Interaction Between Lead and Stress and Associated Epigenetic Changes

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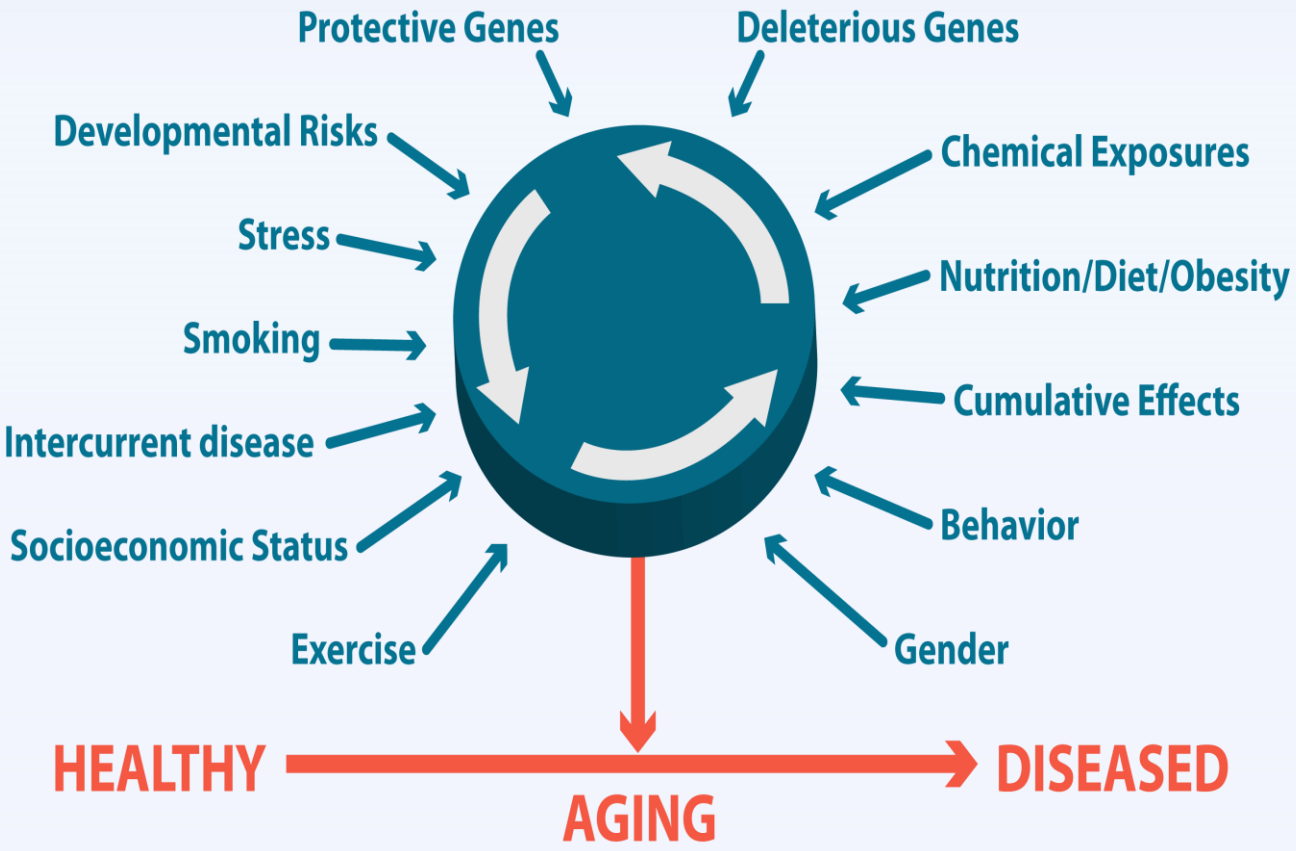
Human Diseases and Disorders Arise From Interactions of Multiple Risk Factors Across Time



Some risk factors may attenuate effects of other risk factors, some risk factors may enhance effects of other risk factors.

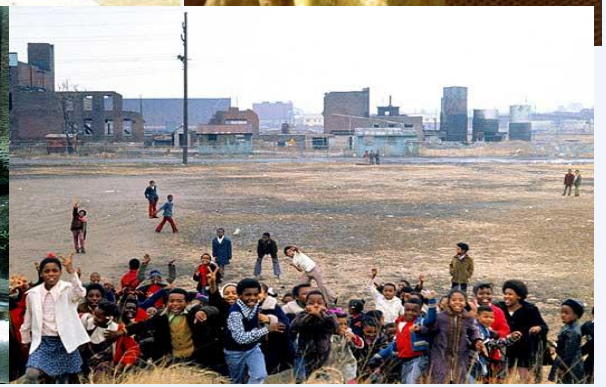
Study Chemical Exposures as Risk Factors in Isolation May Therefore Underestimate Toxicity

- The assessment of neurotoxicity in animal models and epidemiological studies within their relevant human contexts is critical to:
  - Understanding underlying mechanisms
  - Development of behavioral interventions
  - Public health protection

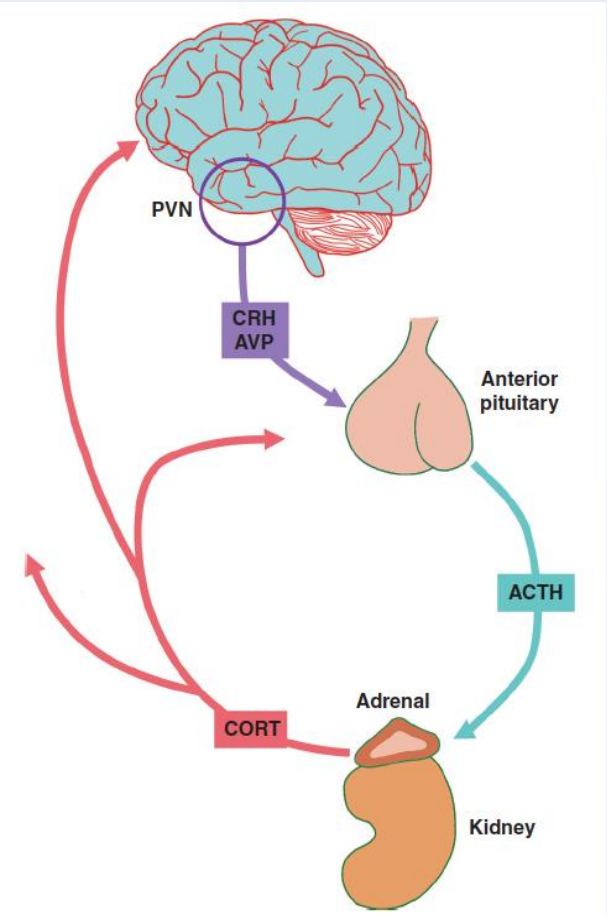
What Considerations Should be Used to Frame Relevant Combinations for Cumulative Risk Assessment?

- Co-occurrence or sequential occurrence of risk factors per se is not enough:
- *Shared Biological Substrates and Common Adverse Outcomes*

Co-Occurring Risk Factors: Neurotoxic Metals and Stress



Stress is Mediated by the HPA Axis

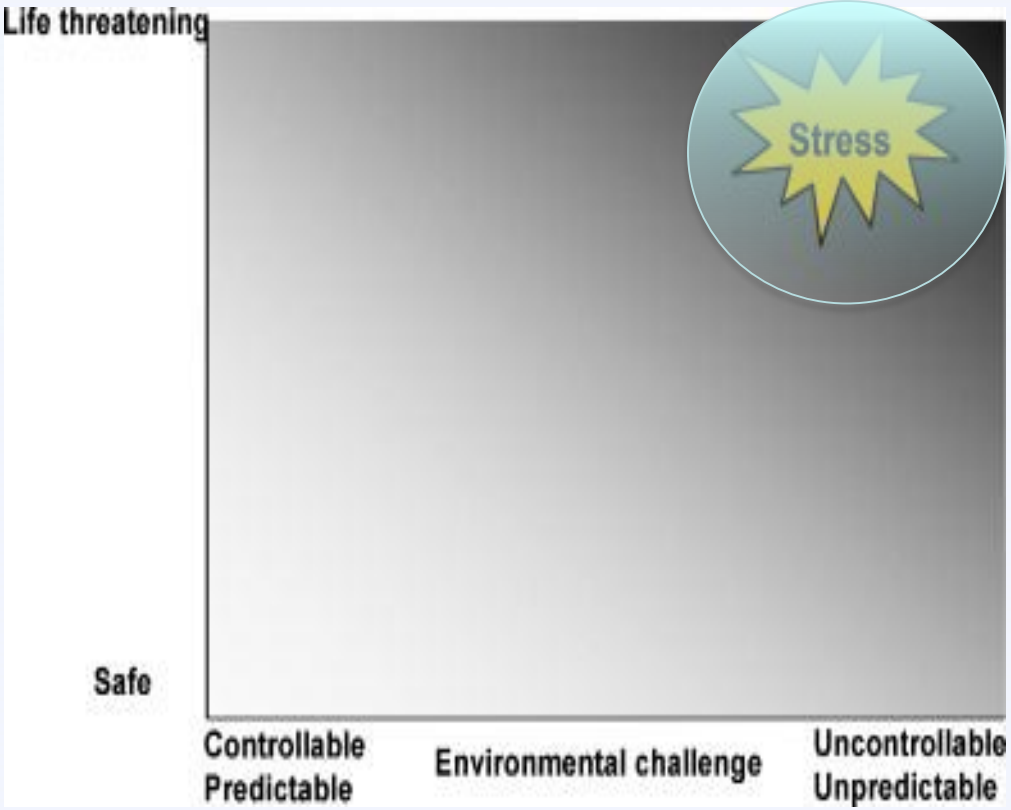


Goel et al., 2014

- Parvocellular neurons in the periventricular nucleus of the hypothalamus produce corticotrophin-releasing hormone (CRH) and vasopressin (AVP)
- CRH and AVP stimulate adrenocorticotrophin (ACTH) synthesis and release from the anterior pituitary corticotroph cells
- This leads to the release of cortisol (rat: corticosterone) from the adrenal cortex.
- Due to the damaging effects of extended glucocorticoid exposure the HPA axis is tightly regulated.
- Glucocorticoids feedback, via glucocorticoid and mineralocorticoid receptors in the limbic (particularly hippocampal) system and glucocorticoid receptors in the PVN and anterior pituitary, to decrease HPA activity.

- All metals appear to alter glucocorticoids.

Not All Stress is Detrimental: Resilience vs. Vulnerability



Schematic illustrating the proposed restriction of the term 'stress' to stimuli that are perceived as uncontrollable, unpredictable and life threatening, whereas those events that are 'controllable and predictable' tend to lead to resiliency phenotypes

Not All Stress is Detrimental: Resilience vs. Vulnerability

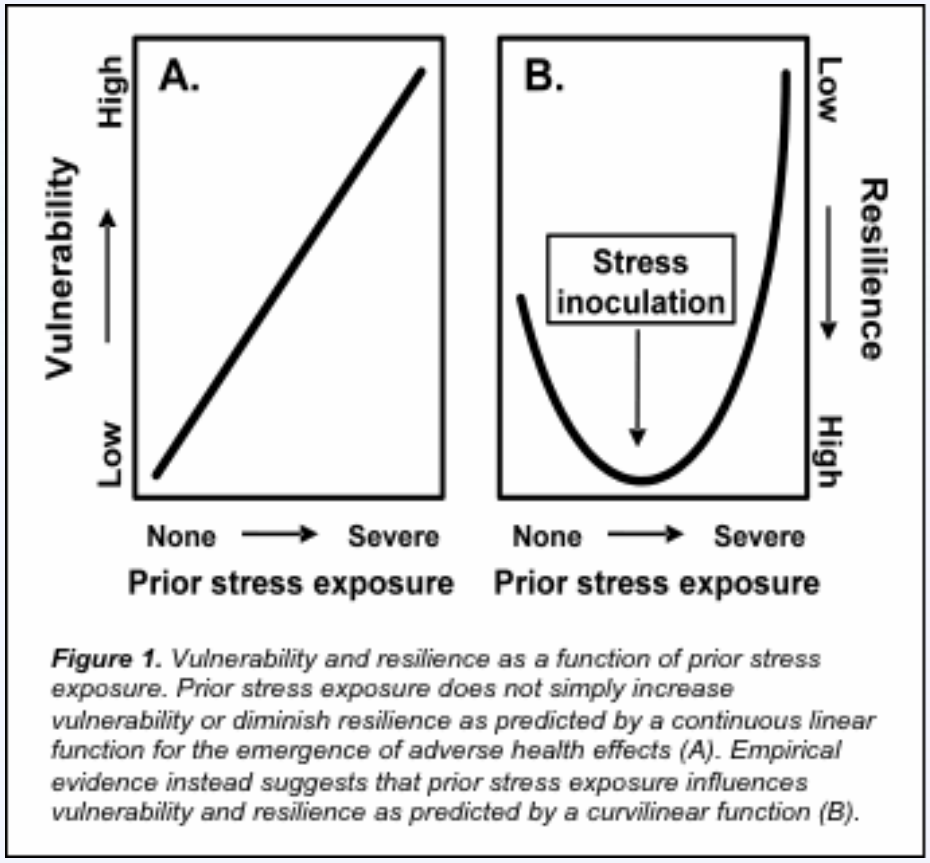
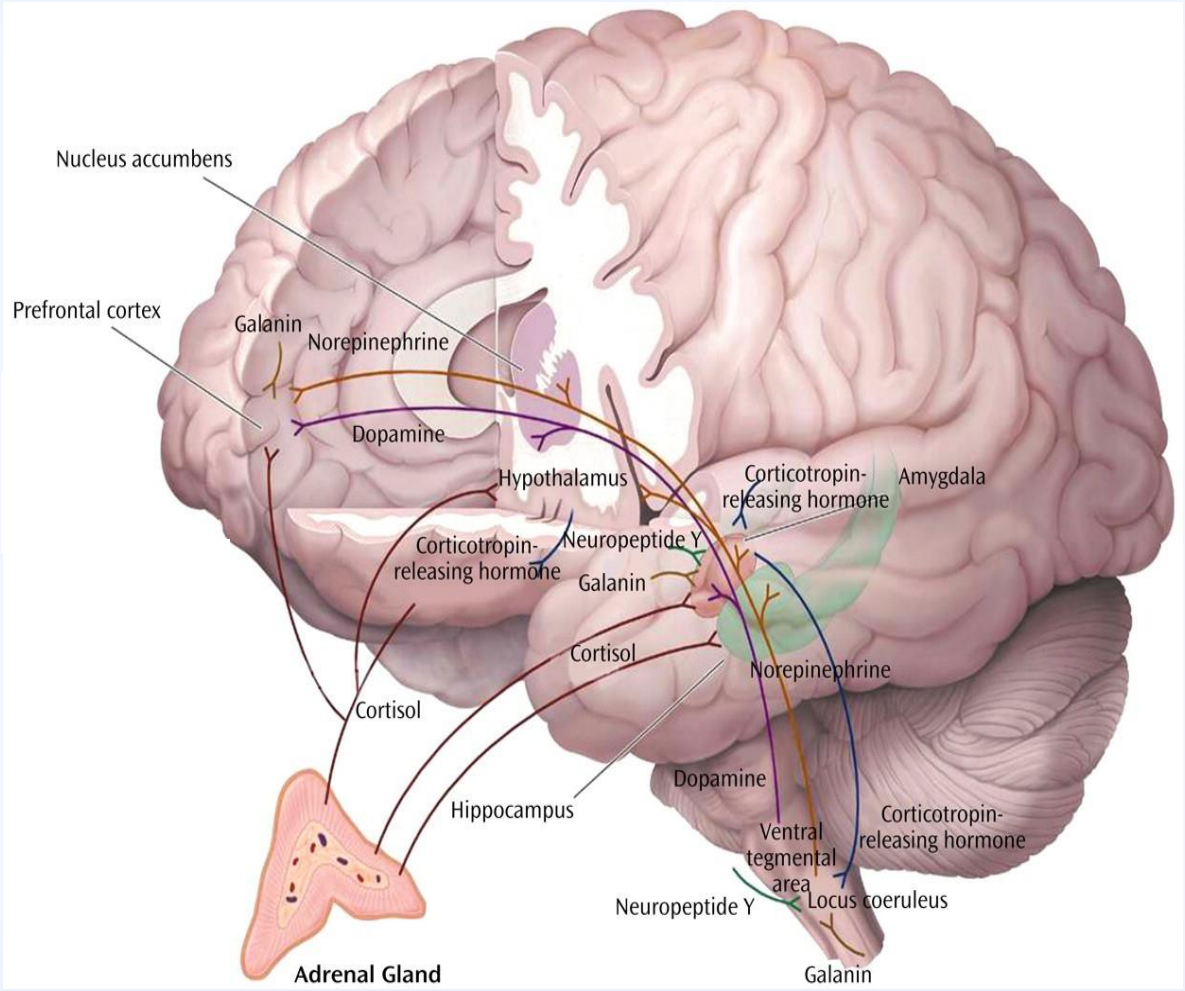


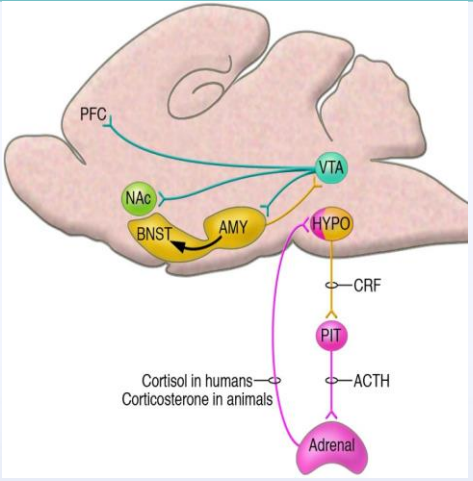
Figure 1. *Vulnerability and resilience as a function of prior stress exposure. Prior stress exposure does not simply increase vulnerability or diminish resilience as predicted by a continuous linear function for the emergence of adverse health effects (A). Empirical evidence instead suggests that prior stress exposure influences vulnerability and resilience as predicted by a curvilinear function (B).*

Stress has always been difficult to define. It has also become increasingly clear that the consequences of stress exposure are dependent upon the conditions of the stressor and can have either beneficial or adverse consequences.

Both Pb and Prenatal Stress Affect the Same Biological Targets: HPA Axis and CNS Mesocorticolimbic System



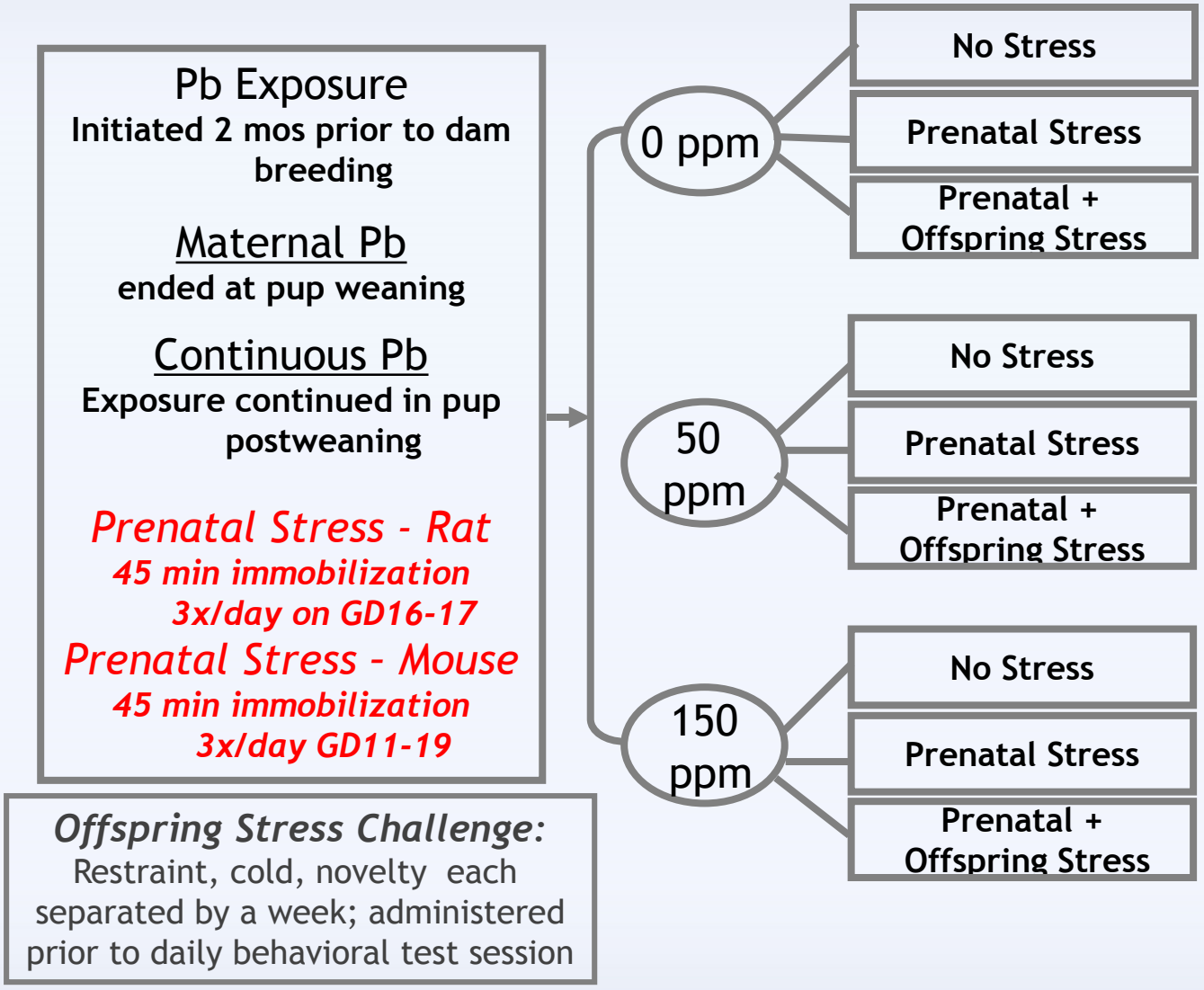
- HPA axis and brain mesocorticolimbic systems interact to mediate behavioral functions, including cognition and aspects of attention deficit disorder



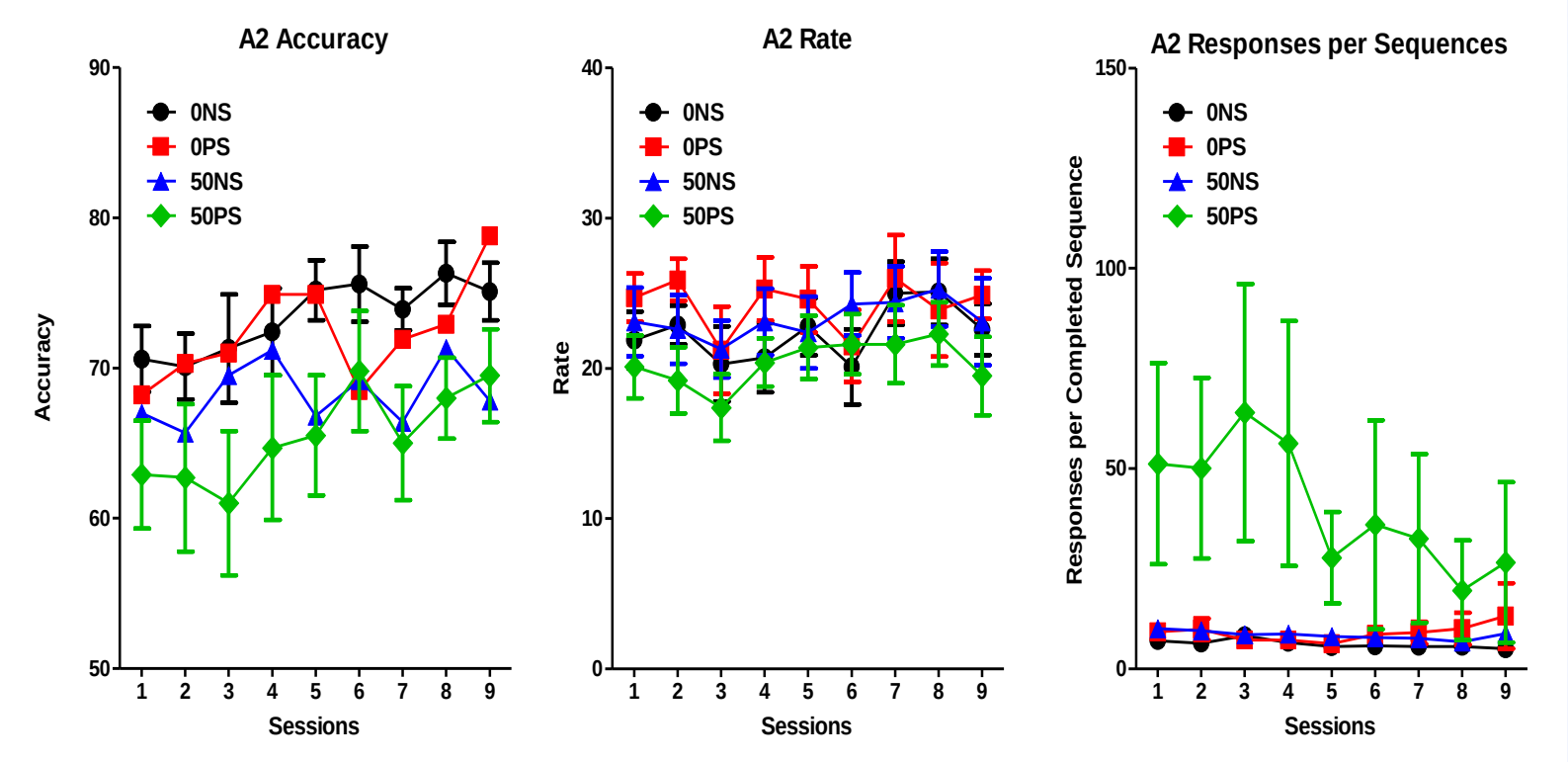
Neurotoxic Metals and Stress Share Biological Substrates and Produce Common Adverse Outcomes

- Metals and Stress Share Biological Substrates:
  - Effects of stress are mediated through the HPA axis and release of glucocorticoids: all metals appear to alter glucocorticoids.
  - Lead and MeHg and prenatal stress all impact brain mesocorticolimbic (prefrontal cortex, nucleus accumbens, hippocampus) dopamine/glutamate circuits
- Lead and MeHg also Share Common Adverse Outcomes with Prenatal Stress:
  - These include deficits in cognitive functions such as learning, memory and attention

Experimental Models of Pb and Stress



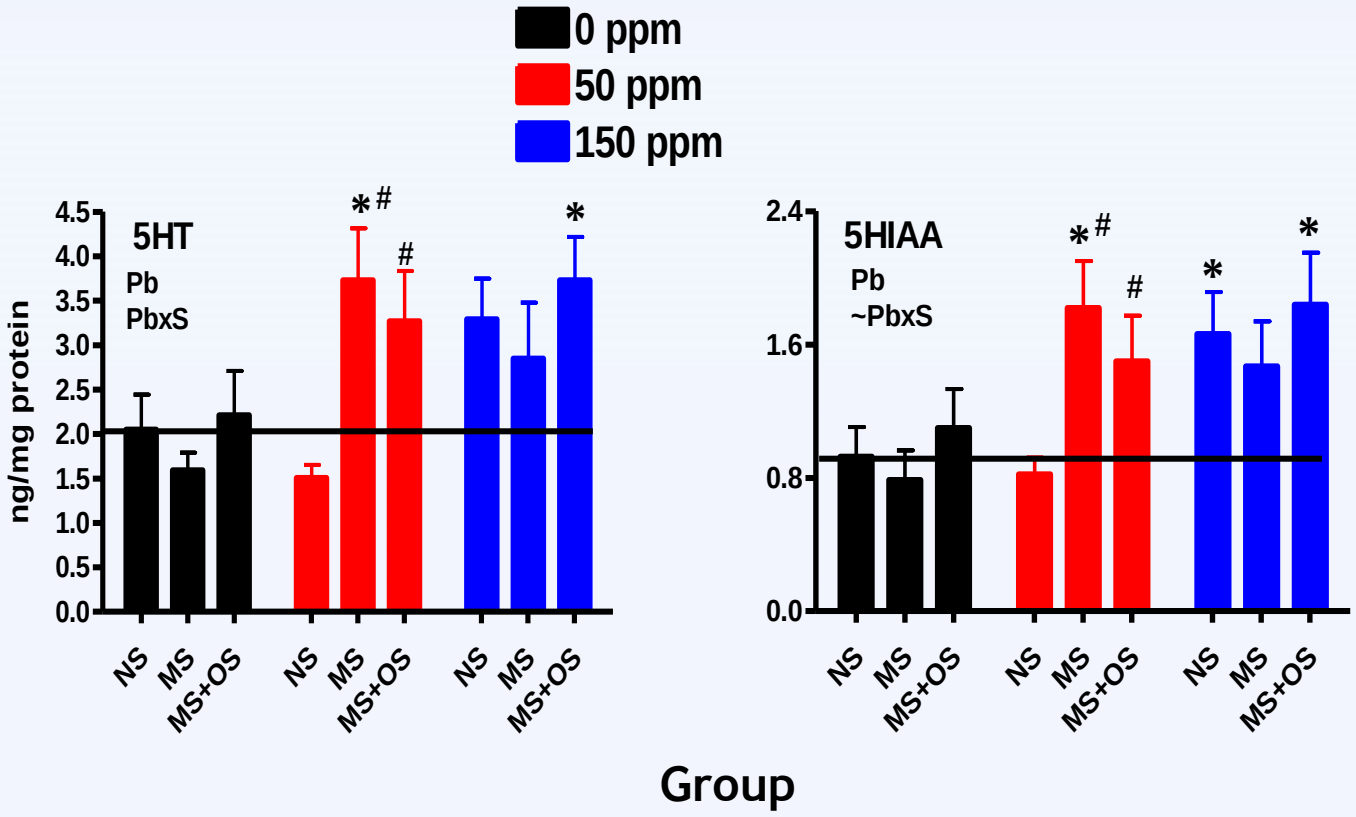
Continuous Pb and Stress Impairs Learning in Female Offspring



Preferential impairments in repeated learning in females: Lower accuracy and delayed improvement over time, requiring more responses to achieve a completed sequence, an effect that does not reflect lowered response rates.

Cory-Slechta et al., 2010

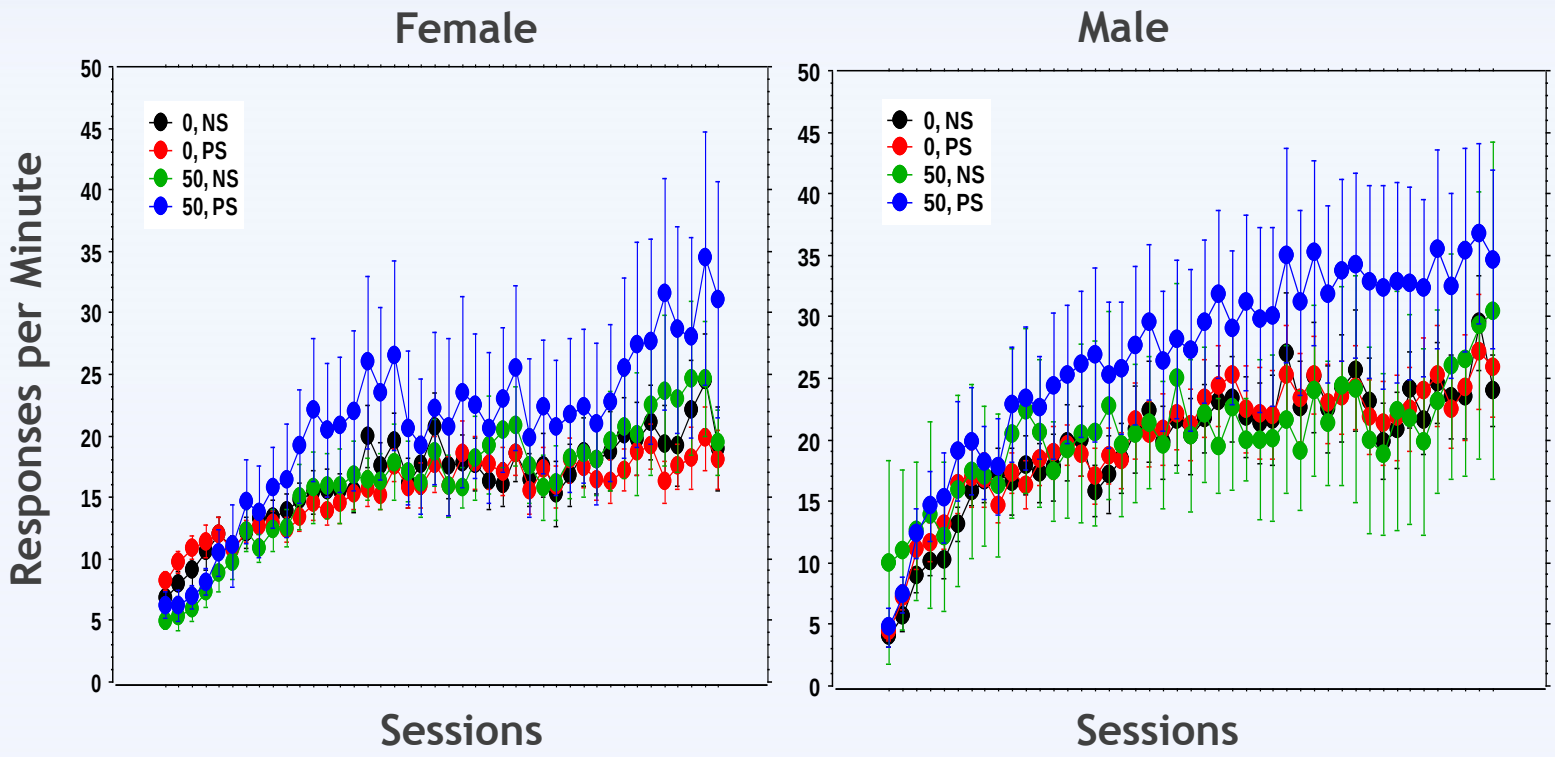
Enhanced Effects of Pb and Stress Condition on Brain Neurotransmitters: Frontal Cortex Serotonin in Females



NS=No stress; MS=maternal stress; MS+OS= maternal and offspring stress

Virgolini et al., 2008

Lifetime Pb and Stress in Mouse Models

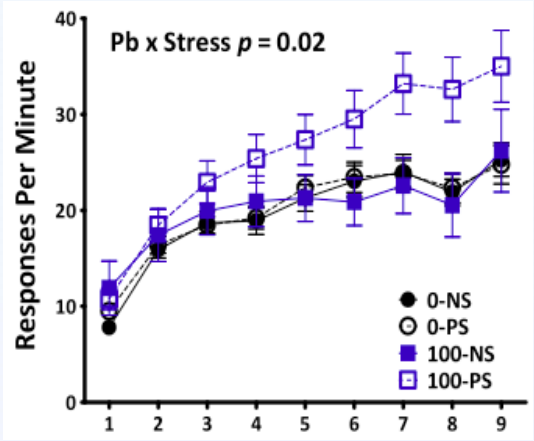


Lifetime Pb and prenatal stress synergistically increase FI response rates in males, with similar, but non-significant trends in females

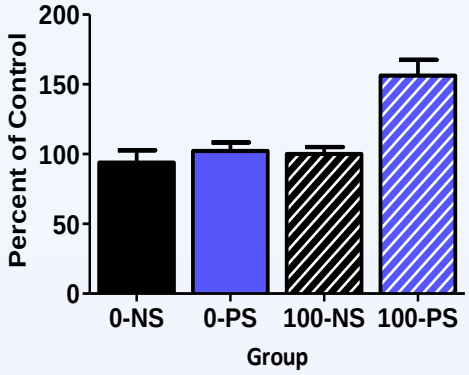
Cory-Slechta et al., 2012

Synergistic Increased FI Response Rates and Hippocampal Nuclear GR Receptors

♂ *Fixed Interval Response Rate*

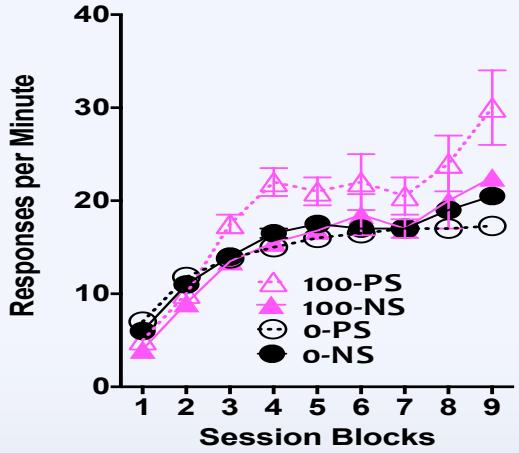


Hippocampal Nuclear GR

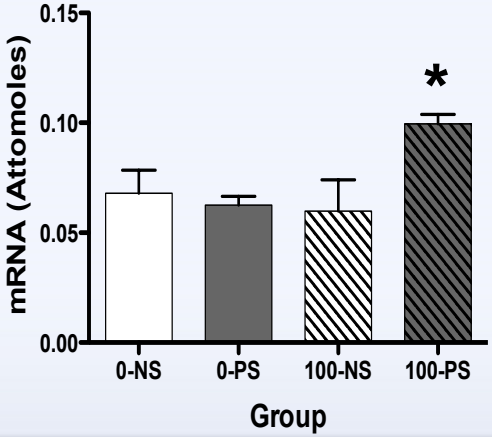


Selective increase in FI response rates in 100-PS males were selectively accompanied by increased levels of nuclear GR receptors in hippocampus determined by Western blot

♀



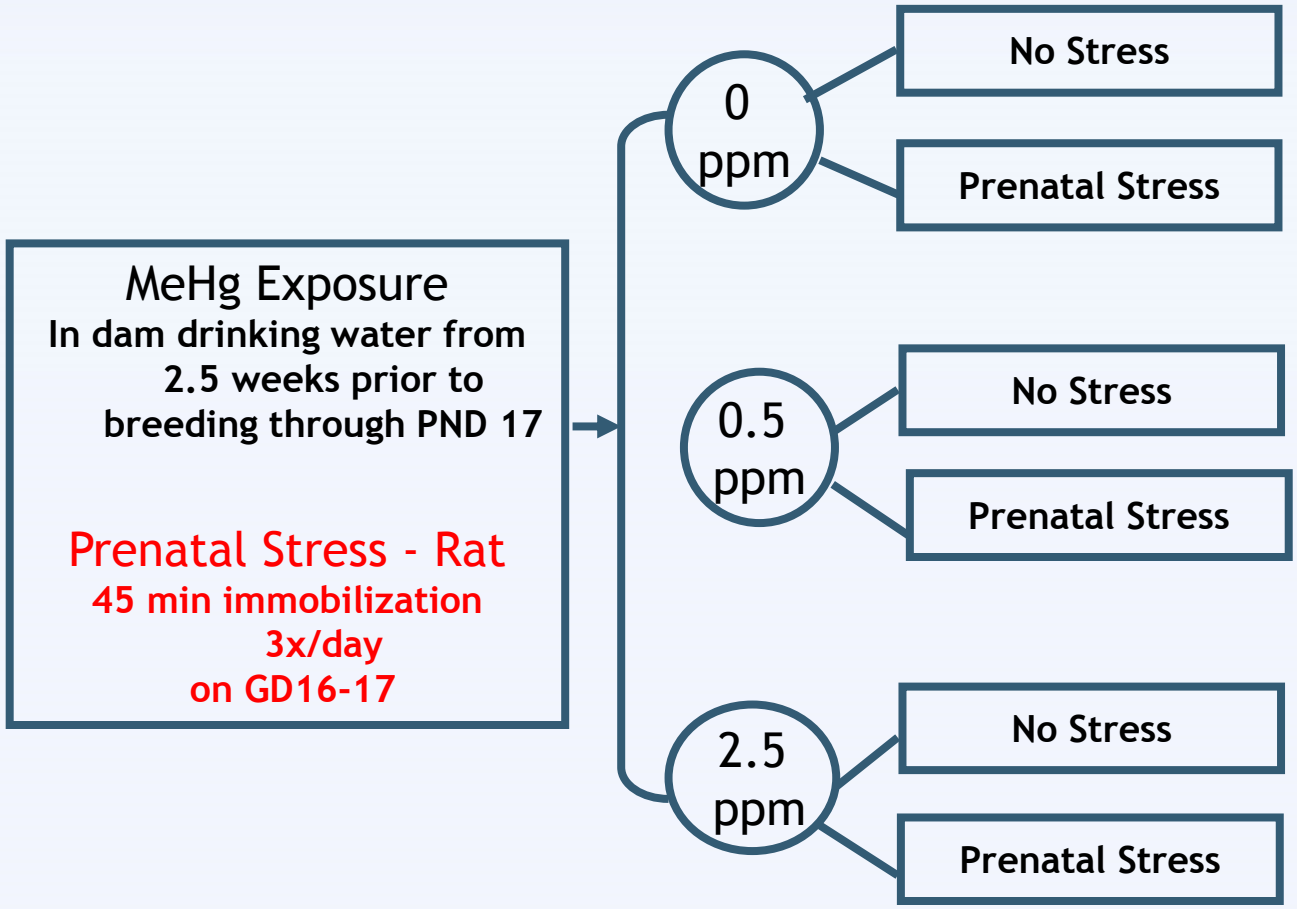
Nr3c1 mRNA



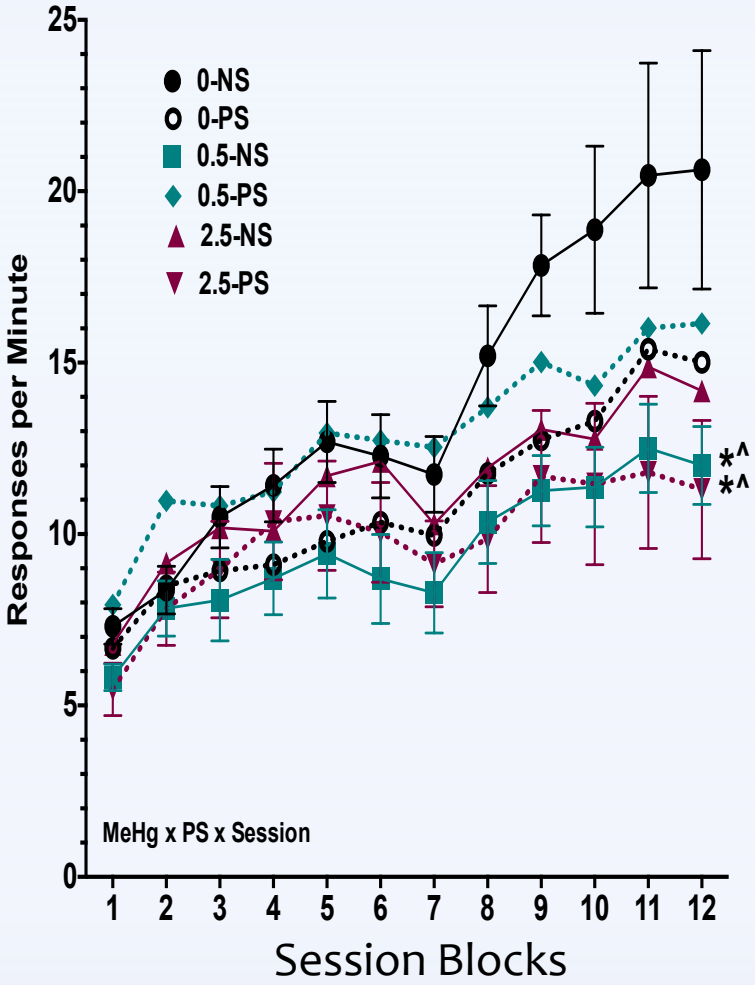
Nr3c1 mRNA expression levels by qPCR in the adult female mouse hippocampus after maternal Pb (0 or 100 ppm), or stress (no stress=NS; prenatal stress=PS or both. Increases were found selectively in Pb + PS females.

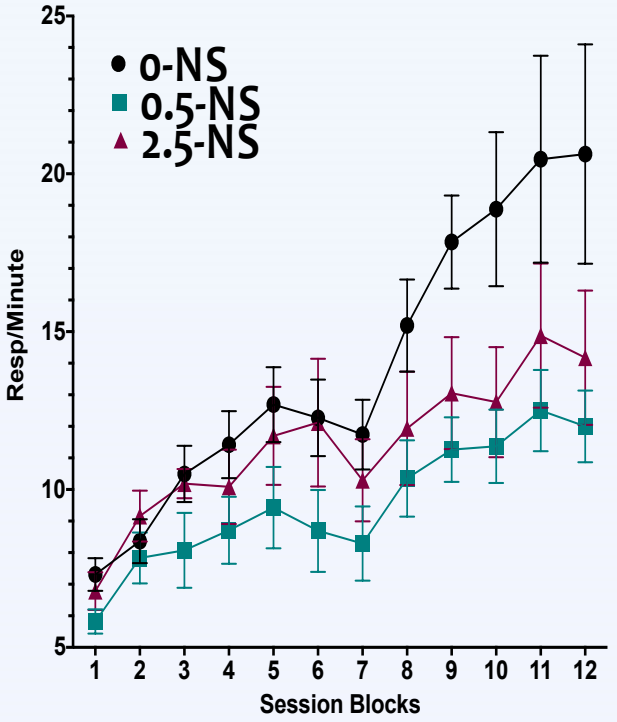
Cory-Slechta et al., 2012

MeHg + Prenatal Stress Experimental Design



MeHg and Prenatal Stress Produce Synergistic Decreases in FI Response Rates in Female Offspring



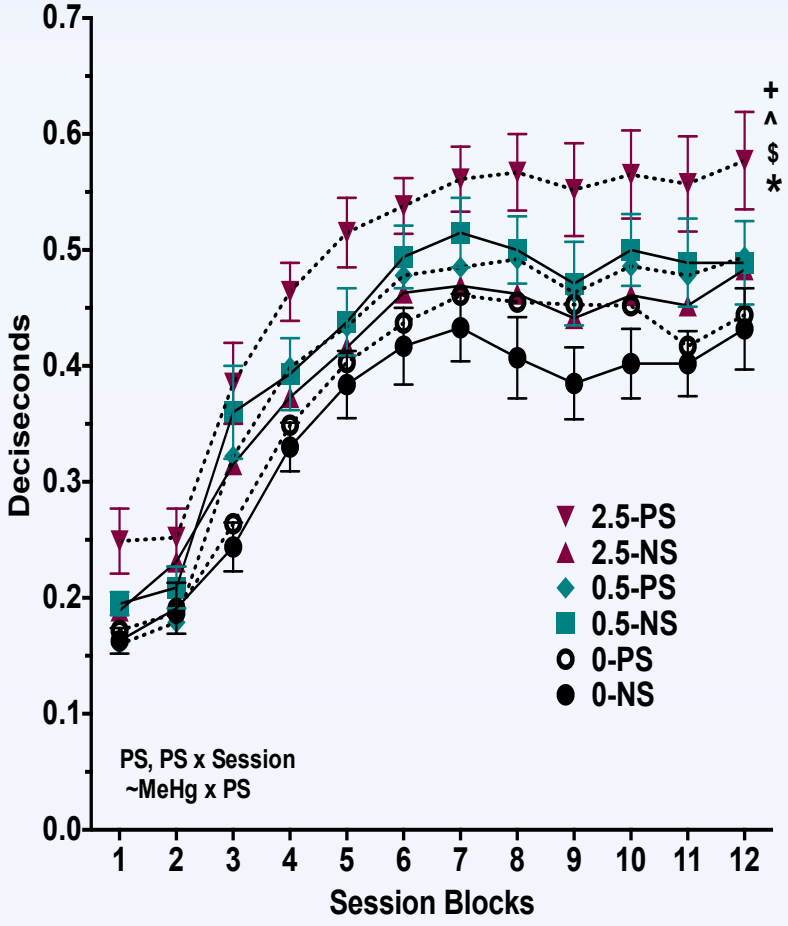


Note U-shaped curve with MeHg alone

No effects in male offspring

Weston et al., 2014

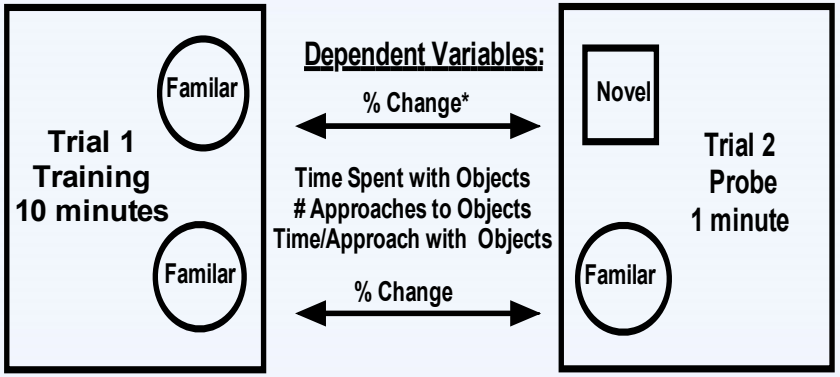
MeHg and Prenatal Stress Produce Synergistic Increases in FI Postreinforcement Pausing in Female Offspring

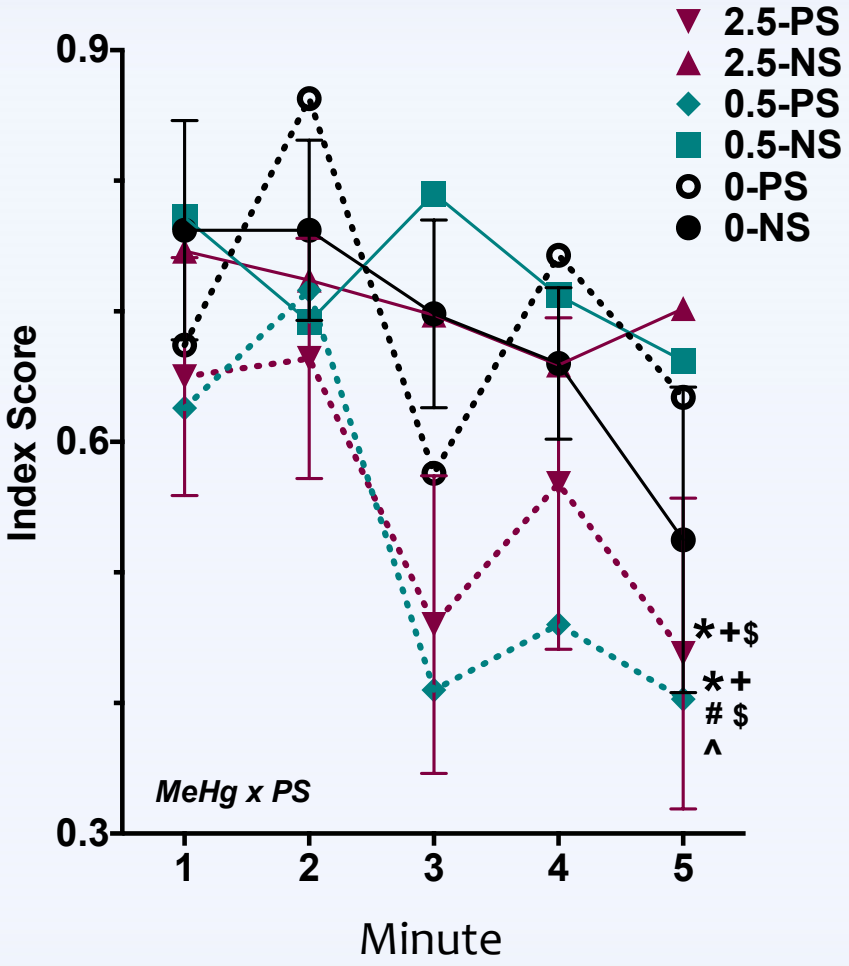


No effects in male offspring

Weston et al., 2014

Only MeHg + Prenatal Stress Impair Short-Term Memory





Weston et al., 2014

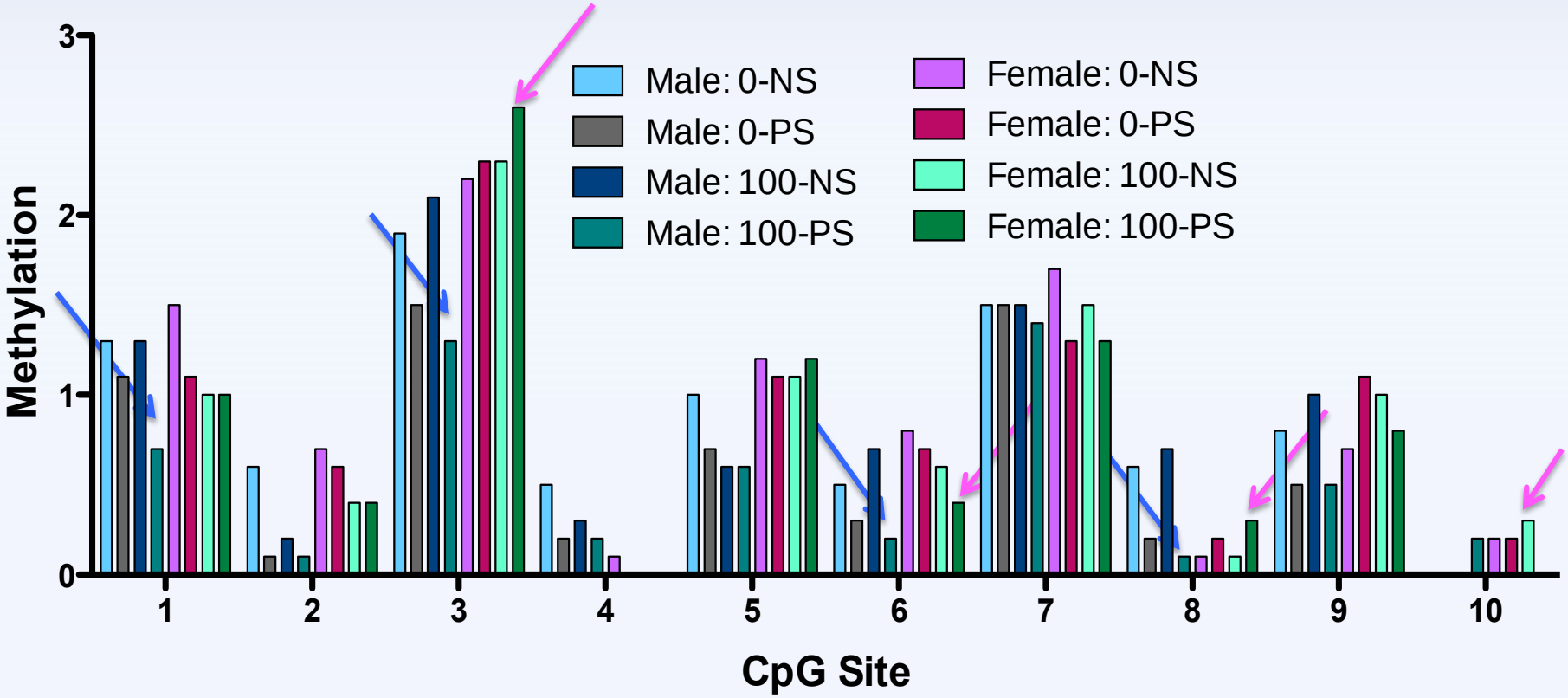
Epigenetic Changes in Response to Pb, Stress and Combined Pb and Stress

SPECIFIC AIMS:

- Developmental Pb and PS will each induce gender-specific epigenetic changes, and combined Pb and PS will lead to enhanced or synergistic changes in epigenetic marks.
- Different behavioral experiences (positive vs. negative) after birth will result in differential profiles of epigenetic marks per se, and, further, these could either mitigate/reverse or even further enhance Pb and/or PS-associated changes.

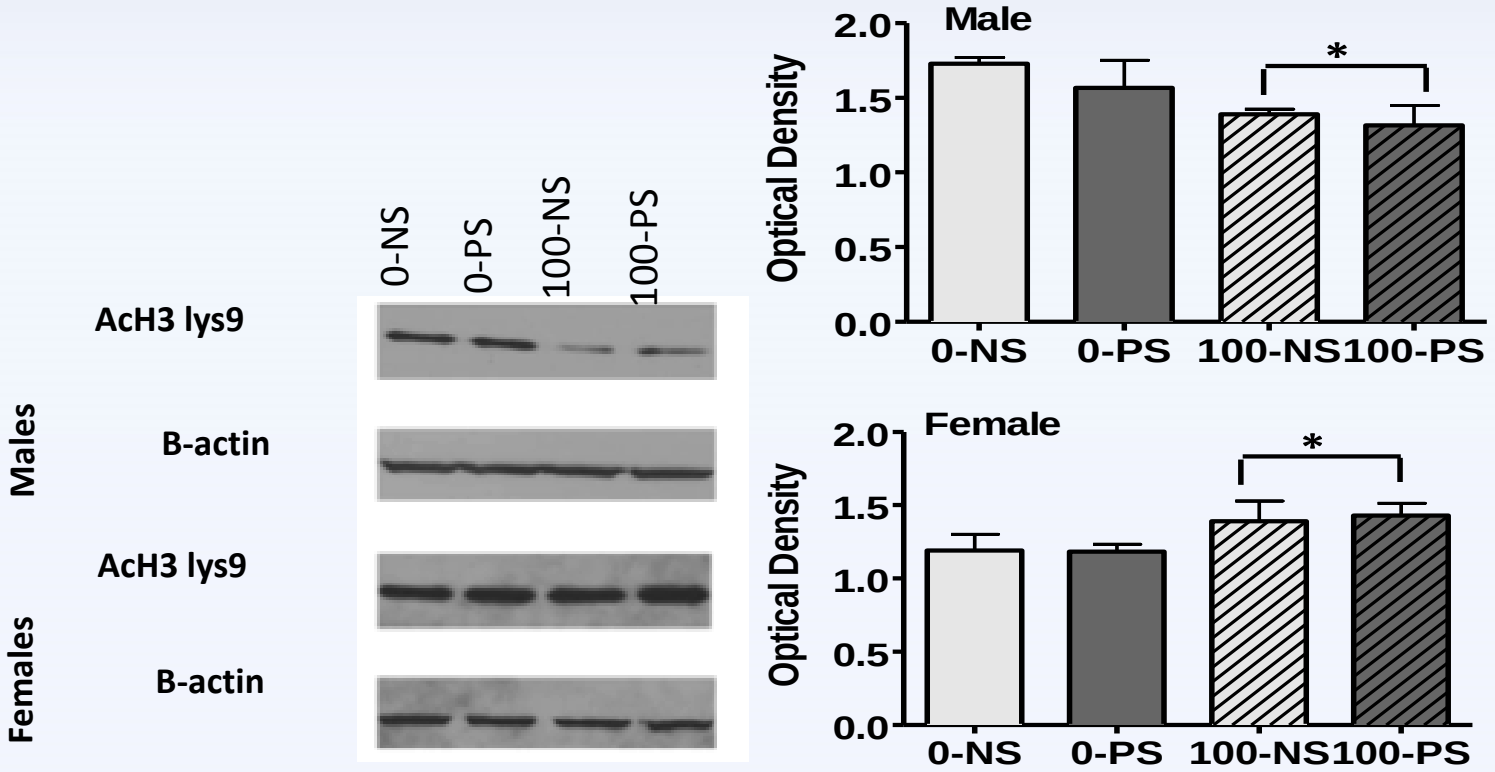
NIH Grant ES021534

Hippocampal *Nr3c1* Methylation Changes



Levels of methylation of hippocampal glucocorticoid receptor CpG islands 1-10 in male and female mice subjected to no Pb (0), no stress (NS) (0-NS); no Pb and prenatal stress (PS) (0-PS), maternal 100 ppm, no stress (100-NS); or 100 ppm and PS (100-PS). While profiles of effects differed at different CpG sites, Pb + PS could produce enhanced effects in both sexes, although often in different directions. Unpublished data.

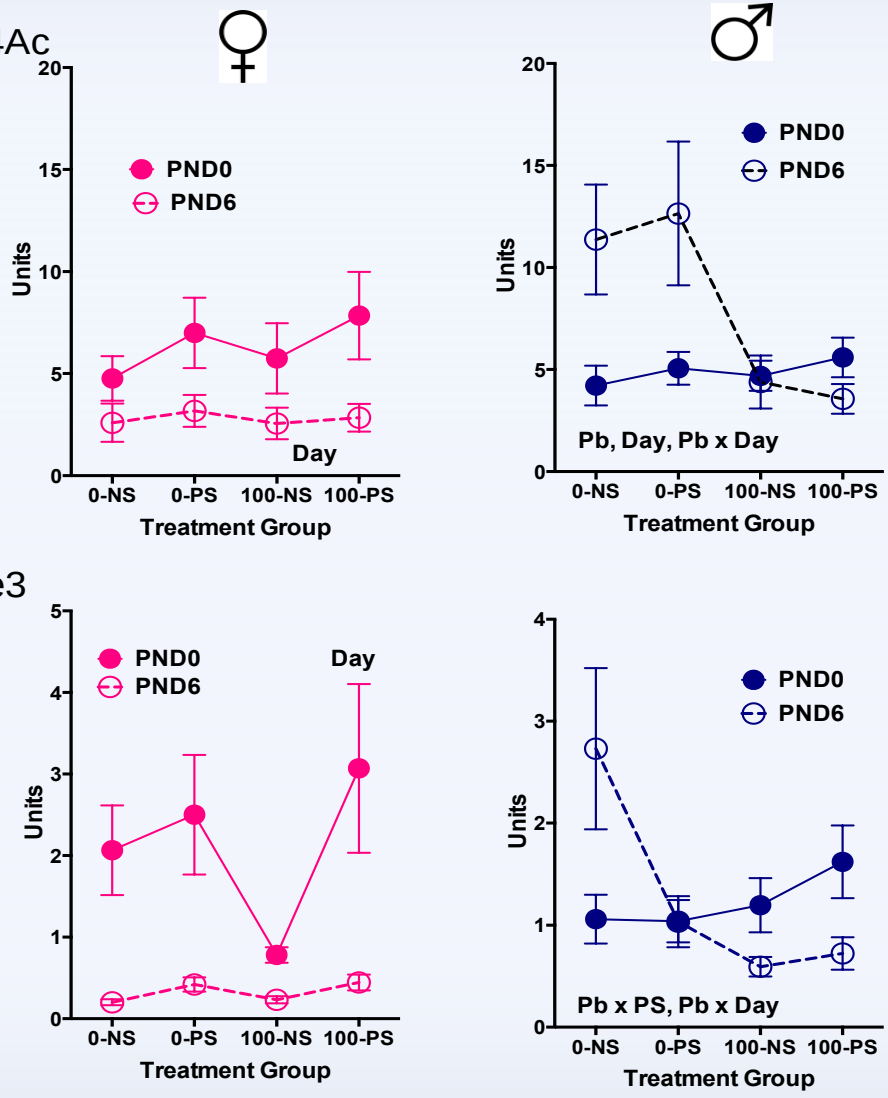
Acetylation of AcH3K9 in Frontal Cortex



Acetylation of histone 3, lysine 9 (AcH3K9; associated with gene activation) in frontal cortex of male and female mice subjected to Pb and/or PS. Pb decreased AcH3K9 levels in frontal cortex of males, whereas levels were increased by Pb in females. These increases were not further enhanced by Pb and may be related to striking gender differences frequently observed in outcomes of developmental exposures to Pb, prenatal stress and the combination. Unpublished data

Hippocampal AcH3K9/14Ac (Gene Activation) and H3K9Me3 (Gene Silencing) Acetylation

H3K9/14Ac



H3K9Me3

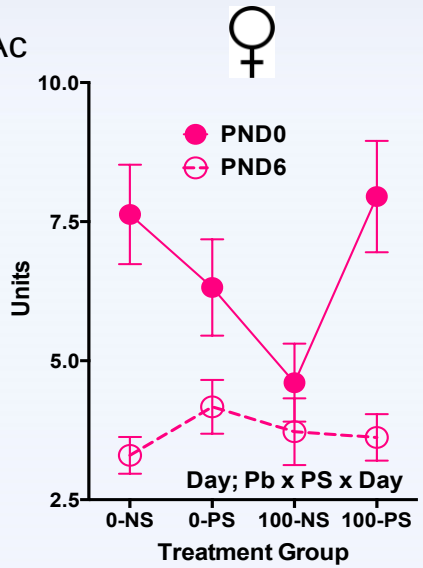
Females: Levels of both histones decline markedly in females between PND0 and PND6, but neither was Influenced by either Pb or PS or the combination

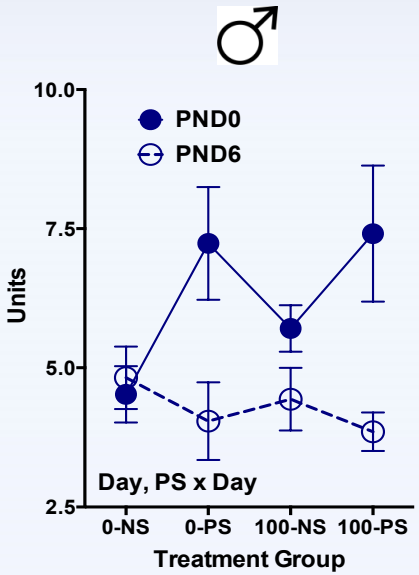
Males: Levels of both histones Increased between PND0 and PND6. Pb and PS had delayed effects on these histones in males: levels of H3K9/14Ac were reduced by Pb by more than 50%, while both Pb and PS reduced levels of H3K9Me3 in hippocampus of females at PND6.

Schneider et al., submitted

Frontal Cortex AcH3K9/14Ac (Gene Activation) and H3K9Me3 (Gene Silencing) Acetylation

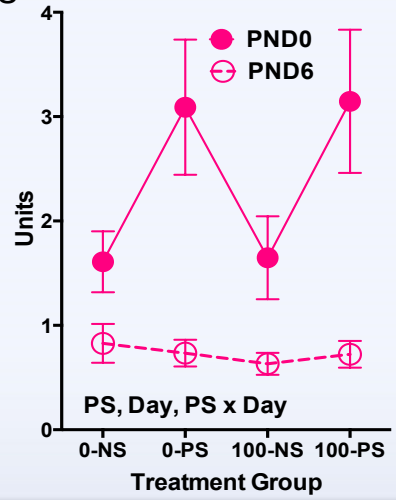
H3K9/14Ac

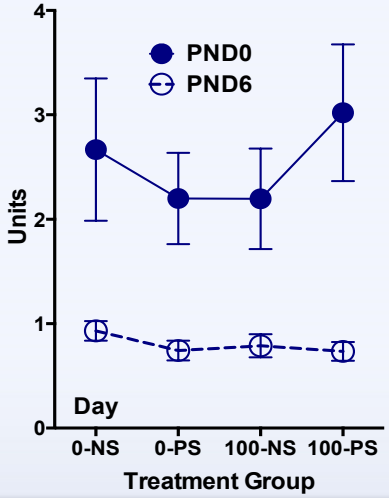




In contrast to hippocampus, levels of both histones were reduced between PND0 and PND6 in both sexes

H3K9Me3



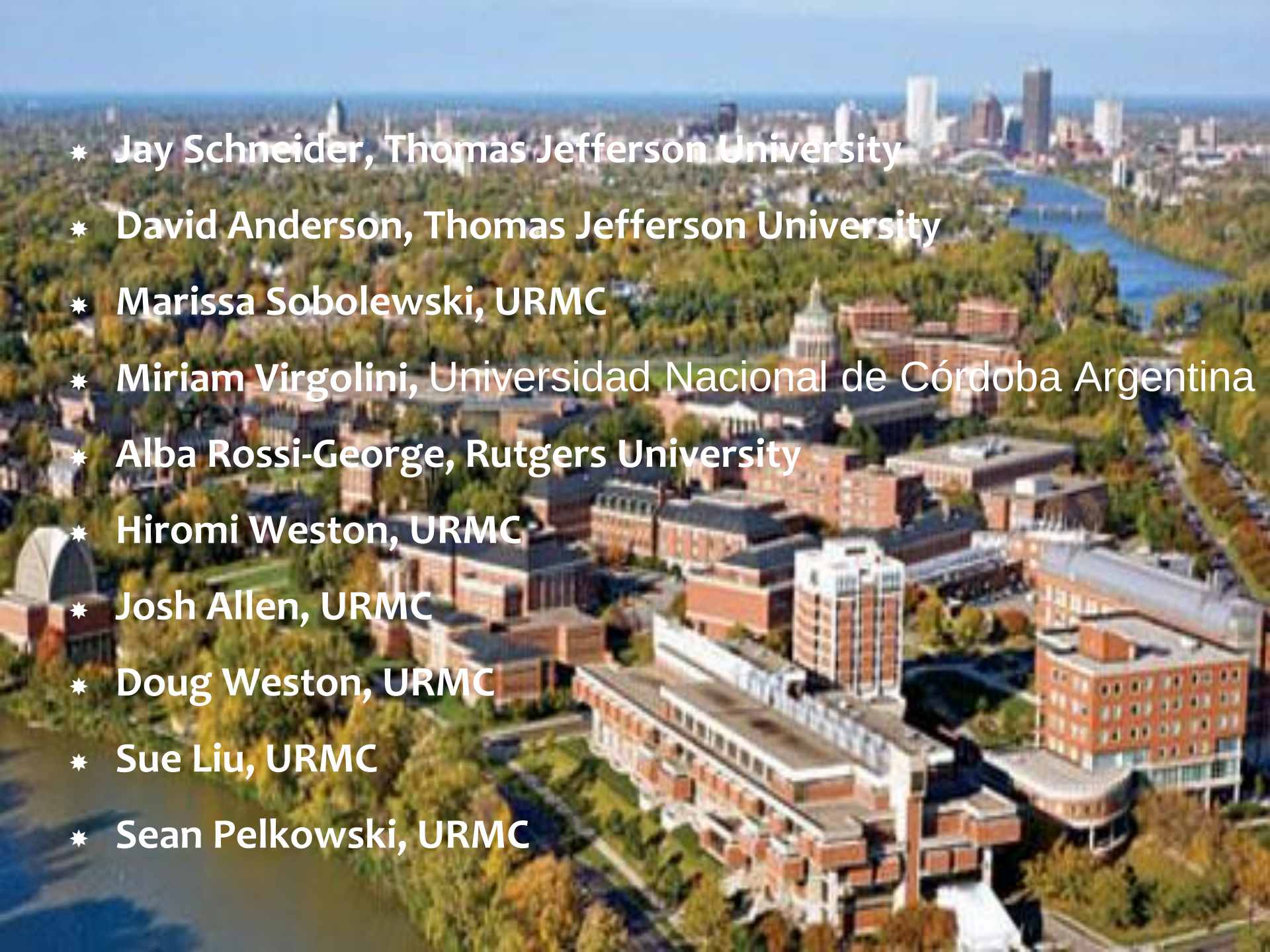


In contrast to hippocampus, effects of Pb and/or PS were seen only at PND0 in frontal cortex, with selective Pb-induced reduction in both histones in females, and of H3K9/14Ac in males

Schneider et al., submitted

Epigenetic Changes, Pb ± PS, and Brain

- Levels of epigenetic marks frequently differ by sex.
- Further, these marks clearly change over the course of early development, and do so in a sex-dependent capacity, suggesting that both timing of insults and gender could be critical to the ensuing consequences.
- It is also clear that epigenetic marks in brain are often regionally specific and may also be dynamic, being acutely influenced by e.g., behavioral experience.
- This is consistent with the need for dynamic behavioral changes in response to rapid environmental changes.
- We saw evidence that Pb + PS can result in enhanced changes relative to either Pb or PS alone in hippocampal Nr3c1 methylation
- As yet unclear is whether positive vs. negative behavioral experience can further modulate Pb ± PS-induced epigenetic marks, perhaps further influencing or even reversing such changes.



- Jay Schneider, Thomas Jefferson University
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- Alba Rossi-George, Rutgers University
- Hiromi Weston, URMC
- Josh Allen, URMC
- Doug Weston, URMC
- Sue Liu, URMC
- Sean Pelkowski, URMC