JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

Pb^{2+} Neurotoxicity: Experiences from Behavior to Molecules

Key Events in the study of Pb^{2+} Neurotoxicity

•Historically, findings from human epidemiological studies that childhood Pb^{2+} intoxication produces impairments in cognitive function formed the basis for experimental animal studies.

•Primate and rodent studies with environmentally relevant Pb^{2+} exposures recapitulated the effects of Pb^{2+} on learning and memory function.

•The ability to use animal models of Pb^{2+} neurotoxicity allowed mechanistic studies on the effects of Pb^{2+} on neuronal systems known to be involved in learning and memory function.

•The hippocampus, a brain structure known to be involved in learning and memory function was shown to be a target for Pb^{2+}.

•The key finding that Pb^{2+} was a potent and selective inhibitor of the NMDA receptor subtype of glutamate receptors opened an important avenue of investigation. NMDA receptors are highly expressed in the hippocampus and are known to play an important role in brain development, synaptic plasticity and neurodegeneration.

Key Events in the study of Pb^{2+} Neurotoxicity

•Subsequent studies showed that developing animals exposed to environmentally relevant levels of Pb^{2+} exhibited profound deficits on NMDA receptor-dependent learning tasks and synaptic plasticity in the hippocampus.

•These basic science findings were driven, for the most part, by advances in the development of methodologies and instrumentation from a number of disciplines.

Are the effects of Pb^{2+} exposure on cognitive function reversible?

•Prevention is the first line of defense-Much more work needs to be done from government, civic and other organizations at the national, state and local level.

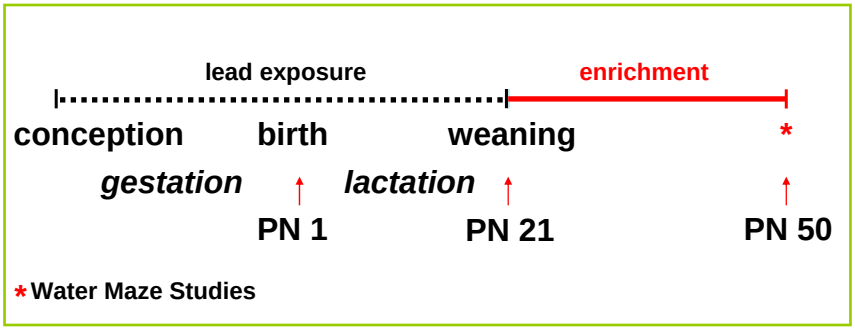
•Chelation therapy-although effective in reducing Pb^{2+} body burden, there is no evidence that it reverses Pb^{2+} -induced deficits in cognitive function once it has occurred.

•NEW APPROACH: Can a change in the living environment in the way of social interaction and stimulation be an useful way to mitigate the effects of environmental neurotoxicants, including Pb^{2+}, on cognitive function in children and developing animals?

•Environmental Enrichment (EE) – an approach being studied as restorative therapy in neurodegenerative diseases may provide important clues about the plasticity of the brain and its ability to recuperate from neurotoxicant-induced dysfunction.

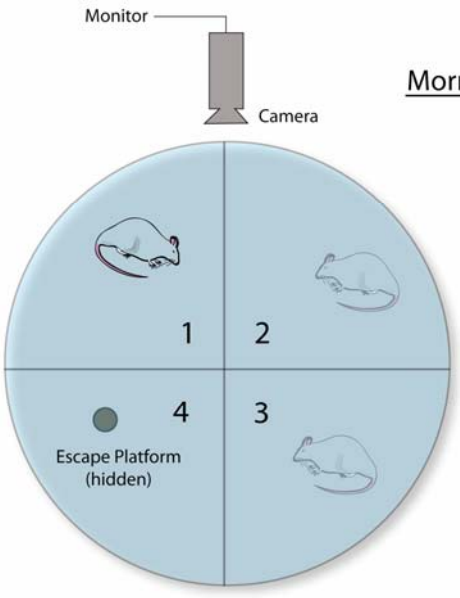
Environmental Enrichment

Pb^{2+} Exposure and Environmental Enrichment Paradigm



Guilarte, et al. *Annals of Neurology* 53:50-56, 2003.

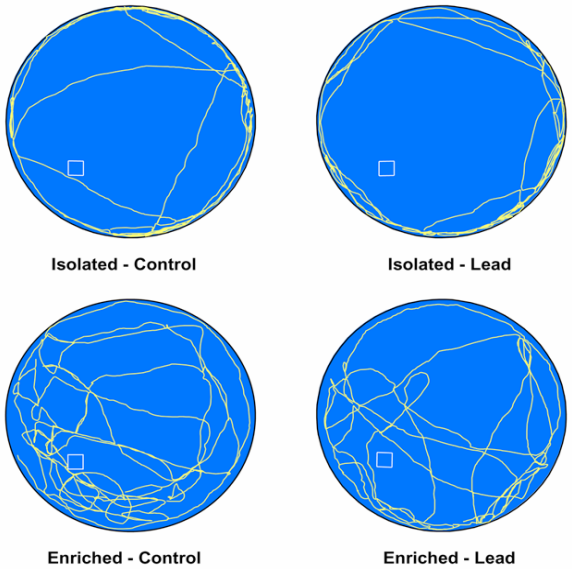
Spatial Learning Task



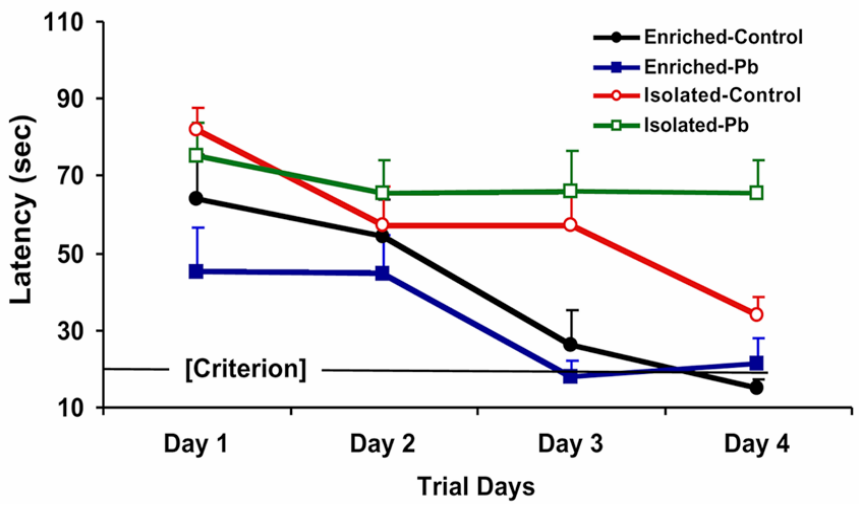
Morris Water Maze

- Pool is filled with opaque water
- Rat placed in pool at 1,2 or 3 (random)
- Allowed to swim for 90 sec
- If goal is found in 90 sec, rat rests on platform for 20 sec
- If goal is not found, rat guided to platform and allowed to rest on platform for 20 sec
- ESCAPE LATENCY=Time to platform discovery

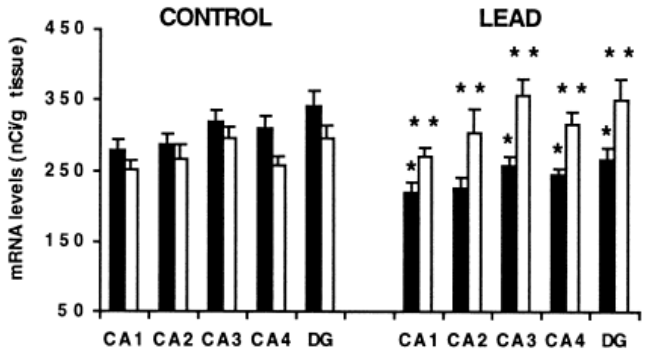
Searching Pattern on First Trial Day



Acquisition of Spatial Learning Task



Environmental Enrichment Reverses Deficits in Gene Expression

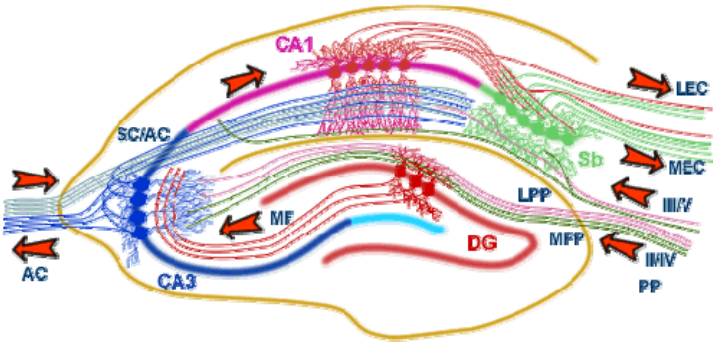


NR1 Subunit of the NMDA Receptor

Adult Neurogenesis: A Target for Environmental Neurotoxicants

- 30 years ago the dogma in was that an individual was born with all the brain cells that he/she was ever going to have.
- Today, we know that through a process known as neurogenesis a constant daily supply of new cells occurs in specific areas of the adult brain.
- One of these areas is in the subgranular zone of the Dentate Gyrus of the hippocampus.
- In the adult brain, new cells are incorporated into the appropriate neuronal circuitry and play an important role in learning & memory.
- Adult neurogenesis is an important modulator of cognitive function and environmental agents may alter neurogenesis (example).
- Neurogenesis can be modified by the living environment (i.e. exercise).

Scheme of Hippocampus and Neuronal Networks



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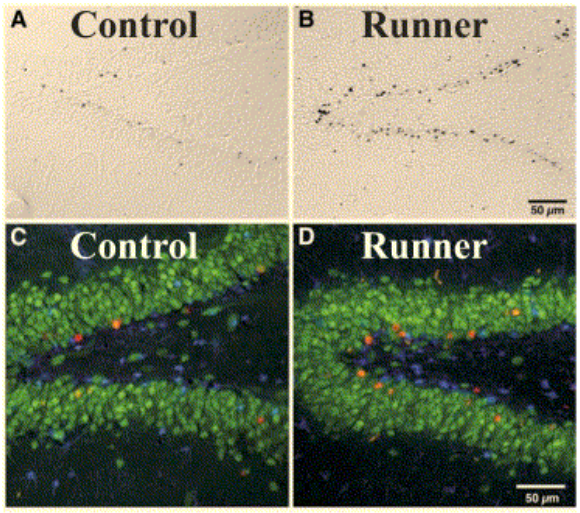
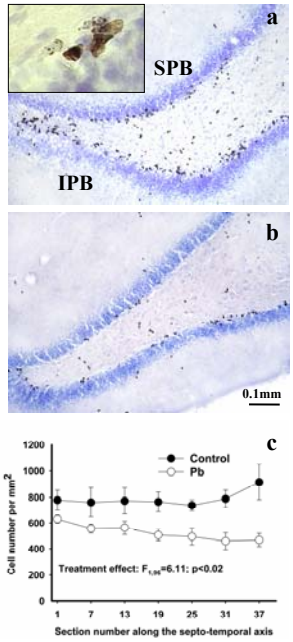
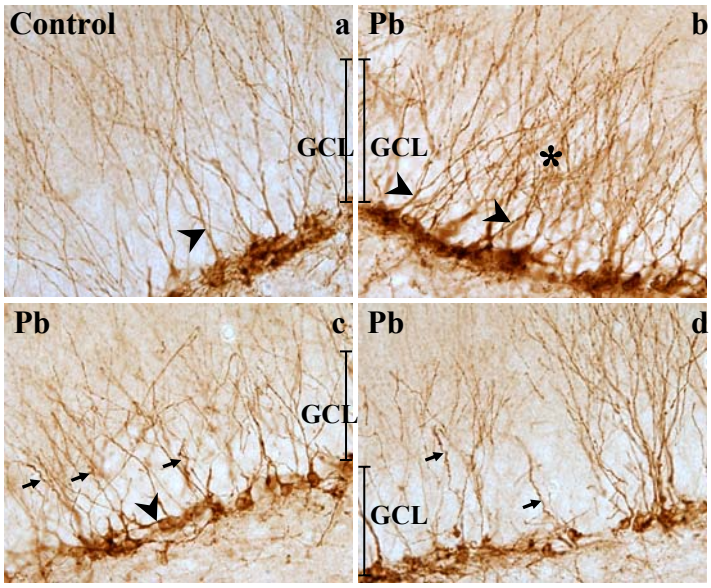


Farmer, et al. *Neuroscience* 124:71-79, 2004.



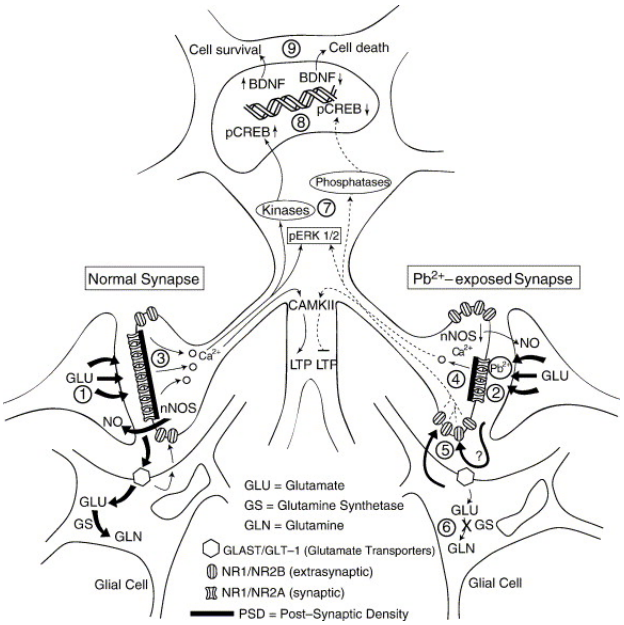
Doublecortin Immunohistochemistry

Doublecortin expressed in newly born neurons

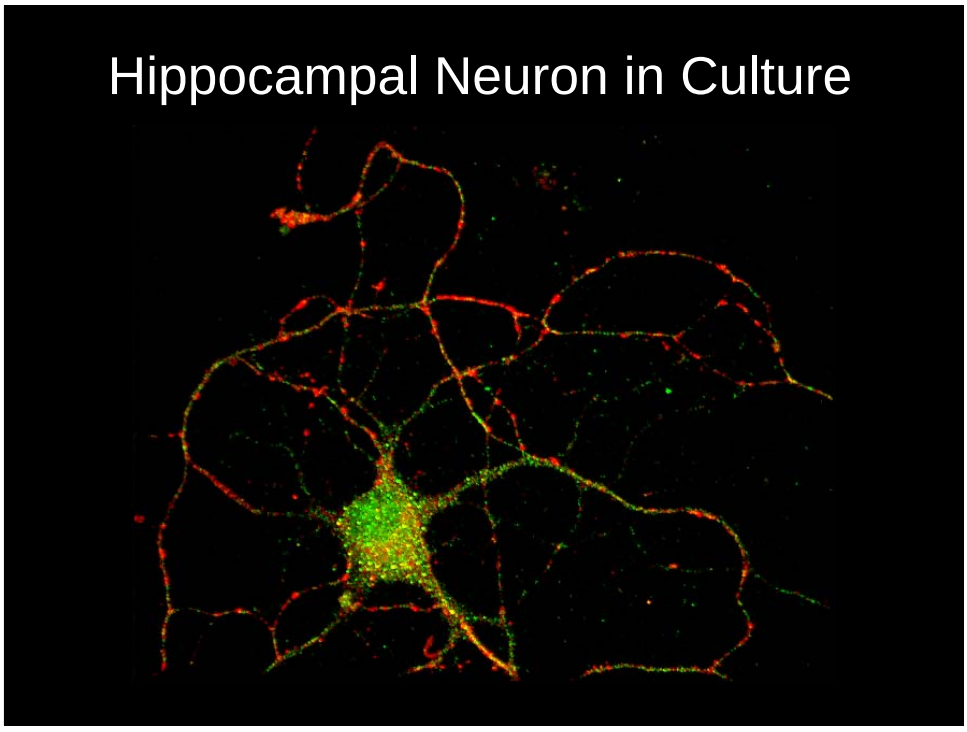


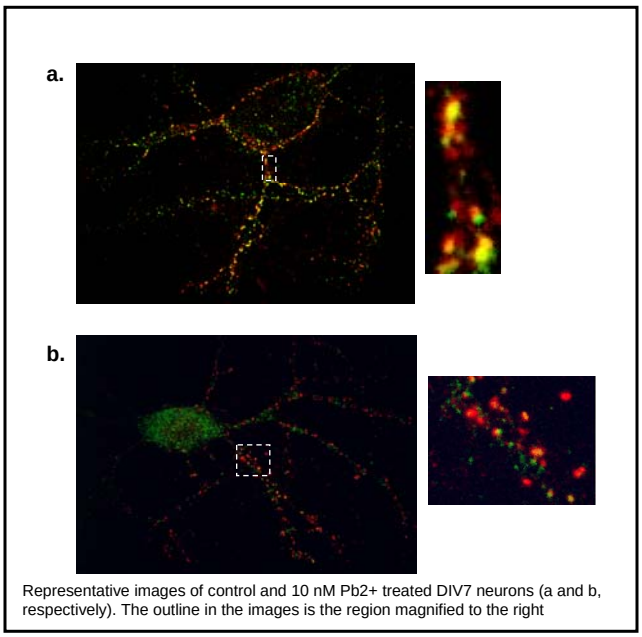
Molecular Studies at the Single Cell Level

- Dysfunction at the synapse, the point of communication between neurons, is known to form the basis for many of the major neurodegenerative diseases.
- Currently, laboratories are able to culture on a routine basis primary neuronal and glial cells to study the molecular effects of environmental chemicals (such as Pb^{2+}) at the single cell level.
- Studies on the effects of Pb^{2+} on the complexity of the dendritic spines are beginning to demonstrate effects at extremely low concentrations.



Toscano & Guilarte. *Brain Res Brain Res Rev.* 49(3):529-54, 2005.





Representative images of control and 10 nM Pb2+ treated DIV7 neurons (a and b, respectively). The outline in the images is the region magnified to the right

What have we learned-can we do it better?

- The development and validation of appropriate experimental animal models in which deficits of cognitive function occurred as a result of developmental Pb^{2+} exposure was an important step in our understanding of Pb^{2+} neurotoxicity today.
- These experimental animal models were used to elucidate cellular and molecular effects of Pb^{2+} neurotoxicity, as well as to test therapeutic strategies to mitigate the effects of Pb^{2+} on cognitive function.
- Some of these approaches now need to be investigated in human populations so that we have information flow from animal studies to human studies and back.
- Unfortunately, most of the new technology such as genomics, proteomics, metabolomics, molecular imaging and mouse genetics have not been used to study Pb^{2+} neurotoxicity. It is almost certain that these approaches would have brought new insights into molecular mechanisms of Pb^{2+} effects on the central nervous system.
- Nevertheless, a great deal of knowledge has been learned and the approaches used could benefit the investigation of effects of new toxicants or combination of toxicants on the CNS.

What Could We Do Different Today?

- Better communication/collaboration amongst basic scientists and population-based scientists. Maybe through conferences sponsored by NIEHS so that there is a rapid flow of information from basic scientists to population-based scientists and vice versa.
- The availability of new brain imaging modalities (i.e. MRI, MRS, DTI) can be used to study the effects of developmental neurotoxicants in children non-invasively.
- From an experimental animal perspective, the application of genomics, proteomics, metabolomics, as well as the newer techniques of molecular imaging and modern behavioral neuroscience, in combination with the ability to genetically modify rodents, provides an extremely powerful approach to modern neurotoxicology.

Acknowledgements

- To the many collaborators, students and colleagues that have made our work fruitful.
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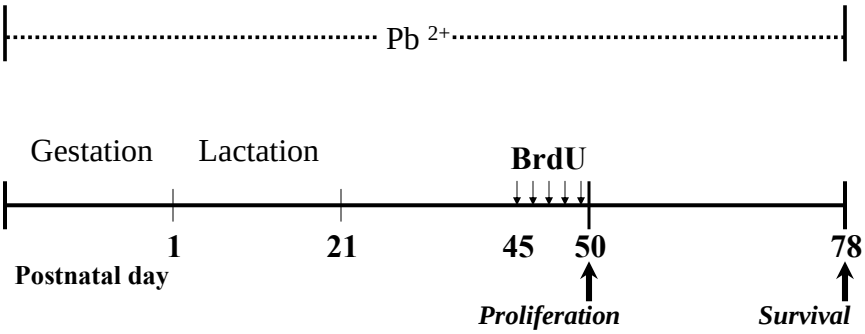
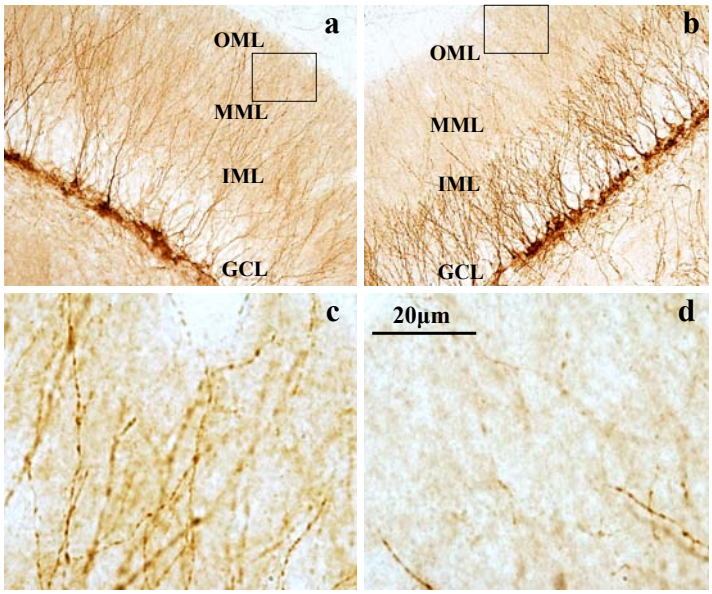


Key Events in Studying Pb2+ Neurotoxicity

- •1960-1970's – Discovered Pb^{2+} exposure produces learning impairments in children (Chisolm, Needleman, & others)
- •Investigation of Pb^{2+} effects on cognitive function in animal models [primates – Rice; rodents – Cory-Slechta (operant behavior), Guilarte (spatial learning)]
- •Hippocampus (HP) was identified as primary brain region targeted by Pb^{2+}
- •HP is important in learning & memory function
- •1990 – A key discovery by Albuquerque (UMD) showed that Pb^{2+} was a selective and potent inhibitor of the N-Methyl-D-Aspartate Receptor (NMDAR)
- •NMDAR is one of the most widely studied receptors in neuroscience and plays an essential role in brain development, synaptic plasticity, learning & memory, and neurodegeneration
- •Our lab and others subsequently confirmed this finding and showed that Pb^{2+} exposure during development produces alterations in the subunits that make up the NMDAR, changing the properties of the receptor and altering the coupling to signaling mechanisms
- •Subsequently, it was shown by Wiegand (Germany) and Gilbert (EPA), later confirmed by our lab and others, that Pb^{2+} inhibits a key cellular process associated with learning & memory function in the HP called long term potentiation (LTP)
- •LTP is a process that is known to be dependant upon the normal functioning of the NMDAR
- •Our lab showed that rodents exposed to environmentally relevant levels of Pb^{2+} (20-25 ug/dL) exhibited deficits in spatial learning and LTP, as well as significant reductions in expression of NMDAR subunits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