boxes represent statistically significant decreases. Patterns of arrows differ for each post-translational histone modification by region and sex. Male control values for FC were 10.7, 14.5, 17.9 and 12.4 for control non-behavior for FC and HIPP and Pb+PS non-behavior control for FC and HIPP, respectively. Corresponding values for females were 14.2, 9.4, 4.9 and 5.5.