Low-Level Gestational Lead Exposure Induces Metabolomic Changes in Developing Mouse Retina

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Purpose: Low-level gestational lead exposure (GLE) increases retinal progenitor cell proliferation and rod photoreceptor and bipolar cell neurogenesis in mice. To determine the GLE-induced changes in cellular metabolism, we surveyed the small molecular metabolic signals in developing control and GLE retinas.

Methods: Female C57BL/6 mice were given tap water or water containing 55 ppm lead two weeks before mating, during pregnancy, and through postnatal day (PND)10 to produce a human-equivalent GLE model. Mice were sacrificed between 1000 and 1200 hours on PND2, PND6, PND10, and 4 months of age. Retinas were fixed with conventional aldehydes, plastic embedded, sectioned and processed for computational molecular phenotyping (CMP). Sections from control and GLE central retinas were examined and compared.

Results: Consistent with our previous studies, GLE mouse retinas had prolonged development compared to control. Between PND2 and PND10 there were metabolic variances in the molecular signals between GLE and controls for virtually every metabolite and protein examined: GABA, glycine, L-glutamate, L-glutamine, glutathione, arginine, L-aspartate, glutamate synthetase, CRALBP, GFAP, rod opsin and taurine. Notable changes in GLE retinas included an increased level of glutathione and GABA in the differentiated cell layer at PND2; an increased level of glutamate and aspartate, a decreased levels of glutamine and a delay in CRALBP expression in the Müller glial cell endfeet at PND6; and greater spacing between the progenitor cell layer and differentiated cell layer, lowered overall taurine levels, and delayed rhodopsin development at PND10. The most pronounced changes in the 4 week-old GLE retinas included isolated cell classes with higher aspartate, glutamine, glutamate and GABA levels: especially in the GCL.

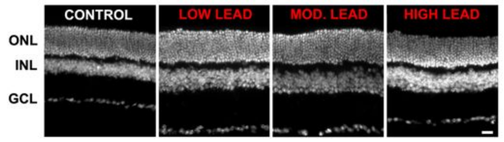
Conclusions: GLE-induced produced distinct metabolic differences during early postnatal retinal development. Differences in the metabolic envelopes of many retinal cell classes including horizontal cells, bipolar cells, amacrine cells, Müller glial and ganglion cells were observed. These findings suggest that alterations in retinal metabolism and the metabolic signatures of individual retinal cells may underlie the increased and prolonged cell proliferation and maturation of late-born rods and bipolar cells.

Commercial Relationship: W.D. Ferrell, None; S. Chaney, None; D.A. Fox, None; RE Marc, Signature Immunologics; BW Jones, None.

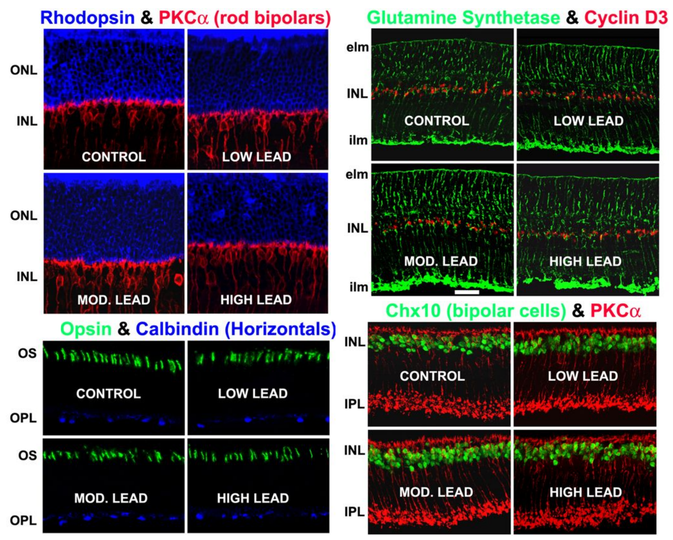
Introduction

Lead is a potent and pervasive developmental neurotoxicant. In comparison to adults, children are more susceptible to lead neurotoxicity. Blood lead concentrations ≤10 µg/dL, CDC's current low level of concern, produce retinal, cognitive and visual-motor deficits in developing children. Children, monkeys and rats with GLE have supernormal scotopic ERGs (Lilienthal et al. 1994; Rothenberg et al. 2002; Fox et al. 2008; Nagpal and Brodie 2009). Adult rats and mice with GLE have a unique retinal phenotype characterized by a selective increase in the number of late-born rods and bipolar cells (Fox et al. 2008; Giddabasappa et al. 2011). Studies during embryonic and postnatal retinal development revealed that GLE increased retinal progenitor cell (RPC) proliferation, did not alter apoptotic programmed cell death, and increased the number of differentiated rods and bipolar cells born during development (Giddabasappa et al. 2011).

The goal of these experiments was to examine cell populations in retina and determine whether GLE produced changes in cellular metabolism via survey of small molecular metabolic signals with Computational Molecular Phenotyping in developing control and GLE retinas.



Low-level, environmentally relevant lead exposure during gestational development results in an increased thickness of the outer and inner nuclear layers (DAPI stain) in the adult mouse. As seen below, this is due to an increase number of rod photoreceptor cells and bipolar cells. Scale bar = 20 m.

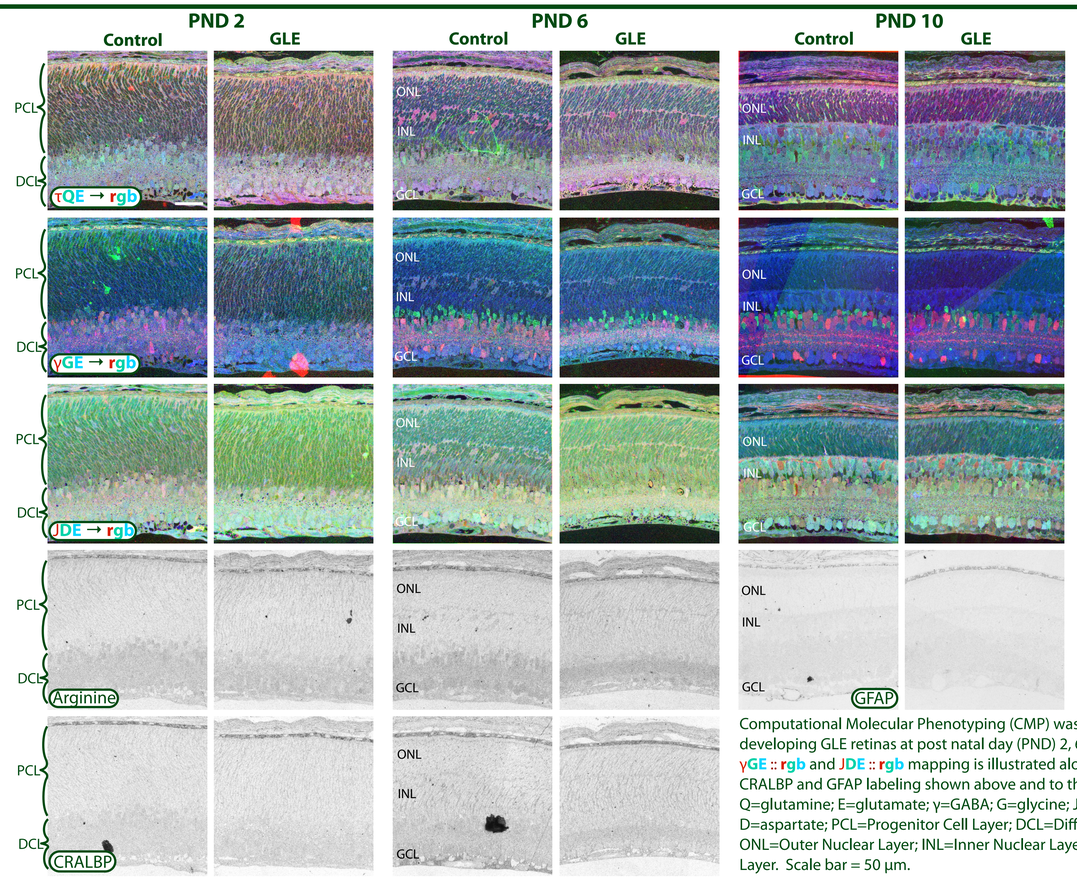


Immunohistochemical (IHC) studies and confocal analysis

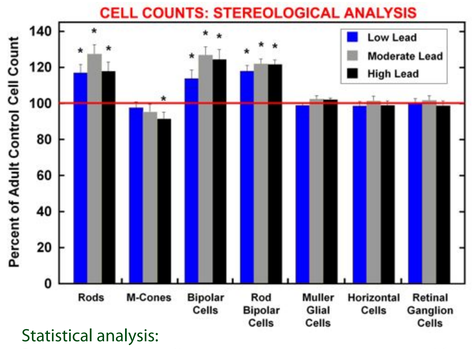
All IHC and confocal procedures were performed as described (Johnson et al. 2007; Giddabasappa et al. 2011). Briefly, enucleated adult control and GLE eyes (n = 4-7 mice/group) were fixed with 4% paraformaldehyde, frozen and sectioned (10 µm). Central retinal sections from control and GLE mice were processed, imaged and examined using identical techniques. Tissues were blocked and incubated overnight with primary antibodies, rinsed and incubated with secondary antibodies, and mounted using Vectashield. All retinae were imaged using a Leica TCS SP2 laser scanning confocal microscope. Images were processed with Adobe Photoshop and counts were made using standard stereological procedures.

Confocal Results

Low-, moderate- and high-level GLE exposures produce an increase in the number of rod photoreceptors, and rod and cone bipolar cells, with no change in the number or distribution of cones, Müller glial cells, horizontal cells or ganglion cells. Scale bar = 20 µm. (Giddabasappa et al. Environ. Health Perspect. 2011)



Computational Molecular Phenotyping (CMP) was performed on developing GLE retinas at post natal day (PND) 2, 6 and 10. τQE :: rgb, γGE :: rgb and JDE :: rgb mapping is illustrated along with arginine, CRALBP and GFAP labeling shown above and to the left. τ=taurine; Q=glutamine; E=glutamate; γ=GABA; G=glycine; J=glutathione; D=aspartate; PCL=Progenitor Cell Layer; DCL=Differentiated Cell Layer; ONL=Outer Nuclear Layer; INL=Inner Nuclear Layer; GCL=Ganglion Cell Layer. Scale bar = 50 µm.



Statistical analysis:
Only one animal per litter per treatment group was used for any measure. Data from 5-7 animals per treatment group, presented as means ± SEMs, were analyzed using an ANOVA followed by post-hoc analysis. The difference from controls was considered significant if $p<0.05$.

Computational Molecular Phenotyping (CMP) Results

CMP reveals retinal cell populations, and their anatomical and metabolomic maturation during development from PND2 through PND10. The onset of expression of several neurotransmitters, neuromodulators and markers of synaptogenesis were delayed in PND2 and PND6 GLE central retinas, compared to controls. In GLE retinas, GABA expression in amacrine and ganglion cells was delayed at PND2 and PND6. Similarly, the expression of VGluT1 (vesicular glutamate transporter) in the plexiform layers of GLE retinas was delayed at PND2 and PND6 (Chaney et al. ARVO 2008). By PND10, these developmental delays in GABA and glutamate expression were no longer present indicating that there was no sustained inhibition of neuronal maturation in GLE retinas. Interestingly, the appearance of glycinergic amacrine cells was similar in PND2, PND6 and PND10 control and GLE retinas.

Developing GLE Müller glia, amacrine and horizontal cells at PND2 and PND6 expressed higher levels of taurine than in control retinas. In contrast, taurine levels in the retinal pigment epithelium (RPE) of GLE retinas at PND10 were lower than in controls. Together, these results suggest altered osmoregulation in the GLE retinas.

Arginine expression was slightly higher in control retinas at PND2 and PND6. CRALBP labeling appeared similar in the RPE of controls and GLE mice, although its appearance was delayed in Müller glial cells of PND6 GLE retinas.

At PND10, there were a larger number of smaller-sized cells in the inner retina of GLE mice, compared to the controls. These are likely immature bipolar cells, as there is an increased and prolonged generation of OTX2-immunoreactive rod and bipolar cell precursors in GLE retinas compared to controls (Mukherjee et al. ARVO 2011, Program #2688). This results in an increased number of rod photoreceptors and bipolar cells in adult GLE retinas (Giddabasappa et al. Environ. Health Perspect. 2011).

Conclusions

CMP and confocal analysis showed that the maturation of the GLE retina is characterized by a delayed onset in the appearance of GABAergic amacrine cells and glutamatergic synaptic terminals. The decrease in retinal GABA and glutamate may underlie the increased and prolonged retinal progenitor proliferation in the GLE retinas. Their decrease, consistent with the findings of Martins and Pearson (Brain Res 2007), would enable progenitor cells to increase proliferation and decrease cell cycle exit at the G1-S transition phase.