2013

Combined effects of rearing environment and lead (Pb2+) exposure on visuospatial learning and memory in rats

Nicholas Tolman
Connecticut College, ntolman@conncoll.edu

Combined effects of rearing environment and lead (Pb^{2+}) exposure on visuospatial learning and memory in rats

A thesis presented by

Nicholas Tolman

to the Department of Psychology

in partial fulfillment of the requirements

for the degree of

Bachelor of the Arts

Connecticut College

New London, CT

May 3, 2013

Acknowledgements

I would like to start by thanking the Neuroscience and Psychology departments for allowing me to carry out this study. Particularly I would like to thank Dr. Schroeder, my Thesis advisor, for investing large amounts of time and effort (even during his sabbatical) to guide me throughout the project. Dr. Schroeder has been a supportive mentor throughout my experiences with undergraduate research. I would also like to thank Dr. Grahn, my Academic Advisor and Thesis reader, for her dedication to research in the Neuroscience department and assistance with my project. Dr. Grahn's charisma for research conducted by the Neuroscience department was a major influence on my decision to become a Behavioral Neuroscience major. Also, I would like to thank Dr. Schneider, my Thesis reader, for taking interest in my project and the time to be one of my readers. I greatly appreciate the enthusiasm, knowledge, and commitment to undergraduate research that was displayed by each of my advising Professors during my project and throughout my time as an undergraduate. I would also like to thank my friends and family for continuing to support me during this entire yearlong project. I would like to give a special thanks to my fellow students that assisted me with my research. For Connecticut College students, Chelsey Louis, Marisa Shields, Timothy Kiprono, and Melanie Arguetta each took time out of their schedules to assist me with multiple aspects of my research. Harsh Patel also took time out of his schedule to commute from the Wheeler High School in order to assist me on this project. All of these students were a huge help to me in completing this study, and I would like to commend all of them for their dedication to my project, as I greatly appreciated it. Finally, I would like to thank my rats because without them, none of this would be possible.

Abstract

Critical periods of neural development occur during early postnatal life that correspond with increases in synaptic plasticity and the formation of neural circuits needed for learning and memory. This development can be profoundly influenced by experience and negatively affected by environmental toxins. Environmental enrichment and lead exposure inversely affect mediators of synaptic plasticity, which suggests that enrichment may have an attenuating effect on lead induced cognitive deficits. A wealth of evidence has indicated that exposure to excessive amounts of inorganic Pb^{2+} during early development can produce long lasting cognitive deficits in humans. Evidence also suggests that children raised in an impoverished environment are at a disproportionate risk for developing Pb^{2+}-induced cognitive deficits compared with peers exposed to an enriched environment. The present study evaluated the effects of both developmental Pb^{2+} exposure and environmental enrichment on visuospatial working and long-term memory in rats. Animals were fed either 1500 ppm Pb^{2+} acetate-laced rat chow or standard chow and exposed to either an impoverished environment (single housed, bedding only) or an enriched environment (4 rats/cage with toys, enclosures, etc.) for 7 weeks following weaning (PN day 25). Long-term and working memory error rates were assessed during a 17 day radial arm maze (RAM) learning task. Results suggest that the quality of the rearing environment but not Pb^{2+} exposure had a significant effect on learning performance. These findings suggest that the detrimental effects of Pb^{2+} exposure on cognitive development may be attenuated by exposure to an enriched environment and that the combination of being reared in an impoverished environment coupled with Pb^{2+} exposure can significantly impair learning performance later in life.

Table of Contents

Tables and Figures

Introduction

1.1 Sources of environmental Pb^{2+} exposure

Lead (Pb^{2+}) is a heavy metal that occurs naturally in the Earth's crust. Pb^{2+} is a transition metal cation, and is almost always found naturally bound to one or more other elements to form Pb^{2+} compounds (Patil et al., 2006). Pb^{2+} is one of the most commonly used metals in human history (Patil et al., 2006), and continues to be released through exhaust emission products and widespread industrial use (Juberg, Kleiman, & Kwon, 1997). As a heavy metal, Pb^{2+} has potential to be toxic for living organisms when found at abnormally high doses. Neural development during "critical periods" that occur in early postnatal life is particularly vulnerable to the physiological stress exerted by Pb^{2+} exposure. During these early postnatal critical periods of neural development, Pb^{2+} exposure can create life-long cognitive deficits (Koller, Brown, Spurgeon, & Levy, 2004; Toscano & Guilarte, 2005). Children in this critical period tend to move by crawling and therefore are more likely to be exposed to different surfaces that could contain Pb^{2+} dust (Koller et al., 2004). In developing countries, especially Asian countries, a large number of people live close to battery factories that use Pb^{2+} to make the batteries (Patil et al., 2006). A number of people in developing countries live close to Pb^{2+} mines (Patil et al., 2006). Developing countries also have little regulation over the use of Pb^{2+} gasoline, which results in elevated exposure of those populations to environmental Pb^{2+} (Juberg et al., 1997). Pb^{2+} mines, industrial plants, and Pb^{2+} based gasoline contaminate their surrounding environments and can cause toxic exposure levels by getting into drinking water and soil.

Pb^{2+} toxicity varies from acute exposure with a short lasting, extremely high environmental dose of Pb^{2+}, to chronic low-level exposure to Pb^{2+} over a longer span of time. Chronic Pb^{2+} exposure is the most prevalent form of Pb^{2+} toxicity. In chronic exposure cases, children and adults are differentially affected by Pb^{2+} exposure (Finkelstein, Markowitz, Rosen, 1998). Children are far more vulnerable to severe and long-lasting symptoms of Pb^{2+} exposure (Lidsky & Schneider, 2006; Ruff, Markowitz, Bijur, Rosen, 1996). In childhood Pb^{2+} poisoning cases, sustained cognitive decline is the most prevalent symptom, which includes fine motor, visuoperceptual, memory, language, and attention deficits (Lidsky & Schneider, 2006). The major environmental source of Pb^{2+} that young children are exposed to is floor dust, which accounts for 50% of the average child's Pb^{2+} intake (Koller et al., 2004). In older housing, Pb^{2+} paint and Pb^{2+} contaminated windowsills are a major risk factor for young children (Koller et al., 2004).

Heightened awareness for the dangers of Pb^{2+} exposure have led to policy changes in developed countries, which has reduced blood Pb^{2+} levels in those developed populations to roughly 3 μg/dL (Koller et al., 2004). Currently the World Health Organization places children with 10 μg/dL or more of Pb^{+} in their blood at risk for Pb^{2+} toxicity (Koller et al., 2004). However, cognitive deficits have been seen in children with smaller concentrations of Pb^{2+} in their blood (Koller et al., 2004). In the United States, 4.4% of all children ages 1-5 are estimated to have unsafe or elevated Pb^{2+} exposure levels (Rabito, Shorter, & White, 2003). However, approximately one quarter of low-income minority children living in older housing have unsafe levels of Pb^{2+} exposure, making Pb^{2+} exposure an important issue in impoverished areas of developed countries

(Rabito et al., 2003). In underdeveloped countries the mean blood Pb^{2+} level is far higher and chronic Pb^{2+} exposure continues to be a major environmental issue (Koller et al., 2004). Lack of regulation on Pb^{2+} mines causes heavy environmental pollution in certain areas. Also, impoverished areas with manufacturing plants that assemble Pb^{2+} are at risk for higher exposure.

1.2 Adverse effects of blood Pb^{2+} levels in humans

Numerous studies have shown cognitive declines in subjects exposed to chronic low-level Pb^{2+} exposure throughout childhood. In Koller et al. (2004), a 1-4% decrease in cognitive decline was observed in children exposed to Pb^{2+} during development. Studies of adults who were exposed to Pb^{2+} before the age of 4 show poorer performance on tests measuring attention, memory, reasoning, and motor speed (White, Diamond, Procter, Morey, & Hu, 1993). Controlled studies using animal subjects have consistently shown Pb^{2+}-induced cognitive deficits that are similar to those observed in humans. Rats that were exposed to Pb^{2+} prenatally through weaning showed significantly decreased performance on the Morris water maze (MWM), which is a test of spatial memory (Kuhlmann, McGlothan, & Guilarte, 1997). Rats tested at postnatal day 21 (PN21) following developmental Pb^{2+} exposure showed significantly decreased spatial memory ability compared with control animals (Jett, Kuhlmann, Farmer, & Guilarte, 1997). The animal studies correspond well with the observations made in children, showing that they are at a differential risk to Pb^{2+} exposure compared with adults.

Pb^{2+}'s interference with neural development is most profound during the critical period, which corresponds to a period of increased synaptogenesis (Hensch, 2004; Toscano & Guilarte, 2005). During this time period, connections between neurons and

neural circuits are first beginning to form. In humans, synaptogenesis occurs during a critical period between the ages of 18-36 months during which children are most vulnerable to Pb^{2+} poisoning (Goldstein, 1990). This period corresponds with heightened levels of synaptic plasticity, where neurons are particularly susceptible to being modified based on environmental factors and experiences (Hensch, 2004). Rats develop their nervous systems much faster than humans do, and synaptic plasticity peaks in the rat nervous system between postnatal days 14-15 (Harris & Teyler, 1984; Toscano & Guilarte, 2005), which corresponds with the critical period of heightened synaptogenesis in rats. Proteins that are particularly involved in memory formation are expressed at their highest levels during this developmental period in both rats and humans (Toscano & Guilarte, 2005). Furthermore, children absorb significantly more Pb^{2+} into their intestines than adults (Toscano & Guilarte, 2005). Increased Pb^{2+} absorption, increased exposure to Pb^{2+} contaminated surfaces, and heightened levels of synaptogenesis and neural circuit formation are major factors in the elevated risk for developing children to Pb^{2+} exposure.

1.3 Physiology of memory formation

Memory and learning are neural processes with one common underlying mechanism, synaptic plasticity. Synaptic plasticity occurs when neurons change or create new connections with other neurons by creating new synapses in a process known as synaptogenesis. A synapse is a small junction where separate neurons send chemical messages to each other called neurotransmitters. Neurotransmitters are packaged in vesicles that remain in the neurons terminal buttons until a signal reaches them to be released. This signal comes from calcium (Ca^{2+}) ions that initiate exocytosis of

neurotransmitter vesicles, releasing neurotransmitter into the synapse. Upon depolarization of a neuron's membranes at the terminal buttons, voltage-gated Ca^{2+} channels open and allow passive influx of Ca^{2+} into the terminals. Action potentials are fired when a neuron's axon hillock becomes depolarized enough to open voltage-gated sodium channels, sending a wave of depolarization down the axon until it reaches the terminal buttons. These physiological events take place in the pre-synaptic neuron leading to neurotransmitter release. Information is passed to the post-synaptic neuron when receptors on the post-synaptic neuron's dendrite bind the released neurotransmitter and elicit a number of different intracellular responses.

Memory can be divided into two independent phases that constitute both short-term and long-term memory formation (Alonso et al., 2002). Short-term memory is independent of protein synthesis and lasts between 1-3 hours, which is different from protein and RNA synthesis dependent long-term memory that can take several hours to several days to occur (Alonso et al., 2002; Izquierdo I., Barros, Souza T., Souza M., Izquierdo, L., & Medina, 1998). This memory formation requires activation of different glutamate receptors and isoreceptors, and also subsequent biochemical cascades, which leads to enhanced activity of protein kinases A, C, G, and calcium/calmodulin-dependent protein kinase II (CaMKII) (Izquierdo & Medina, 1997). Chronically high cellular levels of Pb^{2+} can affect these secondary-signaling pathways in the LTP process of memory formation, causing deficits in memory formation (Finkelstein et al., 1998; Goldstein, 1990; Long, Rosen, & Schanne, 1994).

In order for synaptogenesis to occur, the cellular machinery to create a working synapse must be synthesized and localized in the right places (Bahls et al., 1998).

Neuronal growth cones are responsible for extending axons towards their target areas in order to create neuron-neuron contact (Basarsky, Parpura, & Haydon, 1994). In the hippocampus, Basarsky et al. (1994) has demonstrated that hippocampal neurons undergo several days of contact before synaptic transmission is detected. The beginning of synaptic transmission in these hippocampal neurons is correlated with changes in the localization of specific proteins that are critical to synaptogenesis, including synapsin I, from the soma to the terminal buttons (Basarsky et al., 1994). Synapsins are phosphoproteins that associate with neurotransmitter vesicles and are implicated in neuronal development and synaptic functioning (Valtorta Pozzia, Benfenatib, & Fornasiero, 2011). Synapsin proteins have serine residues that are open for phosphorylation by a number of protein kinases including mitogen-associated protein kinase (MAPK) and Ca^{2+}/CaMK-dependent protein kinase (CaMK) (Valtorta et al., 2011). Phosphorylation of synapsin I modulates the transport and localization of neurotransmitter vesicle precursors along with growth in axons and dendrites during synaptogenesis (Valtorta et al., 2011). Other cellular processes involved in synaptogenesis include synthesis and localization of post-synaptic neurotransmitter receptors and pre-synaptic voltage-gated Ca^{2+} channels (Bahls et al., 1998). Pb^{2+} has the potential to disrupt memory formation by inhibiting any of these physiological processes involved with synaptogenesis.

One prominent mechanism that has been associated with synaptogenesis and memory formation is long-term potentiation (LTP) (Yang, Wu, Liu, & Tung, 1998). When neurons are activated, they undergo physiological changes, and LTP refers to the transcriptional processes that grow and strengthen synapses (Riedel, Wetzel, & Reymann,

1996). Studies have shown LTP is strongly related to inotropic glutamate receptors (Riedel et al., 1996). Glutamate is the major excitatory neurotransmitter in the CNS and is the endogenous ligand at two different inotropic receptors; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and NMDAR (Riedel et al., 1996). AMPARs are ligand gated Na^+ channels that allow Na^+ influx into the cell upon activation by glutamate, causing a cell membrane depolarization. NMDARs require glutamate binding, however, this receptor is also voltage gated. A Mg^{2+} cation blocks the channel so Na^+ cannot enter the cell unless there is subsequent depolarization across the cell membrane. When enough AMPARs are activated by glutamate, NMDARs undergo a conformational change forcing the removal of the Mg^{2+} cation, promoting Na^+ influx.

Activation of NMDA receptors leads to intracellular physiological changes that ultimately cause synapses to form and strengthen (Yang et al., 1998). NMDARs vary based on their composition of subunits, which include the NR1, NR2A, NR2B, NR2C, NR2D, and NR3A subunits (Guilarte & McGlothan, 1998; Prybylowski et al., 2002). Altered expression of these subunits can change the overall composition of NMDARs, and could lead to impairments of NMDAR dependent LTP (Guilarte & McGlothan, 1998). NR2 subunit availability determines the number of functional NMDARs on the cell surface (Prybylowski et al., 2002). NMDAR expression is highest during the critical period (Toscano & Guilarte, 2005), indicating these receptors play a role in synaptogenesis and neural circuit formation. Interfering with NMDARs, in particular NR2 subunit expression, or any part of the intracellular signaling cascade following NMDAR activation, could affect LTP and memory.

Another form of receptors for glutamate that has been shown to affect hippocampal synaptic plasticity is metabotropic glutamate receptors (mGluRs) (Xu et al., 2009). NMDAR activation alone is not enough to explain the process of glutamate-induced LTP (Heidinger et al., 2002). mGluR activation leads to secondary signaling events that cause phosphorylation of a tyrosine residue on NR2B subunits of NMDARs, and phosphorylation of this subunit is known to increase during LTP phases (Heidinger et al., 2002). Ca^{2+} signaling is a part of the intracellular pathway induced upon mGluR activation, as CaMK is activated by mGluR signal transduction (Heidinger et al., 2002). CaMK subsequently activates a number of other kinase families, leading to NR2B phosphorylation (Heidinger et al., 2002). The NR2B subunit is directly coupled to another kinase signaling pathway called the mitogen-activated protein kinase (MAPK) pathway (Krapivinsky et al., 2003). One form of MAPK, the extracellular-related protein kinase (ERK) 1/2, is activated by phosphorylation at both a tyrosine and threonine residue separated by one amino acid (Thomas & Huganir, 2004). Activation of ERK 1/2 signaling pathways is important to synaptic plasticity, and activated ERK is seen in dendrites and cell bodies of neurons that are active due to synaptic activity (Toscano & Guilarte, 2005; Adams & Sweatt, 2002). mGluRs and secondary signaling pathways related to NMDAR activation provide another target for Pb^{2+}-induced LTP inhibition.

Another critical mechanism identified with synaptic plasticity and memory formation is brain-derived neurotrophic factor (BDNF) activity (Richter-Schmidinger et al., 2011). BDNF is a member of the neurotrophin family that is responsible for cell growth and maintenance (Mizuno, Yamada, Olariu, Nawa, & Nabeshima, 2000). BDNF enhances synaptic transmission by causing increases in NMDAR subunit phosphorylation

(Mizuno et al., 2000). BDNF has also been shown to enhance synaptic plasticity by inducing LTP (Mizuno et al., 2000). BDNF expression is another physiological change that affects memory formation.

BDNF polymorphisms have been shown to cause significant working memory impairments is adults compared to those carrying the normal BDNF gene (Richter-Schmidinger et al., 2011). Transgenic mice that overexpress BDNF perform better in spatial memory tasks such as the Morris water maze (MWM) than heterozygote and wild-type BDNF mice, indicating that increased BDNF levels enhance spatial memory and learning (Nakajo, Miyamoto, Nakano, Xue, Hori, & Yanamoto, 2008). Hormones such as β-estradiol that increase hippocampal synaptogenesis can be reversed by both β-estradiol and BDNF receptor antagonists, indicating that BDNF mediates the hormone's effect on synaptogenesis (Sato, Akaishi, Matsuki, Ohno, & Nakazawa, 2007). Increases in BDNF have been consistently associated with improvement in learning and memory tasks.

In the hippocampus, BDNF exerts its multiple effects on memory and LTP through activation of the transcription factor cAMP response element-binding protein (CREB) (Alonso et al., 2002). A higher level of phosphorylated CREB in the hippocampus is associated with better performance in learning and memory in tasks (Kudo, Wati, Qiao, Arita, & Kanba, 2005). Both ERK1/2 and CaMKII are part of biological cascades that act as secondary messengers after NMDAR activation, which leads to enhance CREB activity (Alonso et al., 2002). BDNF increases phosphorylation in both ERK1/2 and CaMKII signaling pathways (Alonso et al., 2002). This effect is possibly caused by BDNF's ability to enhance the responsiveness of NR2B subunit

containing NMDAR's by activating post-synaptic TrkB receptors (Levine & Kolb, 2000). It is clear that BDNF is important to memory formation, as both NMDARs and subsequent signaling pathways that are important to LTP are more active in the presence of BDNF. Increased BDNF expression is important to the enhancing effects of rearing in an EE on memory formation (Schneider, Lee, Anderson, & Lidsky, 2001; Toscano & Guilarte, 2005).

1.4 Role of the hippocampus in memory acquisition

Along with the specific proteins that influence memory formation, certain brain regions are involved in these memory formation processes. The hippocampus is one brain region that is active during several memory forming events (Felix & Levin, 1997; Moser & Moser, 1998). In rodents, the main function of the hippocampus is in spatial memory formation (Moser & Moser, 1998). Lesion of any dorsal hippocampal neurons caused spatial memory recall deficits in rats (Moser & Moser, 1998), indicating that spatial memories are widely encoded throughout the hippocampus. Hippocampal cholinergic neurons have also been shown to impact working memories (Felix & Levin, 1997). In humans, the hippocampus is thought to also encode for declarative or episodic memories (Battaglia, Benchenane, Sirota, Pennartz, & Wiener, 2011). However, the human hippocampus is still organized to process spatial relationships (Kumaran & Maguire, 2005). It is hypothesized that episodic memories are coded in hippocampal neurons and are linked by common features to form an organized network of knowledge (Eichenbaum, 1999).

The hippocampus is crucial to the formation of long-term declarative memories. During inactivity, the hippocampal neurons fire in a pattern of synchronized theta wave

bursts, sending oscillations that propagate to other brain structures (Battaglia et al., 2011). These synchronized neural oscillations encode information that propagates to other cortical and subcortical brain regions, causing this information to be stored as long-term memory (Battaglia et al., 2011). While directly coding for some information, the hippocampus is also critical for information storage in other brain regions. Within the hippocampus, several different neuronal subtypes contribute to memory formation and learning. Farr, Flood, & Morley (2000) demonstrated that drugs targeting acetylcholine, GABA, glutamate, and serotonin containing neurons in the hippocampus all affected memory retention. A number of neurotransmitters and secondary signalers influence memory formation in the hippocampus. However, it is widely accepted that NMDAR activation is most critical to LTP and memory formation in the hippocampus (Toscano & Guilarte, 2005).

The hippocampus is part of a functional system known as the hippocampal formation, and the two terms can be used interchangeably when referring to the functional hippocampus (Andersen, Morris, Amaral, Bliss, & O'Keefe, 2007). There are several different structures within the hippocampal formation including four subfields of the hippocampus called CA1, CA2, CA3, and CA4 (with CA standing for cornu ammonis) (Giap, Jong, Ricker, Cullen, & Zafonte, 2000), along with the dentate gyrus, subiculum, presubiculum, parasubiculum, and entorhinal cortex (Andersen et al., 2007). The CA4 subfield of the hippocampus is also known as a deep polymorphic cell layer in the dentate gyrus (Andersen et al., 2007). The major pathway of information entering the hippocampus is known as the perforant path (Andersen et al. 2007) (figure 1). Input from the neocortex enters the hippocampal formation through superficial cell layers of the

entorhinal cortex and proceeds unidirectionally onto granule cell layers of the dentate gyrus (Andersen et al. 2007). Input next reaches pyramidal cells of the CA3 hippocampal subfield through axons of neurons in the dentate gyrus known as mossy fibers (Andersen et al. 2007). CA3 pyramidal cells next project the input unidirectionally to CA1 pyramidal cells through the axons of Schaffer collateral neurons (Andersen et al. 2007). The unidirectional flow of input continues after CA1 pyramidal cells to neurons of the subiculum and then onto the presubiculum and parasubiculum (Andersen et al. 2007). Prominent cortical projections begin in both CA1 pyramidal cells and the subiculum and are first projected through the entorhinal cortex, completing the unidirectional input loop through the hippocampus (Andersen et al. 2007).

The development of hippocampal connections is critical to the proper circuitry and functioning of the hippocampus. Entorhino-hippocampal projections bring information from the entorhinal cortex into the granule and pyramidal cells of the dentate gyrus (Andersen et al. 2007). Commisural fiber projections send information from the CA3 to the dentate gyrus (Andersen et al. 2007). Finally, septohippocampal projections are cholinergic and GABAergic projections originating in the medial septum and sending input into the hippocampus (Andersen et al. 2007). Afferent neurons that input into the hippocampus are mainly excitatory and contain either the excitatory neurotransmitter glutamate or acetylcholine (Giap et al., 2000). Interneurons of the hippocampus are mostly inhibitory neurons that contain the neurotransmitter γ-aminobutyric acid (Giap et al. 2000). Pb^{2+} can potentially interfere with the formation of hippocampal input pathways including the perforant pathway, or neurotransmission in hippocampal projections, altering learning and memory processes.

1.5 Indirect Effects of Pb^{2+} on memory formation and learning

Pb^{2+} exerts a number of direct and indirect effects on memory once concentrations reach abnormally high intracellular levels. Chronic Pb^{2+} exposure results in cell death (Chao, Moss, & Harry, 2007; Weisskopf et al., 2004), which affects neuron cells in memory related brain regions including the hippocampus. A decrease in neuronal density was observed in Weisskopf et al. (2004) using N-acetylaspartate:creatine ratio to measure hippocampal densities of two 71 year old twins who had received chronic Pb^{2+} exposure. One twin had a history of higher exposure, and therefore showed less hippocampal volume. This finding indicates varying levels of Pb^{2+} exposure correlates with decreases neuronal density in the hippocampus (Jiang et al., 2008). Pb^{2+}'s indirect effect on memory by cell death has multiple physiological causes.

Once Pb^{2+} is inside the body, it negatively affects cellular function in a variety ways. Heme groups are present in multiple enzymes, and heme biosynthesis is inhibited by accumulation of Pb^{2+} in erythrocytes. Heme groups are required for the protein hemoglobin (Patil et al., 2006), which is responsible for moving oxygen throughout the body to respiring tissue. Heme groups have a porphyrin ring system that normally binds an iron cation during synthesis (Counter, Buchanan, & Ortega, 2008). Pb^{2+} induced iron deficiency causes zinc to replace iron in this porphyrin system, and inhibits the synthesis of protoporphyrin IX, which is a metabolic step in heme synthesis (Counter et al., 2008; Fujita, Nishitani, & Ogawa, 2002). Both δ-aminolevulinate dehydratase and ferrochetalase are both enzymes inhibited by Pb^{2+} exposure in the heme synthetic pathway (Fujita et al., 2002). Inhibiting oxygen transportation to neurons required for aerobic respiration is a harmful aspect of Pb^{2+} toxicity.

Some enzymes act as natural antioxidants, clearing up reactive oxygen species (ROS) and radical species that are potential mutagens of DNA (Patil et al., 2006). Ascorbic acid, a potent antioxidant that has been shown to decrease cell death by enhancing ROS pathway enzymes, can ameliorate Pb^{2+} induced oxidative damage in the hippocampus (Chang et al., 2012). This finding indicates ROS enzymes are important for protecting cells against Pb^{2+} induced oxidative stress. Pb^{2+} negatively affects the ROS pathway by inhibiting specific antioxidant enzymes that require a metal cofactor and increasing the formation of highly reactive oxygen species that deplete the supply of antioxidant enzymes (Patil et al., 2006). Significant changes in mitochondrial ROS pathways were observed in glial cells of Pb^{2+} exposed animals (Weisskopf, Hu, Sparrow, Lenkinski, & Wright, 2007). Significant upregulation of ROS pathways ultimately leads to astrocyte swelling and apoptosis (Hu, Sun, Zhao, Cui, & Yang, 2008). These results together indicate that Pb^{2+} exposure puts oxidative stress on neuronal cells, which causes cell death and decreased neural functioning.

In Patil et al. (2006), workers that had been chronically exposed to Pb^{2+} throughout their lives were tested for ROS pathway enzymes. The study showed significantly increased concentrations of ROS pathway enzymes, particularly superoxide dismutase in the Pb^{2+} exposure group. Superoxide dismutase converts reactive superoxide species into water and hydrogen peroxide (Patil et al., 2006). The reactive product hydrogen peroxide is subsequently converted into water and O_2 by another enzyme called catalase, which is a tetrameric protein consisting of four heme groups with a reactive Fe^{2+} cation (Patil et al., 2006). Because heme biosynthesis is inhibited by Pb^{2+}

exposure, reactive intermediates in this ROS metabolic pathway accumulate and cause upregulation of enzymes including superoxide dismutase.

Along with inducing changes in ROS antioxidant pathways, Pb^{2+} has an effect on biological pathways through its reactive properties as a transition metal cation. Pb^{2+} has potential to react with a variety of biological molecules creating highly reactive molecular species. One of these mechanisms that potentially contribute to Pb^{2+}'s negative physiological effects is through inhibition of DNA repair by preventing excision repair at the polymerization or ligation step of DNA synthesis (Finkelstein et al., 1998). Pb^{2+}'s action as a mutagenic agent has potential to cause a wide variety of intracellular problems. In Struzynska, Bubko, Walski, & Rafalowska (2001), an increased immunoreactivity of glial fibrillary acidic protein (GFAP), which is a common astrocyte reaction to CNS damage, was observed in the hippocampus and cerebral cortex following acute Pb^{2+} exposure. ROS pathway upregulation as a mechanism against oxidative stress is seen in both acute (Struzynska et al., 2001) and chronic (Patil et al., 2006) Pb^{2+} exposure. This fact suggests Pb^{2+} reacts with biological species to form highly reactive intermediates that could further react with a number of molecules including DNA.

The blood-brain barrier is unique because unlike the rest of the body's organs, water-soluble molecules such as glucose, amino acids, and important ions cannot passively diffuse into the central nervous system (Goldstein, 1990). Instead, these essential molecules and ions are moved into the central nervous system by active transport, so that all other soluble molecules in the blood plasma are excluded (Goldstein, 1990). This barrier to most molecules is important because many hormones and ions that accumulate in the blood due to diet or stress would have a significant effect on neural

functioning (Goldstein, 1990). By interfering with Ca^{2+} signaling pathways, Pb^{2+} exposure affects the formation of brain capillaries and the blood-brain barrier (Goldstein, 1990; Finkelstein et al., 1998). Endothelial cells have shown increased affinity for Pb^{2+} compared to other cells in the body, and also form the main structural component of the blood-brain barrier by forming tight junction contacts (Finkelstein et al., 1998). Ca^{2+} acts as a secondary signaler in endothelial cells by activating PKC in a signaling pathway critical to homeostasis of the blood-brain barrier (Finkelstein et al., 1998). Pb^{2+} interferes with cell signaling in endothelial cells, disrupting formation of the blood-brain barrier.

Along with homeostatic function, PKC is also critical to the development of brain microvessels (Goldstein, 1990). In particular, a translocation of PKC from the cytosol to the membrane fraction where it associates with cellular membranes between 10-15 days after the beginning of development is critical to microvessel development (Goldstein, 1990). Low-level Pb^{2+} exposure results in premature translocation of PKC, disrupting blood-brain barrier development. Disruptions of blood-brain barrier development due to chronic Pb^{2+} exposure results in a disruption of the barrier between plasma and the CNS, and leads to interstitial plasma accumulation in the CNS (Goldstein, 1990). Fluid accumulation results in CNS edema and an increase in intracranial pressure, which causes decreased perfusion of blood with essential nutrients to neuron cells that require the nutrients (Goldstein, 1990).

Astrocytes form the other major structural component of the blood-brain barrier, and are particularly sensitive to the toxic effects of low-level Pb^{2+} exposure (Finkelstein et al., 1998). Glial cell morphological (Cookman & Regan, 1991) and physiological (Weisskopf et al., 2007) changes are observed in Pb^{2+} exposed animals. Signals between

astrocytes and endothelial cells are important to the development of the blood-brain barrier (Finkelstein et al., 1998). Activation of ROS pathways leads to astrocyte proliferation and cell death (Hu et al., 2008), and Pb^{2+} has been shown to affect ROS pathways in astrocytes (Weisskopf et al., 2007). Interactions between astrocytes and endothelial cells are part of Pb^{2+} toxicity towards blood-brain barrier development. Without the ability to properly obtain nutrients through the blood-brain barrier, it is more difficult for neurons to function. Taken together, Pb^{2+} disrupts formation of the blood-brain barrier during the developmental period, which causes widespread neural deficiencies in nutrient supply.

The actions of Pb^{2+} as an inhibitor of heme biosynthesis, ROS pathway inhibitor, and DNA mutagens result in declined functioning of all biological systems. Together, these actions of Pb^{2+} result in inadequate perfusion along with potential damage to DNA for all cells, including those in memory related brain areas such as the hippocampus. Pb^{2+} also disrupts blood-brain barrier formation, disrupting the normal movement of nutrients and hormones into the CNS. Disruption of blood-brain barrier formation during development can potentially cause more Pb^{2+} to enter the CNS through the blood stream (Toscano & Guilarte, 2005). Inadequate perfusion, DNA damage, and ROS inhibition are all factors that indirectly influence hippocampal cell loss by interfering with normal cellular functioning.

1.6 Direct Effects of Pb^{2+} on Memory

Pb^{2+} exposure interferes directly with the physiological mechanisms that form and strengthen synapses. Along with the inhibition of blood-brain barrier formation through Ca^{2+} signaling pathways, Pb^{2+} has similar effects on Ca^{2+} signaling pathways involved in

memory formation (Goldstein, 1990). Because Pb^{2+} has similar physical properties to other divalent cations including Ca^{2+}, Pb^{2+} characteristically interferes with Ca^{2+} signaling systems (Finkelstein et al., 1998). Intracellular Ca^{2+} is an important regulator of the neural cytoskeleton, making it critical for the modification of neural circuits (Mattson, 1992). Ca^{2+} influx into developing neurons controls axonal differentiation by causing the microtubule-associated protein tau to migrate to the axon, and microtubule associated protein 2 (MAP2) to migrate to the dendrites (Mattson, 1992). Disregulation of intracellular Ca^{2+} leads to neuronal death (Mattson, 1992). All of this evidence supports the idea that Ca^{2+} signaling is important to neuron survival and the physiology of synaptic plasticity.

Extracellular Ca^{2+} enhances both synaptic plasticity and neural excitability upon entry into the cell through actions on second signalers including protein kinase C (PKC) and CaMKII (Yang et al., 1998). PKC is normally activated by rises in intracellular Ca^{2+} concentrations, but can also be activated by subnanomolar concentrations of free intracellular Pb^{2+} (Long et al., 1994). Synaptogenesis is reliant on several kinase pathways, that when blocked, inhibit the induction of LTP (Finkelstein et al., 1998; Klann, Chen, & Sweatt, 1993). A protein in the rodent brain called RC3, which is a third messenger substrate activated by PKC, is involved in synaptic development and remodeling (Watson, Sutcliffe, & Fisher, 1992). PKC activation can also affect the induction and maintenance of LTP by phosphorylating substrates that regulate the activity of NMDARs (Klann et al., 1993). These effects of PKC on synapse development and LTP induction are targets of Pb^{2+}-induced changes in memory and learning at subnanomolar concentrations of free intracellular Pb^{2+}.

When a neurotransmitter binds to its target receptor, it begins the sequence of intracellular events that lead to synaptogenesis (Goldstein, 1990). One important secondary signaling pathway following neurotransmitter induced signal transduction involves the breakdown of a lipid involved in the plasma membrane called phosphoinositide bisphosphate into two signaling molecules called diacylglycerol and inositol triphosphate (Goldstein, 1990). PKC is subsequently activated by diacylglycerol in the presence of Ca^{2+}, leading to the phosphorylation of nerve terminal proteins that enhance neurotransmitter release through increasing Ca^{2+} entry upon depolarization of terminal buttons (Goldstein, 1990). Pb^{2+} replaces Ca^{2+} in this signaling sequence, causing prolonged neurotransmitter release (Goldstein, 1990). This disruption in neural functioning has a wide range of effects depending on the type of neurotransmitter and receptor type, including changes in the threshold of excitation, modulation of neurotransmitter release mechanisms, and losses in neural circuit complexity (Goldstein, 1990).

During splicing of a translated polypeptide chain into functional NMDA subunits, PKC phosphorylation at the C terminus changes the splice variant so that different subunit isoforms are expressed (Toscano & Guilarte, 2005). In hippocampal neurons positive for neuronal nitric oxide synthase (nNOS), a retrograde signaler that is known to affect hippocampal LTP, NMDARs are enriched with a particular NR1 spice variant that is inhibited by PKC phosphorylation (Toscano & Guilarte, 2005). Pb^{2+} inhibits hippocampal LTP by interfering with nNOS retrograde signaling. Some physiological processes involved in hippocampal LTP are independent of NMDARs, and are mediated by dihydropyridine (DHP)-sensitive Ca^{2+} channels (Pourmotabbed, Motamedi,

Fathollahi, Mansouri, & Semnanian, 1998). Pb^{2+} exposure potentially interferes with this non-NMDA LTP by interfering with Ca^{2+} signaling (Pourmotabbed et al., 1998). Pb^{2+} exposure can inhibit hippocampal LTP in multiple ways.

Another protein kinase involved with synaptic transmission that Pb^{2+} interferes with is CaMK, a Ca^{2+}-binding regulatory protein (Goldstein, 1990). Pb^{2+} can activate CaMK directly by binding in place of Ca^{2+}, and indirectly by initiating PKC signaling sequences that lead to elevated intracellular Ca^{2+} concentrations (Goldstein, 1990). Upon activation, the conformation of CaMK changes into an active kinase, allowing CaMK to subsequently activate the protein synapsin (Goldstein, 1990). Pb^{2+}-induced phosphorylation of synapsin leads to enhanced neurotransmitter release, augmenting the disruption of neural functioning caused by the over-release of neurotransmitters (Goldstein, 1990). Conversely, Pb^{2+} has been shown to inhibit synapsin activity by both inhibiting the synthesis of another activator of synapsin, cyclic adenosine monophosphate (cAMP) (Nathanson & Bloom, 1975). Pb^{2+} exposure exerts a number of different effects on kinase pathways and Ca^{2+} signaling that disrupts neurotransmission.

Pb^{2+}'s interference with kinase pathways can affect memory in a multitude of ways. Phosphorylation of voltage-gated Ca^{2+} channels along with other ion channels can change channel conformations to change the permeability of channels to ion passage (Goldstein, 1990). Interfering with ion movement across cell membranes can affect neurotransmission by changing voltages across membranes and causing Ca^{2+} to freely enter the cell and induce exocytosis of neurotransmitter vesicles (Goldstein, 1990). Enzymes that synthesize and break down neurotransmitters are also affected by phosphorylation (Goldstein, 1990). Another kinase pathway affected by Pb^{2+} exposure is

the mitogen-activated protein kinase (MAPK) signaling pathway (Toscano & Guilarte, 2005). ERK 1/2 phosphorylation increases significantly upon Pb^{2+} exposure (Ramesh, Manna, Aggarwal, & Jadhav, 2001), which is a signaling pathway coupled to NR2B subunit containing NMDAR activation (Krapivinsky et al., 2003). By interfering with multiple kinases including PKC, CaMKII, MAPK ,and cAMP, several synaptogenesis and LTP related intracellular pathways are affected upon Pb^{2+} exposure.

Glutamate signal transduction is dependent on Ca^{2+} signaling, which is another target of Pb^{2+}-induced LTP disruption. Pb^{2+} causes a dose-dependent inhibition of both mRNA and protein expression for mGluR5, a member of the mGluR family (Xu et al., 2009). mGluRs signal for NR2B subunit phosphorylation through a secondary signaling pathway involving Ca^{2+}/CaMK, which potentially plays a role in regulating NMDAR function and synaptic plasticity (Heidinger et al., 2002). It is also thought that mGluR activation might trigger the regulation of hippocampal neuronal Ca^{2+} sensor protein (VILIP-1) expression, which would affect neuronal signaling and LTP (Xu et al., 2009). Pb^{2+}'s interference with Ca^{2+} signaling in the mGluR signal transduction pathway affects NMDAR dependent LTP.

Changes in gene expression are another mechanism by which Pb^{2+} causes neurocognitive deficits. CaMKII is expressed at significantly lower levels during low-level Pb^{2+} exposure than control animals (Luo et al., 2011). Decreased CaMKII expression led to impaired cAMP responsive element binding protein (CREB) phosphorylation. CREB is a transcription factor, and impairing its phosphorylation leads to changes in hippocampal gene expression (Ho et al., 2000). Both cAMP and CaMK are Ca^{2+} dependent kinases that activate CREB by phosphorylating a serine 133 residue

(Toscano & Guilarte, 2005). CREB phosphorylation leads to the recruitment of proteins such as CREB binding protein (Chrivia, Kwok, Lamb, Haglwara, Montmlny, & Goodman, 1993), beginning assembly of a transcriptionally active complex that is necessary for hippocampal learning, memory, and synaptic plasticity (Deisseroth, Bito, & Tsien, 1996; Toscano & Guilarte, 2005). As shown in Lau, Saha, Faris, & Russek (2004), NR1 subunit mRNA expression was controlled in a CREB dependent manor, indicating that Pb^{2+} alters the expression of proteins that are critical to LTP. Specific metal-dependent transcription factors, such as the zinc-finger proteins, are also potentially responsible for Pb^{2+}-induced changes in gene expression (Finkelstein et al., 1998). This is consistent with other studies that show differential gene expression upon Pb^{2+} exposure in the hippocampus (Li et al., 2009; Schneider, Anderson, & Vadigepalli, 2011).

The hippocampus is significantly affected by developmental Pb^{2+} exposure. Significant morphological changes in CA3 mossy fiber pathways were observed in animals exposed to Pb^{2+} during development (Alfano, LeBoutillier, & Petit, 1982). In Schneider et al. (2011), male and female rats combined showed 167 hippocampal genes that were differentially expressed upon Pb^{2+} exposure. These differentially expressed genes code for a wide variety of proteins including regulators of GPCR signaling and ROS pathway enzymes such as superoxide dismutase 3 (Schneider et al., 2011). Enzymes that affect the catabolism and synthesis of D-serine, an allosteric modulator of NMDARs, are differentially expressed upon Pb^{2+} exposure (Schneider et al., 2011). Knocking out serine racemase (Srr), the enzyme that synthesizes D-serine, changes the expression of 9 genes involved with the allosteric modulation of NMDARs (Schneider et

al., 2011). Pb^{2+} exposure causes similar changes to 6 out of the 9 hippocampal genes (Schneider et al., 2011), indicating Pb^{2+} induces changes in gene expression that directly affects LTP. Lower concentrations of other neurotransmitter receptors, such as the muscarinic acetylcholine receptor, are also observed in the hippocampus after Pb^{2+} exposure (Jett & Guilarte, 1995). Changes in the expression of both vesicular acetylcholine transporter mRNA and choline acetyltransferase mRNA provides further evidence that Pb^{2+} induces changes in cholinergic signaling (Finkelstein et al., 1998). Hippocampal cholinergic projections are critical to emotional responses along with learning and memory (Finkelstein et al., 1998). These results taken together indicate Pb^{2+} exposure disrupts a wide range of hippocampal physiology including neurotransmission and LTP.

Pb^{2+} interferes with NMDARs in several ways (Toscano & Guilarte, 2005; Zhu et al., 2011; Nihei & Guilarte, 1999), making it an effective NMDA antagonist. NMDAR subunits show decreased expression as a result of Pb^{2+} poisoning (Toscano & Guilarte, 2005; Zhu et al., 2011). When this change in subunit expression occurs during the critical period of development, NMDAR subunit composition undergoes permanent changes, altering the physiology of affected brain regions including the hippocampus (Nihei et al., 1999). Furthermore, changes in NMDAR expression have been shown to be age-dependent, and appear most pronounced during the critical period of development, where induction of LTP is highest (Jett & Guilarte, 1995), contributing to the increased vulnerability of the developing brain to Pb^{2+} exposure.

The NR2A receptor subunit is most inhibited in the hippocampus at low levels of Pb^{2+} exposure (Nihei et al., 1999, Zhu et al., 2011). Inhibition of the NR2A subunit was

correlated with blood-Pb^{2+} levels in the hippocampus, indicating Pb^{2+} directly affects hippocampal NR2A subunit expression (Zhu et al., 2011). In Guilarte & McGlothan (1998), in situ hybridization was used to determine mRNA expression of NMDAR subunits in Pb^{2+} exposed rats at postnatal day 21 (PN21). At PN21, the rat nervous system is undergoing its critical period of development, and NMDAR levels peak in the CNS (Guilarte & McGlothan, 1998). In the hippocampus, NR1 subunit expression was increased in the CA4 subfield and NR2A subunit mRNA expression was decreased across all hippocampal fields, causing altered NMDAR subunit composition that possibly leads to LTP impairment (Guilarte & McGlothan, 1998). Because NR2 subunit expression determines the number of functional NMDARs (Prybylowski et al., 2002), Pb^{2+} induced inhibition of NR2A subunits decreases the number of functional NMDARs throughout the hippocampus. This finding is consistent with Jett & Guilarte (1995), which found a decreased expression of functional hippocampal NMDARs following developmental Pb^{2+} exposure. During development, NMDAR antagonists have been shown cause apoptosis (Dribben, Creeley, & Farber, 2011). Pb^{2+} inhibition of NMDARs has a significant effect on learning, memory, and neuron survival.

Developmental exposure to Pb^{2+} has been shown to increase the expression of amyloid precursor protein (APP) during adulthood (Basha, Wei et al., 2005; Wu et al., 2008; Huang, Bihaqi, Cui, & Zawia, 2011). APP is polypeptide that is cleaved by β and γ secretases at BACE1 sites to form Aβ proteins (Laird et al., 2005). When Aβ-42, a specific protein in the Aβ family, begins to form intracellular and extracellular aggregates, the process results in cell death and is part of Alzheimer's disease pathology (Laird et al., 2005). In Wu et al. (2008), aged monkeys that were exposed to Pb^{2+} during

development showed increased intracellular and extracellular Aβ deposits, which led to higher levels of oxidative damage. In Basha, Murali et al. (2005) Pb^{2+} exposure was shown to cause dose dependent increases in Aβ aggregation. Being a transition metal cation, Pb^{2+} can coordinate several negatively charged species at once, which increases Aβ aggregation and Alzheimer's pathology.

Along with altered APP expression and Aβ aggregation, developmental Pb^{2+} exposure has also been shown to affect other genes and proteins in the Alzheimer's pathology. Neprilysin (NEP), a rate-limiting catabolic enzyme for Aβ metabolism, is downregulated following early life Pb^{2+} exposure (Huang et al., 2011). The low-density lipoprotein receptor-related protein-1 (LRP1) gene has been shown to affect clearance of Aβ from cerebrospinal fluid into the blood plasma, leading to a reduced risk for Aβ deposits to aggregate (Gu et al., 2011). Protein kinase C (PKC) and LRP1 proteins are colocalized in neuron tissue, and chronic Pb^{2+} exposure has been shown to alter PKC activity, leading to a relocalization of LRP1 receptors (Behl, Zhang, Shi, Cheng, Du, & Zheng, 2010).

Amyloidogenesis can affect memory in a number of ways. Oxidative stress and cell death are a result of Aβ aggregation (Wu et al., 2008). In brain regions such as the hippocampus, this cell death can result in decreased memory performance. When β-amyloids were injected into the rat dentate gyrus of the hippocampus, a loss in synaptic transmission and neuroplasticity was observed (Stephan, Laroche, & Davis, 2001). Decreased synaptic transmission was correlated with a decrease in long-term potentiation, and reduced working memory performance (Stephan et al., 2001). Hippocampal deposits of Aβ-40 and Aβ-42 protein was highly correlated with spatial

memory deficits and long-term memory impairment (Zhang et al., 2011). Amyloidogenesis precedes the onset of Alzheimer's disease, and can directly inhibit synaptic plasticity.

Alzheimer's pathology can affect synaptic formation by inhibiting structural components necessary for synaptogenesis. Tau is a protein that helps to stabilize intracellular microtubules. Alzheimer's pathology changes the expression of tau leading to the collapse of microtubules (Zilka et al., 2006). The collapse causes the microtubules and tau proteins to clump together, creating an intracellular neurofibrillary tangle (Zilka et al., 2006). Microtubules are necessary for synaptogenesis, as they stabilize the growth of dendritic spines that lead to synapse formations (Dent, Merriam, & Hu, 2011). Another direct way Alzheimer's pathology affects synapse formation is by interfering with neurotransmitter vesicle exocytosis and subsequent release (Russell, Semerdjieva, Empson, Austen, Beesley, & Alifragis, 2012). Aβ-42 interferes with the interaction between synaptophysin and vesicle-associated membrane protein 2 (VAMP2), increasing the amount of presynaptic neurotransmitter vesicles ready for release into the synapse through exocytosis (Russell et al., 2012). Alzheimer's pathology can directly affect synapses, which leads to potential memory deficits.

Physiological changes in Ca^{2+} signaling pathways, kinase-signaling pathways, expression of proteins critical to memory, and Aβ aggregation all cause direct Pb^{2+}-induced memory and learning deficits. Multiple Ca^{2+} signaling and kinase pathways are involved in the development of synapses during the critical period. Changes in the expression of NMDAR subunits caused by Pb^{2+} exposure are augmented during the critical period as NMDAR expression is normally at its highest (Toscano & Guilarte,

2005). Increased onset of Aβ aggregation and interference with synaptogenesis occurs with developmental Pb^{2+} exposure (Wu et al., 2008). Developmental Pb^{2+} exposure has a severe direct impact on memory functioning and synaptic plasticity.

1.7 Effects of environmental enrichment on memory formation

Exposure to an enriched environment (EE) can positively affect memory formation. EE is an experimental setting in which groups of animals are kept in social cages containing toys and exercise wheels (Piazza, Pinto, Trott, Marcuzzo, Gomez, & Fernandes, 2011). This experimental condition relates to several human activities including physical exercise, social interaction and sensory stimulation, all of which improve learning and memory tasks (Nithianantharajah & Hannan, 2006; Piazza et al., 2011). In Schrijuer, Bahr, Weiss, & Wurbel (2002), both social isolation and environmental stimulus were separate variables, and performance on several memory tasks was analyzed. Animals given toys and physical exercise showed significantly faster habituation to novel environments (Schrijuer et al., 2002). These animals given only an EE preformed better on spatial memory tasks regardless of social background (Schrijuer et al., 2002). Both inanimate environmental stimulus and social interactions contribute to the physiological effects of EE.

EE exhibits its effects on memory in several ways. Morphological analysis of hippocampal layer-III pyramidal neurons showed EE cause increased dendritic growth and arborization while also increasing the density of dendritic spines, which are all critical for synaptic formation (Leggio et al., 2005). EE has also been shown to positively impact neurogenesis. Increased cell counts in the dentate gyrus (DG) of the hippocampus that correlated with improved swim test scores, an animal model of stress,

were found in EE rats (Llorens-Martín, Rueda, Martinez-Cue, Torres-Aleman, Florez, & Trejo, 2007). Rats raised in an EE overcame glial cell morphological changes (Beauquis et al., 2013). Changes in glial morphology are associated with Alzheimer's onset causing decreased synaptogenesis (Beauquis et al., 2013) and disruption in blood-brain barrier formation (Finkelstein et al., 1998). EE improves gliogenesis, or generation of new glia cells and the expression of glial-cell-derived neurotrophic factor (GDNF) (Van Praag, Kempermann, & Gage, 2000), which has potential to ameliorate Pb^{2+} induced disruption of blood-brain barrier formation. Angiogenesis is increased by EE (Van Praag et al., 2000), which can attenuate Pb^{2+} induced decreases in perfusion caused by heme biosynthesis. Positive changes in all types of hippocampal cell counts and morphology are both indicative of improved memory function upon exposure to an EE.

Increased growth in dendritic spines was associated with increased synaptogenesis in CA1 and CA3 hippocampal regions following rearing in an EE (Van Praag et al., 2000). EE rearing is able to exert a wide range of effects on neural physiology by causing epigenetic changes to neuron cell genetic expression (Fischer, Sananbenesi, Wang, Dobbin, & Tsai, 2007). Inhibitors of histone deacylases that induce transcriptional changes within the genome have been shown to increase dendrite sprouting and the number of functional hippocampal synapses (Fischer et al. 2007). Animals reared in an EE show modifications to their chromatin including increased histone acylation (Fischer et al., 2007). These results indicate that enrichment leads to epigenetic changes in expression that improve hippocampal memory acquisition and long-term memory recall.

EE is shown to attenuate disorders and environmental conditions that negatively impact learning and memory. Prenatal stress is known to cause LTP deficits in rat hippocampal synapses, which is reversed by housing in an EE (Yang et al., 2007). Diabetic neuropathy is a disorder that is associated with hippocampal cell proliferation, cell death, and reduced synaptic plasticity (Piazza et al., 2011). In Piazza et al. (2011), an EE increased hippocampal neurogenesis and dendritic branching in diabetic rats, and improved performance on learning tasks compared to the control. Cranial irradiation is a medical treatment that results in impaired memory and cognitive function due to decreased hippocampal neurogenesis (Fan, Liu Z., Weinstein, Fike, & Liu J., 2007). Having an EE can increase neurogenesis in the dentate gyrus of gerbils along with MWM task performance and spatial memory following cranial irradiation (Fan et al., 2007). These findings indicate EE can ameliorate several hippocampal neurodegenerative stressors.

Combined with the positive physiological changes caused by EE, social isolation is known to negatively impact memory formation. Animals in social isolation perform worse on novel object recognition tasks, which is underlined by a loss in synaptic plasticity and function of hippocampal and cortical neurotransmitters (Marsden, King, & Fone, 2011). In Han, Wang, Xue, Shao, & Li (2011) early stage isolation was associated with poorer performance in the MWM, along with decreased expression of BDNF in the nucleus accumbens and dentate gyrus. Rats raised in post-weaning social isolation performed no different from rats given social interactions during development when administered a glutamate receptor agonist during isolation (Jones, Smith, Brown, Auer, &

Fone, 2011). This indicates NMDARs are a potential site that is negatively affected by social isolation.

One physiological mechanism that explains EE's effect on memory is through its role in phosphorylating enzymes critical to LTP (Wang et al., 2007). In Wang et al. (2007), hippocampal ERK1/2 was examined after animals completed a MWM procedure. The study found that the EE group showed higher phosphorylation levels for ERK2, and activation of ERKs is critical to synaptic plasticity (Adams & Sweatt, 2002). Increased ERK activation is correlated with improved spatial memory in the MWM (Wang et al., 2007). This effect of EE has potential to attenuate MAPK signaling disruptions caused by Pb^{2+} exposure, overcoming adverse effects on LTP. In Duffy, Craddock, Abel, & Nguyen (2003), a PKA inhibitor attenuated learning improvements caused by EE; indicating EE affects memory through PKA-dependent signaling pathways. Multiple memory-related kinase pathways are positively influenced by EE

1.8 Environmental enrichment attenuates the adverse effects of Pb^{2+} exposure

When combined with Pb^{2+} exposure, EE can counteract the adverse effects of Pb^{2+} exposure on memory. In Schneider et al. (2001), animals raised in an EE were protected against Pb^{2+} induced memory inhibition, and showed elevated BDNF levels. Electrophysiological studies of hippocampal neurons also show an increase in LTP that correlates with memory task performance for Pb^{2+} exposed animals given an EE compared with Pb^{2+} exposed animals (Cao Huang, & Ruan, 2008). In Zhu et al. (2011), spatial cognitive impairment due to hypoperfusion, which mimics possible effects created by heme synthesis inhibition, was reversed by an EE. NR1 NMDAR subunit mRNA expression was increased in EE animals exposed to Pb^{2+} compared to animals only

exposed to Pb^{2+} (Guilarte, Toscano, McGlothan, & Weaver, 2003). Giving rats an EE during development reverses Pb^{2+}'s negative effects on memory by enhancing hippocampal BDNF expression, NMDAR expression, and LTP.

Enhanced growth factor expression and improvement in neurotransmitter signaling are both central to EEs ability to overcome Pb^{2+}-induced deficits in synaptic plasticity (Toscano & Guilarte, 2005; Van Praag et al., 2000). EE increases expression for neurotransmitter receptors including the serotonin 1A receptor (Van Praag et al., 2000). Physical exercise can increase levels of neurotransmitters by increasing the expression of mRNA for enzymes that synthesize monoamine neurotransmitters such as tyrosine hydroxylase, which is involved in the synthesis of norepinephrine (Toscano & Guilarte, 2005). Physical exercise is also known to increase choline uptake in the hippocampus, leading to enhanced hippocampal cholinergic signaling (Fordyce, 1991). Cholinergic signaling is negatively impacted by Pb^{2+} poisoning (Finkelstein et al., 1998), which can be ameliorated by EE. EE enhances the expression of growth factors that are suggested to affect memory and synaptic plasticity, including BDNF and nerve growth factor (NGF) (Guilarte et al., 2003; Toscano & Guilarte, 2005; Van Praag et al., 2000). In Guilarte et al. (2003), BDNF expression reversed deficits in NR1 subunit expression in the hippocampus, but not NR2B, mGluR, or CaMK gene expression. This change in NR1 expression is important for EE's effect on memory and synaptic plasticity.

It was previously thought that Pb^{2+}-induced cognitive deficits that occurred during the critical period were permanent, however, EE has provided a paradigm for the reversal of the cognitive symptoms of developmental Pb^{2+} exposure. Studies such as Schneider et al. (2001) have proven that cognitive deficits induced by developmental Pb^{2+} exposure

can be overcome by an EE. Many of the physiological effects of Pb^{2+} exposure and EE appear to directly counteract each other. Both ERK and cAMP-dependent protein kinase are inversely affected by developmental Pb^{2+} exposure and EE. Increased levels of BDNF caused by EE exert a neuroprotective effect that counteracts Pb^{2+} exposure's neurodegenerative effect. Finally, EE causes transcriptional changes that enhance some of the physiological mechanisms involved in synaptic plasticity that are disrupted by developmental Pb^{2+} exposure.

1.9 Testing working memory using the radial arm maze

The radial arm maze (RAM) is a behavioral test used to study both working memory and spatial memory (Olton & Samuelson, 1976; Kolata & Kolata, 2007). Animals are introduced into the middle of a maze with multiple arms in a circular arrangement similar to the spokes of a wheel (figure 1). Each arm radiates outward from the center, and half of the arms are baited with a food reward. Animals must use spatial cues on each wall of the room in order to successfully navigate the maze by finding the food rewards. However, rats must also use working memory to keep a record of which arms they have already entered during the task (Kolata & Kolata, 2007). Working memory is a transient form of memory allowing animals to maintain task relevant information about the conditions for task completion (Baddeley, 2010).

The RAM and other maze tasks require animal subjects to build a cognitive map of their environments (Hodges, 1996). Using stable visual cues distributed throughout the environment, or allocentric visuospatial cues, rats develop cognitive maps in order to navigate their environments (Hodges, 1996). The cognitive mapping theory states that animals such as rats use their hippocampus to encode a representation of the environment

using relative external stimuli or landmarks (an allocentric frame of reference), but not when a location is identified by its relative position to the observer (an egocentric frame of reference) (Holdstock, Mayes, Cezayirli, Isaac, Aggleton, & Roberts, 2000). In Olton, Becker, & Handelmann (1980), extramaze visual cues were reversed, which caused an increase in both short-term reference-memory errors and long-term working memory errors. Increased working memory errors indicated rats solved the task using associative learning between baited arms and the reward, which relies on both forming a cognitive map from extramaze and intramaze spatial cues and associating arms with their reward when forming the cognitive map (Hodges, 1996; Olton et al., 1980). In Jarrard (1983), RAM tasks that required visuospatial learning, or place tasks, and tasks requiring associative learning through intramaze cues, or cue tasks, were separated and compared after hippocampal legions. Both reference-memory and working memory errors were increased in the place tasks, whereas only working memory errors increased for the cue tasks (Jarrard, 1983). This finding indicates rats form a visuospatial map for the location of cues they associate with baited arms. Together, these findings indicate that rats build cognitive maps in their hippocampus by using visuospatial cues, which is critical to reducing long-term working memory errors in RAM tasks.

In Kolata & Kolata (2009), mathematical models are used to compare animal working memory in the RAM model to working memory tasks for humans. The working memory model is based on two parameters, number of maze arms and a fixed limit of memory capacity. Both mathematical models for uniform search strategies and non-uniform search strategies that factor in primacy and recency bias were compared by simulation, and did not differ significantly from each other (Kolata & Kolata, 2009). The

result of these models based on experimental data from both rat and human RAM trials indicates a working memory capacity between three and nine objects, or arms that had previously been entered (Kolata & Kolata, 2009). Human subjects in an 8-arm RAM with no other variables almost never re-enter an arm before entering all eight arms first (Glassman, Leniek, & Haegerich, 1998). These subjects also report using spatial cues in order to keep track of the arms they entered. This strategy of completing the task by human subjects appears similar to rodent methods of completing the RAM task using working spatial memory (Olton & Samuelson, 1976). Both human and animal subjects appear to have similar strategies and working memory capacity when completing RAM tasks, providing this animal model with solid face validity for measuring working memory.

Human working memory has traditionally been studied using memory tasks about discrete entities such as numbers or visual cues. The human working memory capacity has been shown in repeated studies to be between four and seven different objects (Cowan, 2001). Because these types of verbal and visual memory tasks are unable to be run with rodents, developing valid animal models of working memory is challenging. In Olton & Samuelson (1976), animals showed a recency effect, meaning their likelihood of making a short-term error increased with the number of different arm choices since the initial instance. Animals chose an average of seven different arms with their first eight choices (Olton & Samuelson, 1976). These results indicate that animals succeed at the RAM task by storing spatial information rather than using odor trails or stereotypic response patterns (Glassman et al., 1998). Working memory in these animals also shows bias for recently acquired task-relevant information just as in human models (Glassman et

al., 1998). Furthermore, a "task-completion pause" was observed in rats that completed the RAM task (Dale, 1986), indicating the use of working memory to complete the maze task rather than a reward-based response pattern algorithm. These studies provide evidence for construct validity in the RAM model, or the validity of inferences made about human working memory from studying animal's RAM task performance.

The RAM can be used to study the mechanisms behind memory formation. Drugs that decrease BDNF levels, such as prenatal opioid exposure, decrease memory performance in the RAM (Schrott, Franklin, & Serrano, 2008). Elevated BDNF mRNA levels are associated with improved memory on RAM tasks (Mizuno et al., 2000). Blocking NMDARs inhibits their involvement in LTP and memory formation, which translates to decreased RAM performance (Levin, Bettegowda, Weaver, & Christopher, 1998). When administered a NMDA antagonist that is known to cause memory deficits, animals showed decreased performance in working and reference memory in the RAM (Levin et al., 1998). Nicotine agonists can overcome these memory deficits at high enough doses (Levin et al., 1998). However, after lesion of the ventral hippocampus, no nicotine-induced improvements in working memory were found (Levin, Christopher, Weaver, Moore, & Brucato, 1999). α7 and α4β2 nicotine receptor subunit antagonists induce similar memory impairment on RAM tasks when administered into the hippocampus (Felix & Levin, 1997; Levin, Bradley, Addy, & Sigurani, 2002). Because hippocampal cholinergic neurons are involved in working memory function (Felix & Levin, 1997), these studies indicate the RAM has predictive validity as physiological changes that are known to interfere with memory performance translate to decreased RAM task performance.

The hippocampus is clearly involved when animals build cognitive maps during RAM tasks; however, it is unclear whether the hippocampus is involved in visual working memory (Baddeley, 2011). In Olson (2006), humans with medial temporal lobe damage, which includes the hippocampus, showed impairment in visual working memory tasks. However, in Baddeley, Jarrold, & Vargha-Khadem (2011), a human subject with significant reduction in bilateral hippocampal cells showed no impaired performance in visuospatial working memory tasks. One explanation for this discrepancy is that participants in Olson, Moore, Stark, & Chatterjee (2006) performed visual working memory tasks that involved allocentric visual processing, as opposed to tasks in Baddeley et al. (2011) that involved egocentric visual processing. Because the RAM is a task that requires a greater amount of allocentric visual processing (Hodges, 1996), working memory tasks in the RAM are likely to involve hippocampal neurons.

Several studies have used the RAM to examine the hippocampus' role in memory. Activation of neurons in the dorsal hippocampus and medial prefrontal cortex were measured after a RAM task, and the dorsal hippocampus was more active after the time-window for short-term memory range was exceeded, and the memory starts becoming consolidated (Lee & Kesner, 2003). Knocking out aryl hydrocarbon receptor (AhR), which are expressed at relatively high levels in the hippocampus and are involved in the development and regulation of the hippocampus, caused decreased performance on RAM tasks by female rats (Powers, Lin , Vanka, Peterson, Juraska, & Schantz, 2005). Decreased RAM performance was correlated with decreased size of female rat hippocampal intra and infrapyramidal mossy fiber fields (Powers et al., 2005). This study shows drugs that decrease hippocampal volume correlate with worse performance

in RAM tasks. In Munoz, Garbe, Lilienthal, & Winneke (1988), dorsal hippocampal lesions showed no affect on RAM acquisition, however, memory recall of the RAM several weeks later was significantly worse in animals with a hippocampal lesion. It is clear that hippocampal activity is required for many aspects of working memory required for RAM tasks.

The effects of EE can be studied using RAM tests. The RAM and MWM were used to study how allowing rats social cages, physical exercise, and interactions with toys improved latency and maze error scores compared to rats with no EE (Leggio et al., 2005). Animals with a developmentally EE displayed improved performance on the RAM, which was blocked by a cholinergic antagonist (Tees, 1999). These findings are consistent with previous findings that hippocampal activation is required for RAM tasks (Stackman & Walsh, 1995), and the hippocampus contains cholinergic neurons important in working memory (Levin et al., 1999; Levin et al., 2002). In Langdon & Corbett (2012), animals were subjected to different forms of EE including habitual physical activity and cognitive activity. These animals showed improved RAM performance, along with elevated levels of hippocampal BDNF (Langdon & Corbett, 2012). EE causes animals to improve memory function on the RAM, which appears to be related to physiological changes in memory related proteins and brain regions.

1.10 Testing hippocampal volume using NeuN

In order to measure cell death in the hippocampus, the neuronal nuclear antigen (NeuN) is used to quantify the number of neurons in each of our treatment groups. NeuN was first described in Mullen, Buck, & Smith (1992), as a soluble nuclear protein that is expressed in mostly all vertebrate neurons, and is not found in other cells besides

neurons. NeuN begins expression right after a neuron initially develops following differentiation from a pluripotent cell, and continues to be expressed in mature adult neurons (Mullen et al., 1992). This makes NeuN antigens a good way to determine the overall number of neurons in different brain regions. Since Pb^{2+} is known to cause neuronal death, particularly in the hippocampus, we can use NeuN to measure this neurodegenerative effect along with the neuroprotective effect of EE.

The NeuN antigen can be used to show neuroprotective or neurodegenerative effects of drugs or other environmental factors. These effects can be measured in specific brain regions such as the hippocampus, and correlated with behavioral data to show involvement of these brain regions in behavioral outputs. Carboxypeptidase E knockout mice show fewer neurons than the wild type in the CA3 region hippocampal subfield (Woronowicz et al., 2008). This result was correlated with a significantly increased latency to complete the MWM task during memory acquisition for knockout mice. In Collombet et al. (2011), the neuroregenerative effect of cytokine treatment was determined using NeuN antigens after soman poison-induced brain lesions. NeuN staining indicated cytokine treatment significantly increased neural regeneration in the hippocampus. However, this did not significantly correlate with improved memory task performance, which indicates the neuroregenerative effects were not enough to overcome deficits caused by the lesions.

Physiological mechanisms that influence memory formation can be studied for their neuroregenerative effects using NeuN. Following stroke-induced neuron death, neurogenesis in the hippocampus was blocked using an NMDAR antagonist, indicating NMDARs are involved in neurogenesis (Arvidsson, Kokaia, & Lindvall, 2001).

Intrahippocampal BDNF injections have also been shown to increase neurogenesis in the hippocampus using the NeuN antigen (Scharfman, Goodman, Macleod, Phani, Antonelli, & Croll, 2005). In Boscia, Esposito, Di Crisci, Franciscis, Annunziato, & Cerchia, (2009), the neuroprotective effects of GDNF, a neurotropic factor increased by EE exposure (Van Praag et al., 2000), was examined in CA1 and CA3 hippocampal sections after excitotoxic cellular injury evoked by NMDA exposure. NeuN immunohistochemistry revealed that GDNF protected to a greater extent in the CA3 subfield of the hippocampus (Boscia et al., 2009). The NeuN antigen is an accurate marker of neuroprotective and neurodegenerative effects in specific brain regions.

NeuN antigens have also been used to show how an EE can have neuroprotective effects against agents that cause cell death such as Pb^{2+}. EE can enhance hippocampal neurogenesis and memory task performance following ischemia-induced cell death (Matsumori et al., 2006). One physiological change that occurs in depression is hippocampal cell loss, which can be ameliorated by an EE (Hattori et al., 2007). In animal models such as chronic stress that cause hippocampal cell death, EE can overcome the loss of hippocampal neurons (Veena, Srikumar, Raju, & Shankaranarayana Rao, 2009). NeuN staining revealed BDNF knockout mice with an EE show no significant increases in neurogenesis compared to BDNF heterozygotes with the same environment, which indicates BDNF is critical to the physiological affects of EE (Rossi et al., 2006). The affect of enriched EE on hippocampal neuroprotection following Pb^{2+} induced cell death will be measured by the NeuN antibody.

1.11 Testing hippocampal activation using c-Fos

In order to measure cellular activation in the hippocampus, c-Fos immunohistochemistry was used. The c-Fos protein is an immediate early inducible transcription factor (Beckmann & Wilce, 1997). Expression of these immediate early transcription factors increases rapidly following administration of neurotransmitter analogues (Beckmann & Wilce, 1997), indicating neurotransmitter activity controls the expression of c-Fos. Electrical activity throughout hippocampal subfields also induces c-Fos expression (Beckmann & Wilce, 1997). These results indicate c-Fos expression correlates with neural stimulation and communication. Because RAM tasks require hippocampal activation, we used c-Fos to examine the differences in hippocampal activation between rats in different treatment groups.

c-Fos immunohistochemistry can be used to assess how chronic Pb^{2+} exposure effects cellular activation. In Lewis & Pitts (2004), rats were chronically exposed to Pb^{2+} during development prior to administration of amphetamine as adults. Following amphetamine administration, c-Fos immunohistochemistry revealed Pb^{2+} exposed animals had significantly less activation in their striatum, a brain region that is activated following amphetamine administration (Lewis & Pitts, 2004). This result indicates developmental Pb^{2+} exposure leads to decreased neural activation in specific brain regions compared to animals not exposed to developmental Pb^{2+}. Similar to how amphetamine administration induces striatal activation, rat's exposed to a RAM task will have increased hippocampal activation. Our present study used c-Fos immunohistochemistry to determine if developmental Pb^{2+} exposure leads to decreased hippocampal activation following the RAM task.

Environmental enrichment could also cause differential patterns of neural activation. Several brain regions are more active when animals are exposed to EEs, including the dentate gyrus and CA3 hippocampal subfields (Ali, Wilson, & Murphy, 2009). Increased activation in these hippocampal regions could potentially strengthen synaptic connections in response to enrichment, which would lead to elevated c-Fos expression during tasks that involve hippocampal activation including the RAM. Hippocampal neurons are also involved in the response to stress, and socially isolated animals have elevated stress levels compared to control animals (Ali et al., 2009). Because enrichment and isolation both induce hippocampal activation for different reasons, we would expect enriched and isolated rats to show differential neural activation throughout hippocampal subfields.

1.12 Objectives of present study

The goal of our study was to examine the effects of developmental Pb^{2+} exposure and EE on working and reference memory acquisition and performance and hippocampal neuron survival. We hypothesized that developmental Pb^{2+} exposure would cause decreases in RAM performance, which is indicative of visuospatial working memory deficits. We further hypothesized that the Pb^{2+} induced deficits in RAM performance would be ameliorated for rats reared in an EE. NeuN was used to examine hippocampal cell density in three hippocampal regions (CA1, CA2, and CA3). We hypothesized Pb^{2+} exposure would decrease the number of mature neurons across all subfields of the hippocampus, and that rearing in an enriched environment would ameliorate Pb^{2+} induced decreases in mature neurons across all hippocampal subfields. During the course of our study, Pb^{2+} exposure caused no behavioral deficits in rats during RAM testing. Because

of this result, we changed the aim of our study to investigate hippocampal activation by using c-Fos immunohistochemistry instead NeuN immunohistochemistry. We hypothesized Pb^{2+} exposed animals would show decreased hippocampal activation. We further hypothesized that rearing in an EE would cause differential patterns of hippocampal activation. Our results confirm that enriched rats perform significantly better during RAM testing, indicating that rearing in an EE improves working visuospatial memory in rats.

Figure 1. Hippocampal Subfields

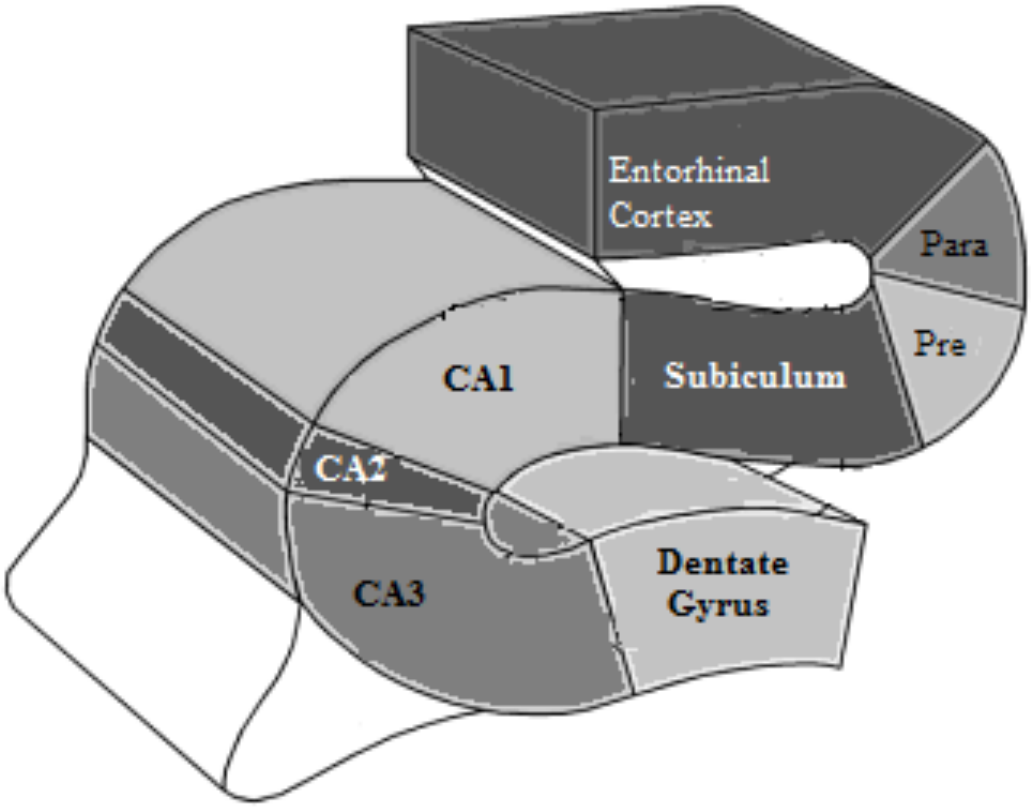

Figure 1. A layout of the different hippocampal subfields (adapted from Andersen et al., 2007). The major hippocampal pathway, or perforant pathway, first receives input at the entorhinal cortex, and is projected to the dentate gyrus. The dentate gyrus then projects axons into the CA3 subfield, which is subsequently sent to the CA2 and CA1 subfields. The information then is sent to various places including the subiculum, presubiculum, and parasubiculum, before being projected to other brain regions including the cortex.

Method

Animals and Environment

Thirty-two male Sprague-Dawley rats (Charles River Labs) were used as subjects in this experiment. Immediately upon arrival on post natal day 21 (PN21), rats were randomly separated into a housing condition with either an impoverished environment or an enriched environment. Impoverished condition rats were housed in single cages and had no access to stimulus objects within their cages. Enriched condition rats were housed in group cages with four rats to each cage and had stimulating toy objects in their cages including balls, wheels, tunnels, and a housing structure to lie underneath. Both groups had ad lib food and water access for the entire time they were housed in the facility, except for the RAM testing days where animals were food deprived. Rats were given ad lib food for one hour after they completed the RAM task. After one hour, food was removed and rats were deprived of food until their next day of testing. All animals were kept in identical cages on housing racks in the same room. The room was temperature controlled and kept on a 24-hour light/dark cycle (12 hours light/12 hours dark) so rats received identical conditions. On the start of testing (PN54), rats weighed an average of 267.34 g.

Within each housing condition, rats were further divided into different Pb^{2+} exposure conditions. The Pb^{2+} exposed group received 1500ppm Pb^{2+} acetate in the food pellets they were given to eat throughout the experiment, while the non-Pb^{2+} control group was given normal rat chow without Pb^{2+} acetate. Pb^{2+} exposed rats were not housed at the same time with non-exposed rats to prevent airborne and other forms of inadvertent Pb^{2+} exposure. The concentration of Pb^{2+} given to rats in this experiment is the highest dose or only dose given in the chow of animal subjects in many other

experiments (Jett & Guilarte, 1995; Munoz et al., 1988; Nihei & Guilarte, 1999). Our study consisted of four groups and two treatment conditions (EE and Pb^{2+} exposure) (Table 1). These conditions were maintained throughout the experiment until the rats were sacrificed on PN78.

Animals were immediately separated into the one of the two housing conditions upon arrival to the facility. Following the first day of acclimation in the lab, rats were given 30 days post weaning exposure to their group's dietary conditions. During this time, cages were cleaned every 5 days. Following the 30 days of exposure, rats began testing on the radial arm maze (RAM) on PN54. Rats were tested for 18 days on the (RAM) before being allowed another week of exposure to their environments. On PN78, the rats were tested once more on the RAM for memory recall. Following this test on PN78, rats were sacrificed 30 minutes following their completion of the RAM task. All parts of this experiment were approved prior to the beginning the research by the Connecticut College Animal Care and Use Committee (IACUC).

Radial Arm Maze Apparatus

The Radial Arm Maze (RAM) procedure is adopted from Olton & Samuelson (1976) as a test of visuospatial working memory and reference memory. Animals were placed in the middle of an apparatus that consists of eight arms with walls and a central octagonal platform (26 cm in diameter). The arms (50 cm long, 10 cm wide and 13 cm high) are made of grey Plexiglas mounted on an opaque platform (figure 2). The apparatus was placed in the center of one side of a room with three surrounding walls. The three walls surrounding the apparatus had three separate symbols, a circle, square, and triangle, made from black tape for the rat to use as spatial cues. The fourth side was

empty space with a constant backdrop of supplies against the far side of the room. Each arm was numbered differently, and kept consistent for the webcam recording device. The webcam was positioned above the maze in the center and connected with the webcam recording program to analyze trial errors and latency.

Radial Arm Maze Procedure

At PN52, rats in each experimental group began acclimation to the RAM apparatus. Food rewards (chocolate flavored rice cereal) were placed in each of the eight arms and rats were allowed to explore the arms for 5 minutes. Non-enriched control animals were exposed individually to the apparatus, while enriched condition animals were exposed to the apparatus in pairs. At PN53, rats were each placed individually in the center of the apparatus and were given 10 minutes to explore and retrieve food rewards located in each of the eight arms. Each rat was able to complete at least four arms before progressing to testing. On PN54 rats in each group began testing individually. Rats were randomly assigned four arms that a food reward was placed in, and this was kept consistent throughout experimentation. Rats were given ten minutes to complete the maze activity, which occurred when the rat was able to find all four of the food rewards. Rats were video recorded, and recorded for latency to complete the task, short-term reference memory errors, and long-term working memory errors. Working memory errors were recorded when a rat reentered a baited arm that the rat had already been too during the trial. Reference memory errors were recorded any time a rat entered an arm that did not contain a food reward. All rats continued to perform the task throughout the 18-day period to eliminate the number of previous trials rats completed as

a variable for the recall test. At PN76, following the 7-day period without testing, rats were tested once more using the same procedure as the 18-days of prior RAM testing.

Tissue Preparation

After completing the recall phase of testing (PN78), rats were sacrificed for tissue harvest between 30-40 minutes after completing their task in the RAM apparatus. Rats were placed in an enclosed glass chamber and asphyxiated using carbon dioxide. This was followed immediately by transcardial perfusion with 400-500 mL of 0.1 M phosphate buffer saline (PBS), followed by 400-500 mL of 4% paraformaldehyde in 0.1 phosphate buffer (PB). Using ronguer forceps, the brain was extracted, and stored in the same 4% paraformaldehyde solution for one day. The following day, brains were removed and transferred to a 30% sucrose/PBS with sodium azide solution until tissue sectioning. Hippocampal sections were taken at approximately 3.14 mm posterior to Bregma, (Paxinos & Watson, 1998) from each animal, and were sliced at 40µm and kept in a PBS solution.

c-Fos Immunohistochemistry

Rabbit anti-Fos polyclonal (c-Fos) primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, 1:8000) was used to identify neurons that contained the c-Fos protein, a member of the activating protein-1 (AP-1) transcription factor family (Monje, Hernandez-Losa, Lyons, Castellone, & Gutkind, 2005). c-Fos is usually expressed when neurons fire an action potential (Dragunow & Faull, 1989), making it a marker of neural activation. An enhanced DAB (3,3'-diaminobenzidine) detection step was used along with a horseradish peroxidase and biotinylated goat anti-mouse secondary antibody

(Jackson Laboratory, Bar Harbor, ME, 1:200) for the reaction step of immunohistochemistry (IHC) staining of sections.

For each animal, three tissue sections (falling between -3.60 and -3.80 from Bregma) were prepared for IHC. The tissue was first washed three times for ten minutes each in a 0.01M PBS. Sections were next transferred to a solution containing the c-Fos primary antibody (1:8000) dilution buffer in a blocking solution to prevent non-specific binding of immunoglobin. This tissue was left with the primary antibody in a shaker overnight. Approximately 24 hours later, the tissue was washed in 0.01M PBS three separate times for ten minutes each. During this wash, the biotinylated goat anti-rabbit polyclonal secondary antibody (Jackson Laboratory, Bar Harbor, ME, 1:200) dilution buffer was prepared in a blocking solution. The tissue was then washed in the secondary antibody for two hours while left on a shaker.

The tissue was again washed three times for ten minutes each in 0.01M PBS. During this wash, Avidin-biotin complexed with horseradish peroxidase (ABC Kit, Vector Laboratories) was prepared in PBS. The tissue was next left on a shaker in the horseradish peroxidase solution for one hour. Before the reaction step, the tissue was washed three times for ten minutes each in PB. During these washes, enhanced DAB solution was prepared and used as the final reagent in the series of enzymatic reactions that creates tissue staining. One DAB tablet (10mg) was placed in 50 mL of 0.01 M PB with 25µL glucose oxidase, 800µL nickel ammonium sulfate solution, 500µL of cobalt chloride solution, and 20mg of ammonium chloride and sonicated for ten minutes. 25 mg of D-glucose in 2mL of ddH_2O was added to the enhanced DAB solution immediately before the tissue was transferred into it. The staining was developed while the tissue was

washed on a shaker in the enhanced DAB solution for fifteen minutes. Finally, the tissue was washed three times for ten minutes each in PBS.

Sections were positioned on glass slides and dried overnight. The following day they were dehydrated through a number of graded ethanol washes (50%, 75%, 90%, 95%, and 100%) and placed in a clearing solution before permanent mounting. The slides were coverslipped using a mounting medium for microscopic evaluation.

c-Fos analysis

One of three c-Fos stained sections of the hippocampal region (between -3.6 and -3.8 mm from Bregma) of each animal was selected and examined using the Olympus BX41 Microscope under 20X magnification. Using IPLab 3.6 software, pictures of CA1, CA2 and CA3 hippocampal subfields were taken. A fixed rectangular area was placed on each of the three brain regions and applied uniformly to the captured pictures, which was consistent for each animal. c-Fos positive neurons were identified by their dark-brown stains. The experimenter remained blind to what condition the animal was in while counting c-Fos positive neurons.

Table 1. Housing Conditions

Animals	Diet	Housing
1	Pb^{2+}	Isolation
2	Pb^{2+}	Isolation
3	Pb^{2+}	Isolation
4	Pb^{2+}	Isolation
5	Pb^{2+}	Isolation
6	Pb^{2+}	Isolation
7	Pb^{2+}	Isolation
8	Pb^{2+}	Isolation
9	Pb^{2+}	Enriched
10	Pb^{2+}	Enriched
11	Pb^{2+}	Enriched
12	Pb^{2+}	Enriched
13	Pb^{2+}	Enriched
14	Pb^{2+}	Enriched
15	Pb^{2+}	Enriched
16	Pb^{2+}	Enriched
17	Control	Isolation
18	Control	Isolation
19	Control	Isolation
20	Control	Isolation
21	Control	Isolation
22	Control	Isolation
23	Control	Isolation
24	Control	Isolation
25	Control	Enriched
26	Control	Enriched
27	Control	Enriched
28	Control	Enriched
29	Control	Enriched
30	Control	Enriched
31	Control	Enriched
32	Control	Enriched

Table 1. Housing and dietary condition for each of the 32 animals. These animals were divided into 4 groups (Isolation/Pb^{2+}, EE/Pb^{2+}, Isolation/Control, EE/Control).

Figure 2. Layout and Dimensions of the Radial Arm Maze

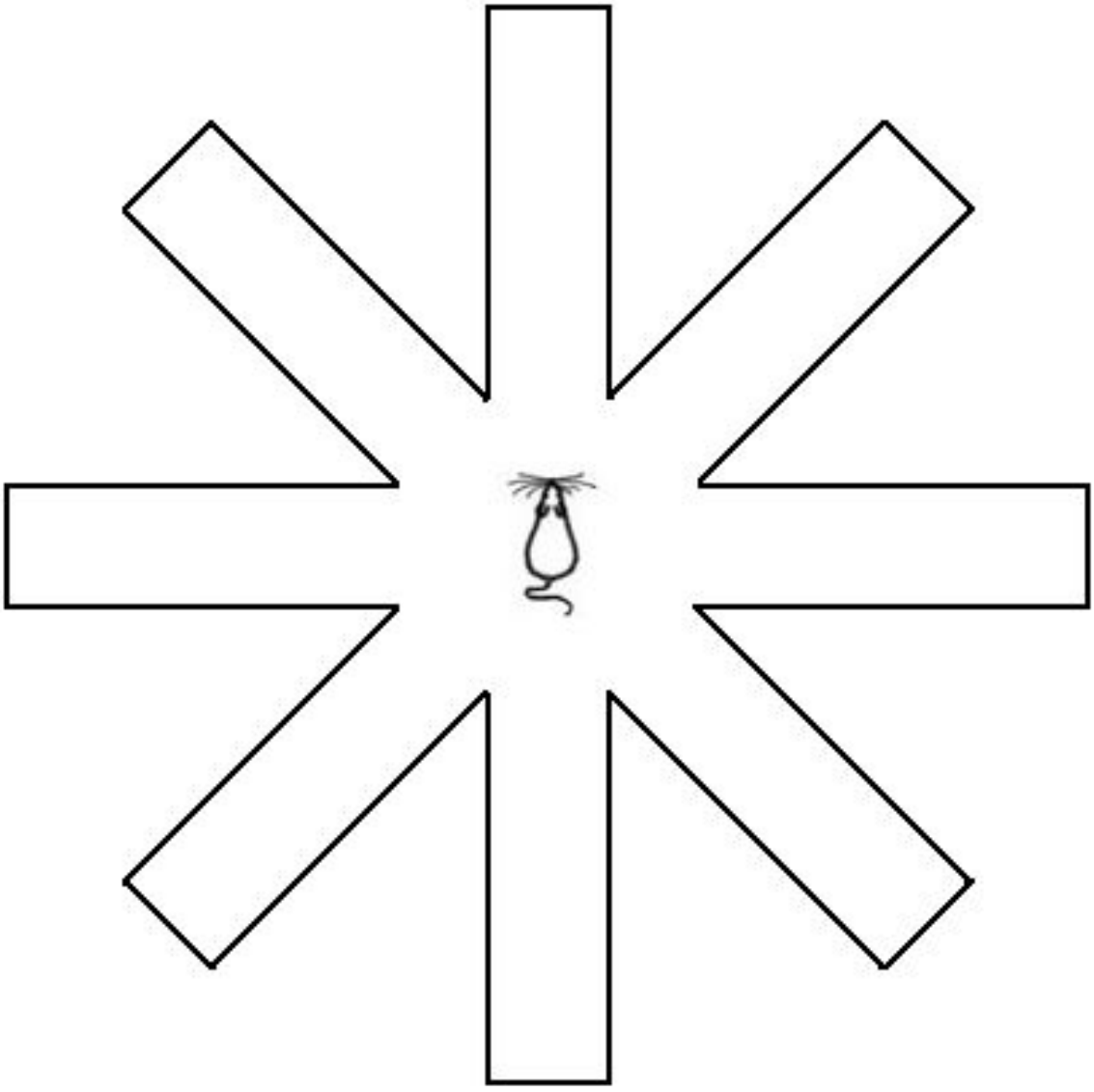

Figure 2. A diagram representing the layout of the Radial Arm Maze. Each wall of the maze was constructed with grey plastic and the base was also grey. The arms (50 cm long, 10 cm wide and 13 cm high) are made of grey Plexiglas mounted on an opaque platform. The maze was located in a room surrounded by three walls, each with a separate symbol (circle, square, and triangle) made out of black duct tape. Rats were placed in the maze in the middle as shown in the figure. The rat's movement was tracked using a video recorder placed directly above the maze.

Results

During radial arm maze testing, animals reared in an EE consistently performed better during their testing than animals reared in isolation. A significant main effect, $F(3,31)=7.242$, $p<0.001$ was seen for the overall latency to complete the RAM task across each treatment group over the entire testing period. Tukey's post hoc tests revealed control diet animals reared in isolation showed significantly more latency to complete the RAM task over the 18 days of testing compared with isolated animals, $p<.05$ (Figure 3). Animals fed Pb^{2+} laced chow during testing and given an EE also completed the RAM task with significantly less latency compared to Pb^{2+} exposed animals that were isolated, $p<.05$ (Figure 4). For short-term working memory errors, a significant main effect was seen between the four treatment groups, $F(3,31)=6.226$, $p<0.05$. Tukey's post hoc tests revealed animals fed a control diet throughout testing and raised in an isolated environment committed significantly more errors than animals fed a control diet and raised in an enriched environment, $p<.05$ (Figure 5). A significant main effect was also seen in the long-term reference memory errors, $F(3,31)=3.228$, $p<0.05$. Tukey's post hoc tests indicated the control diet and socially isolated group committed significantly more errors when completing the radial arm maze task than the control diet and enriched environment group, $p<.05$ (Figure 6).

Animals reared in an EE performed significantly better during memory retention testing than animals reared in isolation. Significant main effects across the four treatment groups was also seen when comparing RAM task completion latency during memory recall testing, $F(3,31)=4.142$, $p<0.05$. Tukey's post hoc tests showed that animals fed a control diet and reared in isolation completed the RAM task with significantly higher

latency during memory retention testing compared with animals reared in an enriched environment, $p<.05$ (Figure 7). Significant overall differences were also seen when testing reference memory errors during memory recall testing, $F(3,31)=4.030$, $p<0.05$. Tukey's post hoc tests revealed animals fed a control diet and reared in an enriched environment had significantly more reference memory errors compared with socially isolated animals during memory recall, $p<.05$ (Figure 8). No significant differences were seen in working memory errors between socially isolated and enriched animals (Figure 9). No significant effects were seen between control diet and Pb^{2+} exposed animals during any memory retention radial arm maze tasks.

No significant main effect was seen for either treatment group when comparing radial arm maze testing on the last day of testing (day 18) and memory retention testing (day 25) for both working and reference memory errors or latency. Comparison of the first 5 days of testing is indicative of the animal's initial learning curve. A significant main effect is seen for latency to complete the RAM task during the first five testing days, $F(1,28)=14.011$, $p<.001$. Tukey's post hoc tests revealed that animals fed a Pb^{2+} diet and reared in an EE were significantly more latent completing the RAM task compared to animals fed a control diet and reared in social isolation. A paired t test between the beginning of the radial arm maze testing (day 2) and the end of testing (day 18) revealed that animals significantly improved their latency to complete the RAM task during the initial testing period $t(31)=6.3759$, $p<.001$, indicating that across groups, animals improved their ability to complete the RAM task. Overall our behavioral results indicated Pb^{2+} exposure had no effect on RAM task performance. Due to our results, we did not expect NeuN to reveal significant findings in the hippocampus. Instead, we

proceeded by investigating if there were different levels of hippocampal activation by using c-Fos immunohistochemistry.

Figure 3. Latency of radial arm maze task acquisition: control diet animals

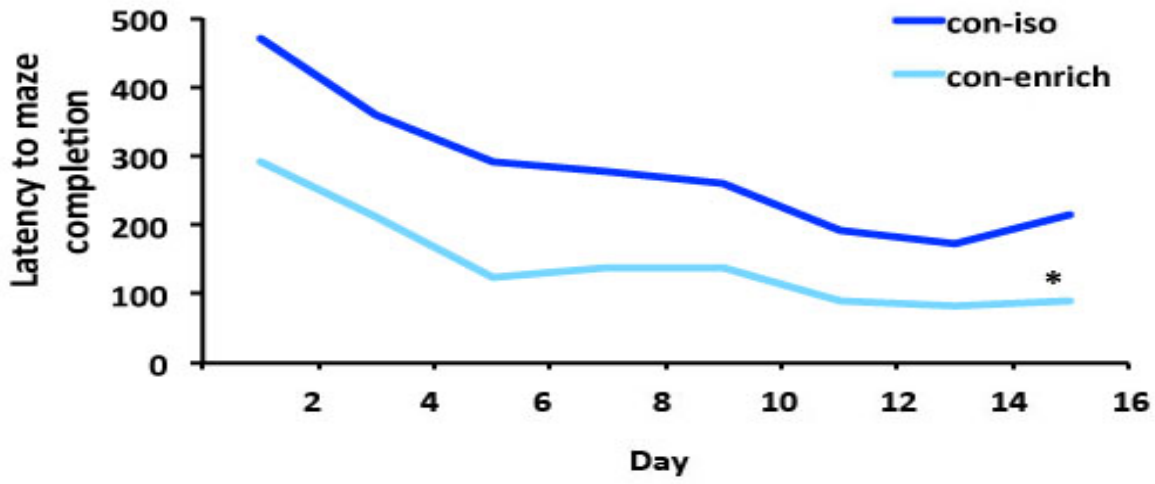

Figure 3. Learning acquisition of discovering all for baited arms as measured by the latency (seconds) to complete the radial arm maze task. Rats that were fed a control diet are compared for differences in the group reared in isolation and the group reared with an enriched environment. The overall latency between the groups is summed for every two-day interval starting on day 2 and ending on day 18. The area under the curve was calculated for each animal and the group average compared for statistical significance. A one-way ANOVA revealed a significant main effect across conditions $F(3,31)=7.242$, $p<0.001$. A Tukey's HSD test revealed enriched animals in the control diet condition had significantly lower completion latency $p<0.05$.

Figure 4. Latency of radial arm maze task acquisition: Pb^{2+} exposed animals

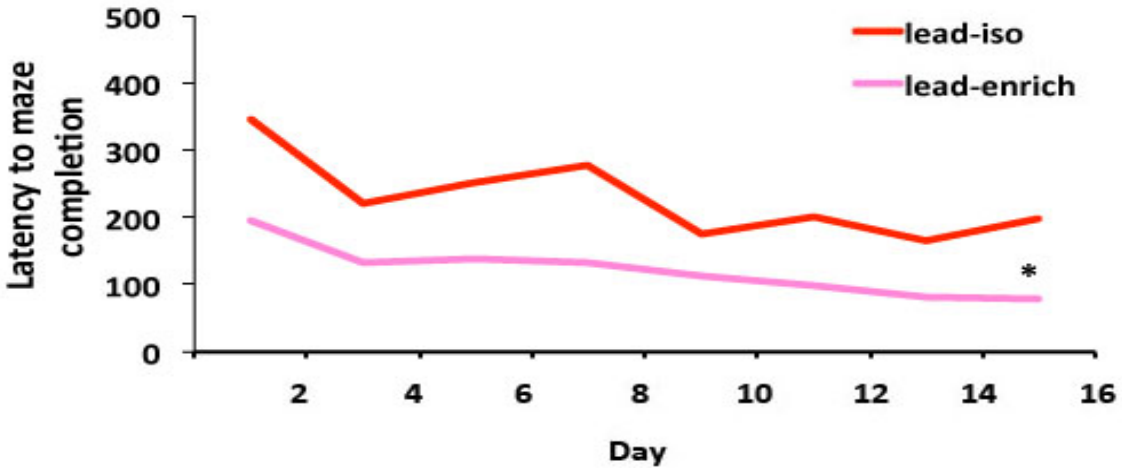

Figure 4. Pb^{2+} exposed animals were also compared after significant latency (seconds) differences were found across dietary Pb^{2+} and housing condition variables, $F(3,31)=7.242$, $p<0.001$. Animals reared in isolation and fed dietary Pb^{2+} showed significantly more latency when completing the radial arm maze task compared with Pb^{2+} exposed animals reared in an enriched environment over the entire 18-day testing period ($p<.05$).

Figure 5. Working memory errors during radial arm maze testing

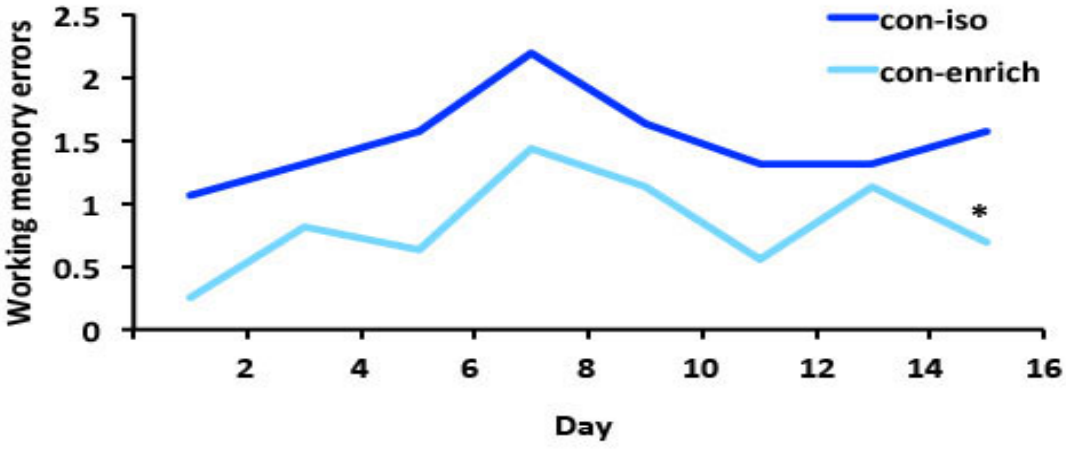

Figure 5. The number of working memory errors was compared between each animal between day 2 and day 18 of testing. The total number of errors between each 2-day testing interval was graphed, and the average area under each graph was compared for each group. A one-way ANOVA found a significant main effect across conditions, $F(3,31)=6.226$, $p<0.05$. A Tukey's HSD test revealed that animals fed a control diet and reared in isolation made significantly more working memory errors during RAM testing compared to animals reared in an enriched environment $p<.05$.

Figure 6. Reference memory errors during radial arm maze testing

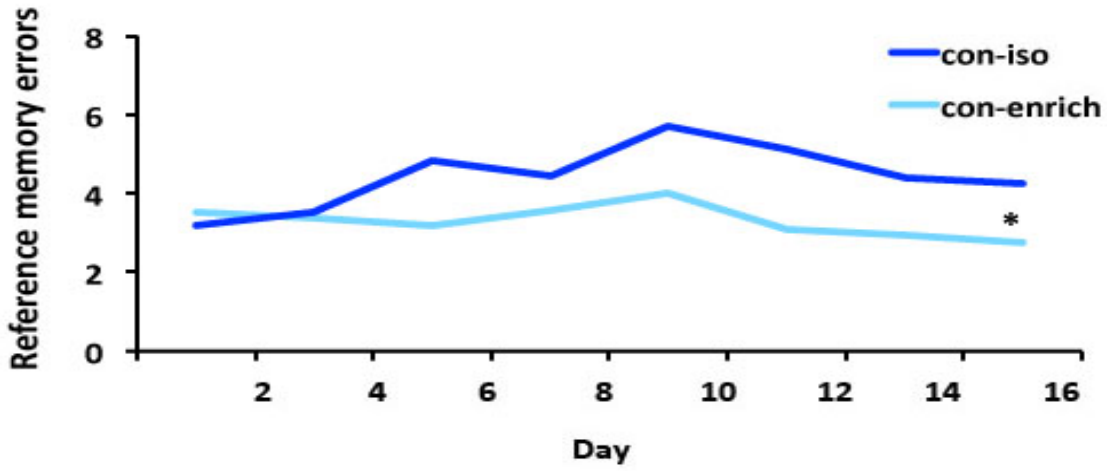

Figure 6. The number of reference memory errors was compared between each animal between day 2 and day 18 of testing. The total number of errors between each 2-day testing interval was graphed, and the average area under each graph was compared for each group. A one-way ANOVA found a significant main effect across conditions, $F(3,31)=3.228$, $p<0.05$. A Tukey's HSD test revealed that animals fed a control diet and reared in isolation made significantly more reference memory errors during RAM testing compared to animals reared in an enriched environment $p<.05$.

Figure 7. Latency to complete memory retention task

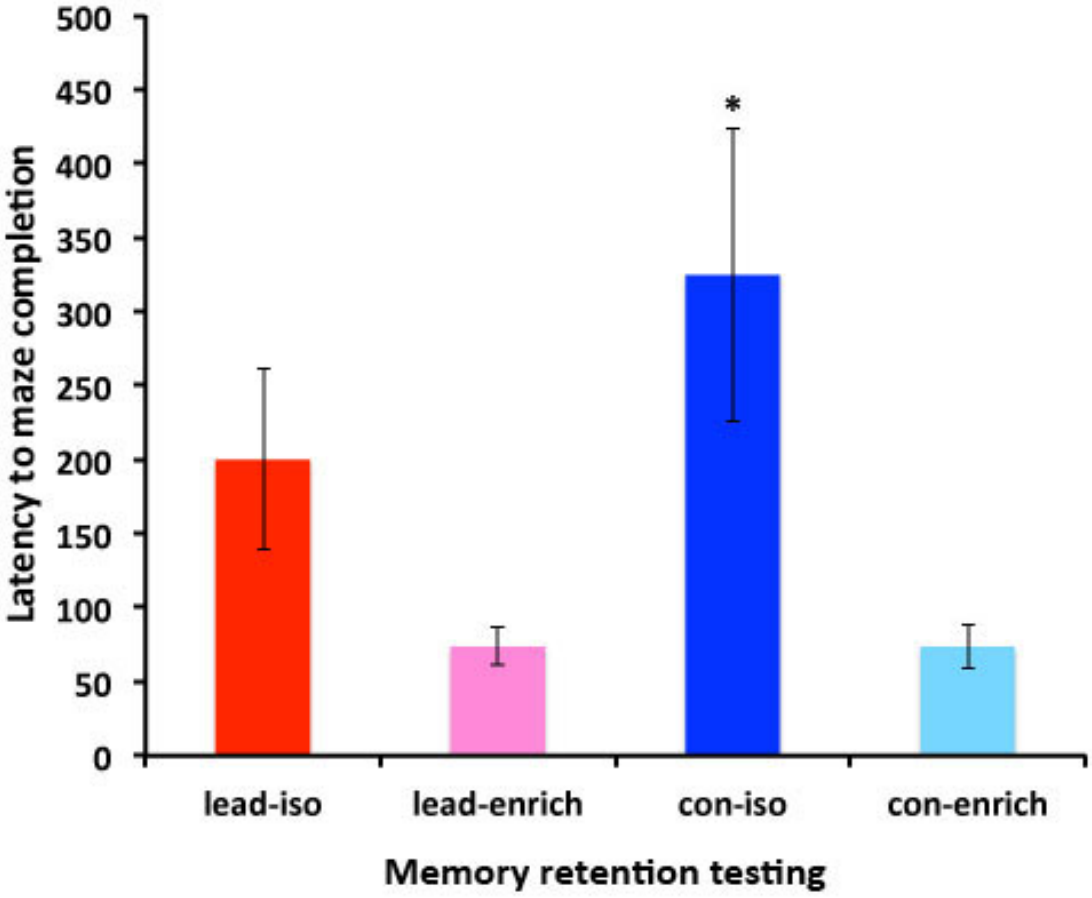

Figure 7. Memory recall of the radial arm maze task on testing day 25 was measured by comparing the rats overall latency of animals completing radial arm maze task on day 25. A one-way ANOVA revealed a significant main effect across conditions, $F(3,31)=4.142$, $p<0.05$. A Tukey's HSD test indicated that control animals reared in isolation showed significantly more latency to complete the maze compared to animals reared in an EE, $p<.05$.

Figure 8. Reference memory errors during memory retention testing

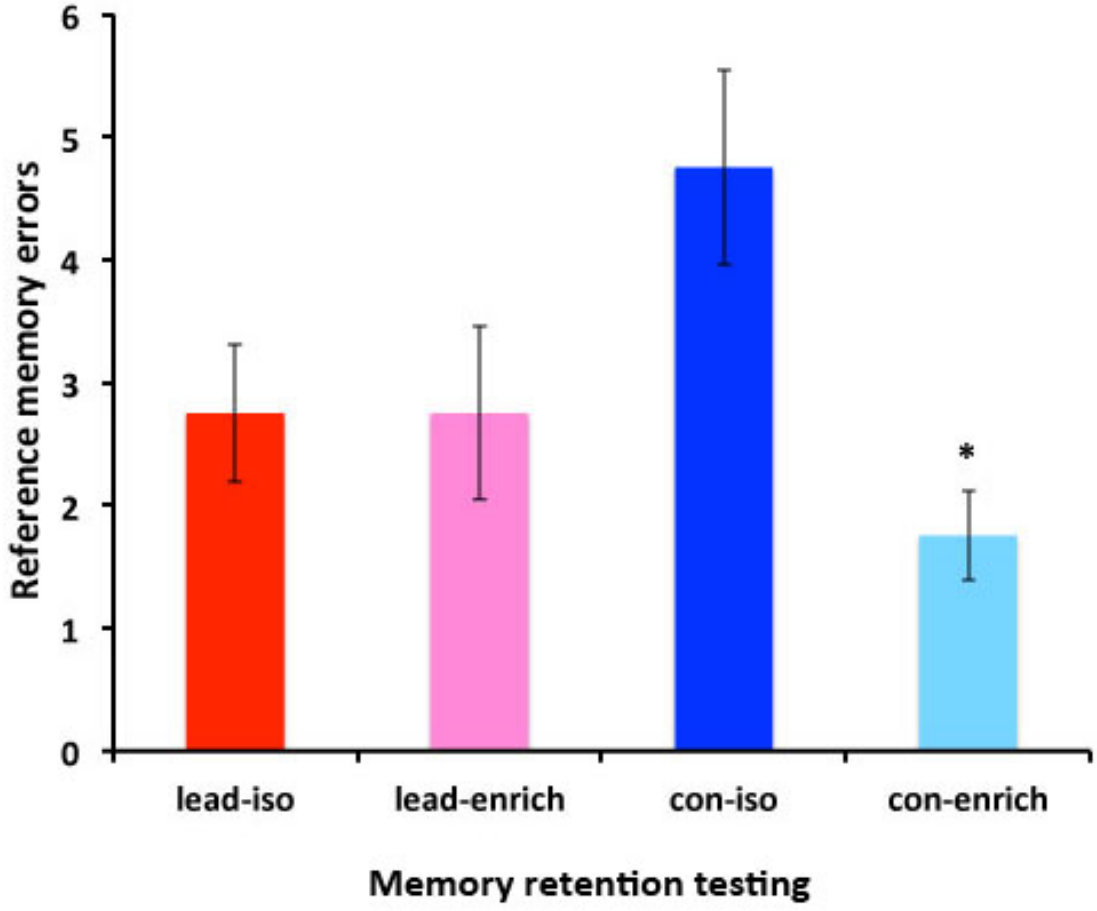

Figure 8. Reference memory errors were compared on testing day 25 to examine if rats were able to equally recall the visuospatial and other long-term memories about the radial arm maze task. A one-way ANOVA revealed a significant main effect across conditions, $F(3,31)=4.030$, $p<0.05$. A Tukey's HSD test indicated that animals fed a control diet and reared in an enriched environment had significantly fewer reference memory errors compared to animals fed a control diet and reared in an isolated environment, $p<.05$.

Figure 9. Working memory errors during memory retention testing

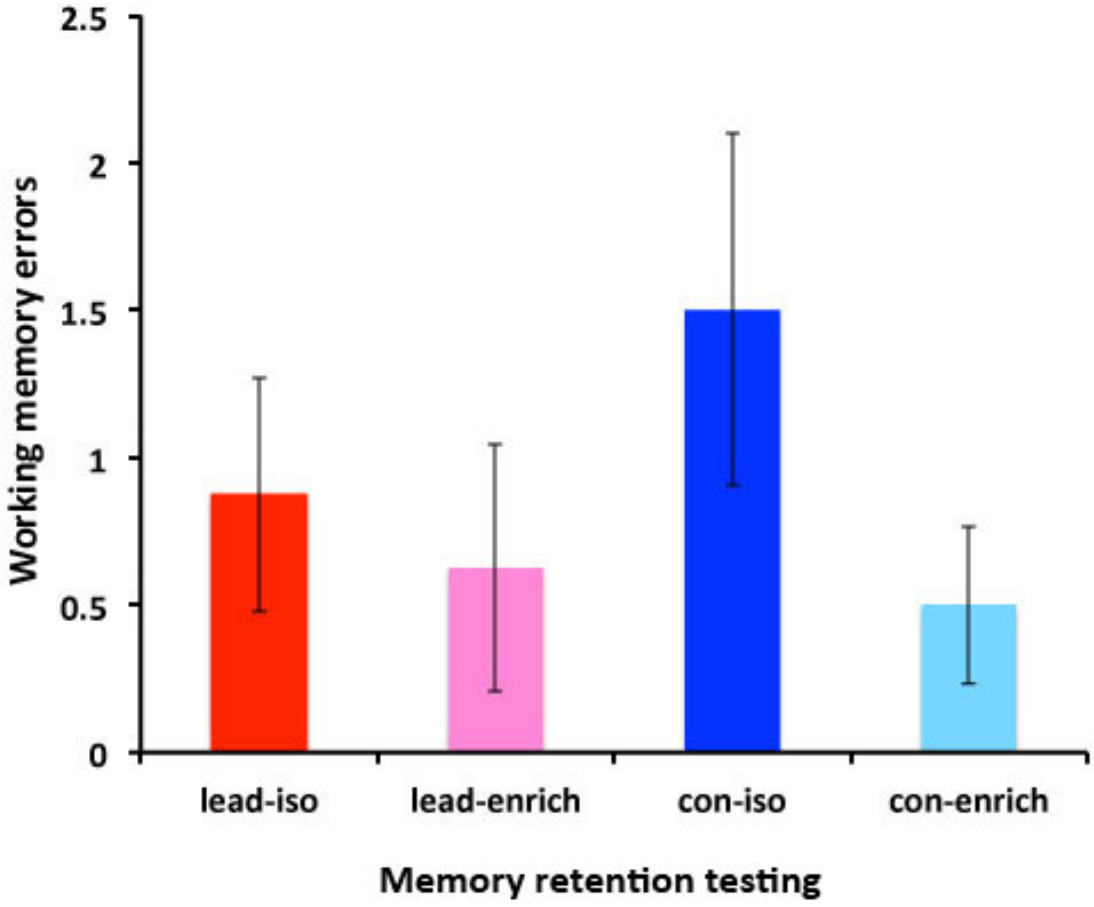

Figure 9. Working memory errors were compared on testing day 25 to examine if rats retained any aspect of their differences in working memory performance during the 18-day testing period. A one-way ANOVA did not reveal a significant main effect $F(3,31)=0.876$, $p>0.05$.

Discussion

Results of this study provide evidence in support of EE enhancing working and reference memory acquisition. Findings also indicate that EE enhances memory recall of the RAM task after a week without performing the task. Significant effects were not seen between Pb^{2+} exposed animals and control diet animals. There were no significant effects during memory retention testing for the treatment variable of Pb^{2+} exposure either. Pb^{2+} exposed animals did not have significantly more working or reference memory errors during the 18 days of testing compared with animals on a control diet. These results indicate Pb^{2+} exposure did not significantly affect visuospatial working or reference memory acquisition or retrieval. In Jarrard (1983), rats exposed to different aspects of the RAM task showed performance consistent with the use of an allosteric hippocampal map. Since hippocampal volume is positively correlated with RAM task performance (Powers et al., 2005), and Pb^{2+} exposed rats in our study did not show RAM task performance deficits, we expected to find that Pb^{2+} exposure in our study did not decrease the number of hippocampal cells compared with control diet animals.

Our data indicates animals reared in isolation performed significantly worse compared with animals reared in an EE, regardless of whether or not they were exposed to Pb^{2+}. The effect of enrichment on memory acquisition in our study is consistent with previous reports that EE enhances memory acquisition (Leggio et al., 2005; Llorens-Martín et al., 2007, Van Praag et al., 2000). Elevated expression of BDNF has been shown to improve RAM performance (Mizuno et al., 2000), and increases in BDNF expression are seen in rats reared with an EE (Schneider et al., 2001; Guilarte et al., 2003). Social isolation can also decrease hippocampal BDNF levels (Han et al., 2011),

which is associated with decreased performance on the RAM. Our results also indicate that enriched rats performed better at recalling long-term reference memories in RAM tasks after a week of no testing compared with isolated rats. This result is consistent with Fischer et al. (2007) where rats exposed to an EE were able to re-establish long-term memories following brain atrophy induced hippocampal cell loss. Working memory performance was not affected by enrichment, which is consistent with our definition of working memory as a transient form of task-relevant information that is not committed into long-term memory. Our results provide further evidence that EE enhances memory acquisition and retrieval.

Our conclusion that Pb^{2+} exposure does not inhibit hippocampal-dependent memory acquisition is different from other studies where Pb^{2+} exposure significantly decreases hippocampal spatial memory (Kuhlmann et al., 1997). Working memory is different from other forms of hippocampal memory because working memory also involves other brain regions such as the medial prefrontal cortex (Lee & Kesner, 2003). In Jett et al. (1997), a working memory paradigm was developed and tested on the Morris water maze. Pb^{2+} exposed animals showed no differences in escape latency compared with control animals in the working memory paradigm. Like our present findings, this study indicates developmental Pb^{2+} exposure does not have a robust effect on working memory.

Memory recall was not affected by Pb^{2+} exposure, which also appears to conflict with previous results. In Munoz (1988), memory recall was tested in RAM tasks weeks later after animals were given dorsal hippocampal legions. Hippocampal legions decrease the number of neurons in the hippocampus, which is analogous to our

hypothesis for Pb^{2+} exposure. Pb^{2+} exposed animals did not show impaired memory retrieval during any RAM task. Performance between the final trial during the 18 days of RAM testing and the memory recall trial were not significantly different in Pb^{2+} exposed rats. Our results indicate Pb^{2+} did not decrease hippocampal cells that are involved in memory recall.

One explanation for our Pb^{2+} exposure results is that our Pb^{2+} exposure period was too late into the rat pup's neural development. In humans, the brain undergoes elevated periods of experienced-based synaptic plasticity and neural circuit formation between 18-36 months after birth (Goldstein, 1990). Evidence suggests that rat nervous systems undergo a corresponding critical period of synaptogenesis much earlier in life during weaning from their mother's care (Toscano & Guilarte, 2005). Peak hippocampal LTP activity in the rat nervous system occurs at approximately day 14-15 after the rat was born (Harris & Teyler, 1984). This time period is also when NMDAR expression is at highest levels in the hippocampus (Toscano & Guilarte, 2005). Because our present study design began Pb^{2+} exposure at PN21 of the rat's lives, our subject's brains could have already undergone periods of increased synaptogenesis and neural circuit formation. If our rat subjects went through critical periods of neural development prior to their period of Pb^{2+} exposure, memory performance would not be disrupted by Pb^{2+} exposure.

In Kuhlmann et al. (1997), different groups of rats were exposed to Pb^{2+} at varying points in their lifetime. Some animals were exposed to Pb^{2+} in utero through their mother's diet, which continued through weaning, as the pups were breast-feeding. Other groups received Pb^{2+} after they were finished weaning at PN 21. Rat's exposed to Pb^{2+} in utero showed a significantly slower escape latency from the Morris water maze as

adults compared with the control group. On the other hand, rats given Pb^{2+} after PN21 did not show any significant spatial memory impairment in the Morris water maze task compared with control animals. This finding indicates that rats respond differently to Pb^{2+} exposure depending on the time period in which they are exposed to environmental Pb^{2+}.

Because of our conclusion that Pb^{2+} did not affect hippocampal volume based on behavioral data, we concluded NeuN IHC analysis of mature hippocampal neurons was unnecessary considering there was not a significant effect for Pb^{2+} exposure. Since our behavioral data indicated that EE does affect RAM performance, we decided to pursue cFos staining in the hippocampus instead of NeuN staining. The effects Pb^{2+} exposure had on the death of hippocampal neurons were not great enough to cause memory impairments. Instead, we became interested in how EE elicits neurological changes that leads to enhanced memory performance. c-Fos staining measures cell activation (Dragunow & Faull, 1989), and we were interested to see if any significant changes in hippocampal activity occurred between the enriched and socially isolated animals.

When c-Fos immunohistochemistry staining was performed on hippocampal sections from our animals, no specific staining was seen for the c-Fos antigen. There are several reasons why our c-Fos staining could have failed to specifically target the c-Fos protein. Endogenous peroxidase activity can react with the enhanced diaminobenzidine (DAB) solution to create staining that is not specific to the c-Fos protein. Antibody binding is another reaction step that can disrupt specific staining for the c-Fos protein. Enzyme-substrate reactivity can be altered, resulting in non-specific staining. There are a multitude of potential reasons why our c-Fos staining was disrupted.

After the hippocampal tissue was sliced by cryostat and stored in phosphate buffer, the freezer to keep the tissue stored at low temperatures was disrupted. It is unlikely that any c-Fos proteins became denatured during this time because tissue was not subjected to temperatures above the physiological range. However, keeping tissue in cold temperatures prevents proteins and other biological molecules from undergoing conformational changes that alter the reactivity of those molecules. If biomolecules in hippocampal slices were to change their conformations, antibodies used for our c-Fos staining could possibly react with a number of these biomolecules besides the c-Fos protein. Sliced tissue not kept at cold temperatures are also subject to reactions between biomolecules within the hippocampal slices. Reactions directly with c-Fos protein would inhibit the primary antibody from binding to c-Fos. Reactions between other proteins could create molecules that react as a substrate during any of the enzymatic steps in the IHC procedure, causing staining that is not specific to the c-Fos protein.

If c-Fos immunohistochemistry were to show specific staining in our tissue, we would expect animals treated with an EE to show higher levels of cellular activation after completing the RAM task. EE improves the number of functional hippocampal neurons by increasing hippocampal neurogenesis (Piazza et al., 2011), which we would expect to result in higher levels of hippocampal activation in animals treated with an EE compared to socially isolated animals. Neurotransmitter signaling improves after animals are reared in an EE (Toscano & Guilarte, 2005; Van Praag et al., 2000). Enhanced neurotransmitter signaling would cause enriched animals to have higher levels of cellular activation. Hippocampal cholinergic signaling impacts working memory (Felix & Levin, 1997), which can be enhanced by aspects of environmental enrichment such as physical exercise

(Fordyce, 1991). Septohippocampal cholinergic pathway activation is critical to visuospatial working memory in RAM tasks (Stackman & Walsh, 1995). Because animals given an EE performed significantly better in tasks that require visuospatial working memory, we would expect more c-Fos labeled cells in hippocampal cells that receive septohippocampal axon projections from the medial septum in enriched rats compared with isolated rats. Because enriched rats outperformed isolated rats in all aspects of RAM testing, we would expect them to show higher levels of hippocampal cellular activation.

A BDNF val66met genetic polymorphism affects the trafficking and secretion of BDNF, which is associated with decreased functional activity of hippocampal neurons during working memory tasks (Dennis, Cabeza, Need, Waters-Metenier, Goldstein, & LaBar, 2011). Based on this result we would expect increases in BDNF as a result of EE rearing to enhance hippocampal functional activity during the working memory RAM task. Increased NMDAR subunit mRNA expression is a result of EE (Guilarte et al., 2003), which would enhance glutamatergic signaling. Glutamate signaling results in depolarization of the post-synaptic membrane, increasing the likelihood a neuron will be activated and fire an action potential. The ability of EE to enhance BDNF and NMDAR expression are major reasons why our enriched animals would have probably shown higher levels of hippocampal cellular activation.

Our present study was limited in a number of ways. Because all of the rats were either reared in an EE or socially isolated, there was no control group for comparison to either of our housing conditions. In Lukkes, Mokin, Scholl, & Forster (2008), animals were reared in isolation beginning on PN21. These isolated animals were tested in

conditioned fear paradigms as adults and displayed elevated levels of anxious behavior compared to control animals (Lukkes et al., 2008). During the first 2 hours after the isolation restraint was given, isolated animals had elevated levels of corticosterone, a natural response to environmental stressors, compared to control animals (Lukkes et al., 2008). These isolated animals also had decreased corticosterone levels 2 hours after the isolation restraint was given (Lukkes et al., 2008). This finding is consistent with previous studies that show chronic early life stress causes the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for releasing corticosterone in response to environmental stressors, to adapt and modulate the release rate of corticosterone (Miachon, Rochet, Mathian, Barbagli, & Claustrat, 1993). Because rats reared in isolation exhibit more anxious behavior as adults, we cannot use isolated animals as a control to compare with enriched animals. Therefore, we are limited in our conclusions regarding enrichment, and further research is required to determine exactly how enrichment and isolation individually effect working visuospatial memory. Our present study was also limited in drawing conclusions about our hypotheses on the ameliorating effects of enrichment on developmental Pb^{2+} exposure. Because no significant results were seen for developmental Pb^{2+} exposure, we could not assess our hypothesis that rearing in an EE would ameliorate Pb^{2+} induced working visuospatial memory deficits.

In future studies concerning developmental Pb^{2+} exposure and working memory, rat pups exposed to Pb^{2+} should be exposed in utero and throughout weaning. Studies should aim to have animals chronically exposed to Pb^{2+} by postnatal days 14-15 because peak hippocampal LTP occurs during the period (Harris & Teyler, 1984), which indicates neural circuits are forming. More research is required on the differential impacts of Pb^{2+}

exposure on working memory and spatial memory acquisition. Because our study did not expose rats to Pb^{2+} during the critical period, our results are not valid to draw conclusions about how developmental Pb^{2+} affects working memory. Future studies should determine if other neurons outside the hippocampus such as the medial prefrontal cortex (Lee & Kesner, 2003) are active during working memory in rats with an impaired hippocampus following developmental Pb^{2+} exposure.

Future studies should address the different components of enrichment including physical exercise, social interactions, and inanimate object stimuli, to determine their individual impacts on memory performance. Enrichment causes epigenetic changes (Fischer et al., 2007), however it is not known what impact each component of enrichment has on memory performance. Knowledge about the individual components of enrichment has implications in child development. Each component of EE represents environmental aspects of human development, and knowledge about how these different environmental components influence the expression of memory-related genes in the hippocampus would affect how children are developed.

Intervention programs with different approaches of adding enrichment to the development of children should be applied to improve cognitive function of developing children. These programs should be applied to children subject to Pb^{2+} exposure or other environmental settings that are known to impact cognitive function. Social networking programs, exercise programs, and programs that expose children to different objects in the environment for them to play with are three human correlates to what rats experience in an EE setting, and these settings should be explored in humans to improve cognitive functioning. Continued research into how developmental Pb^{2+} exposure specifically

affects different types of memory is required. Research into how these effects of Pb^{2+} exposure can be ameliorated by rearing in an EE has implications to human development.

References

Adams, J. P., & Sweatt, J. D. (2002). Molecular psychology: Roles for the ERK MAP kinase cascade in memory. *Annu. Rev. Pharmacol. Toxicol*. *42*, 135–163.

Alfano, D. P., LeBoutillier, J. C., & Petit, T. L. (1982). Hippocampal mossy fiber pathway development in normal and postnatally lead-exposed rats. *Experimental Neurology, 75*(2), 308-319.

Ali, A. E. A., Wilson, Y. M., & Murphy, M. (2009). A single exposure to an enriched environment stimulates the activation of discrete neuronal populations in the brain of the fos-tau-lacZ mouse. *Neurobiology of Learning and Memory, 92*(3), 381-390.

Alonso, M., Vianna, M. R. M., Depino, A. M., Mello E Souza, T., Pereira, P., Szapiro, G., Medina, J. H. (2002). BDNF-triggered events in the rat hippocampus are required for both short- and long-term memory formation. *Hippocampus, 12*(4), 551-560.

Andersen, P., Morris, R., Amaral, D., Bliss, T., O'Keefe, J. (2007). The Hippocampus Book. *Oxford University Press*. New York, NY.

Arvidsson, A., Kokaia, Z., & Lindvall, O. (2001). N-methyl-D-aspartate receptor-mediated increase of neurogenesis in adult rat dentate gyrus following stroke. *European Journal of Neuroscience, 14*(1), 10-18.

Baddeley, A., Jarrold, C., & Vargha-Khadem, F. (2011). Working memory and the hippocampus. *Journal of Cognitive Neuroscience, 23*(12), 3855-3861.

Baddeley, A. (2010). Working memory. *Current Biology, 20*(4), R136-R140.

Bahls, F. H., Lartius, R., Trudeau, L., Doyle, R. T., Fang, Y., Witcher, D., Haydon, P. G. (1998). Contact-dependent regulation of N-type calcium channel subunits during synaptogenesis. *Journal of Neurobiology, 35*(2), 198-208.

Basarsky, T. A., Parpura, V., & Haydon, P. G. (1994). Hippocampal synaptogenesis in cell culture: Developmental time course of synapse formation, calcium influx, and synaptic protein distribution. *Journal of Neuroscience, 14*(11 I), 6402-6411.

Basha, M. R., Wei, W., Bakheet, S. A., Benitez, N., Siddiqi, H. K., Ge, Y., Zawia, N. H. (2005). The fetal basis of amyloidogenesis: Exposure to lead and latent overexpression of amyloid precursor protein and β-amyloid in the aging brain. *Journal of Neuroscience, 25*(4), 823-829.

Basha, M. R., Murali, M., Siddiqi, H. K., Ghosal, K., Siddiqi, O. K., Lashuel, H. A., Zawia, N. H. (2005). Lead (pb) exposure and its effect on APP proteolysis and Aβ aggregation. *FASEB Journal, 19*(14), 2083-2084.

Battaglia, F. P., Benchenane, K., Sirota, A., Pennartz, C. M. A., & Wiener, S. I. (2011). The hippocampus: Hub of brain network communication for memory. *Trends in Cognitive Sciences, 15*(7), 310-318.

Beauquis, J., Pavía, P., Pomilio, C., Vinuesa, A., Podlutskaya, N., Galvan, V., & Saravia, F. (2013). Environmental enrichment prevents astroglial pathological changes in the

hippocampus of APP transgenic mice, model of alzheimer's disease. *Experimental Neurology, 239*(1), 28-37.

Beckmann, A. M., & Wilce, P. A. (1997). Egr transcription factors in the nervous system. *Neurochemistry International, 31*(4), 477-510.

Behl, M., Zhang, Y., Shi, Y., Cheng, J., Du, Y., & Zheng, W. (2010). Lead-induced accumulation of β-amyloid in the choroid plexus: Role of low density lipoprotein receptor protein-1 and protein kinase C. *Neurotoxicology, 31*(5), 524-532.

Boscia, F., Esposito, C. L., Di Crisci, A., de Franciscis, V., Annunziato, L., & Cerchia, L. (2009). GDNF selectively induces microglial activation and neuronal survival in CA1/CA3 hippocampal regions exposed to NMDA insult through Ret/ERK signalling. *Plos One, 4*(8)

Cao, X., Huang, S., & Ruan, D. (2008). Enriched environment restores impaired hippocampal long-term potentiation and water maze performance induced by developmental lead exposure in rats. *Developmental Psychobiology, 50*(3), 307-313.

Chang, B., Jang, B., Son, T. G., Cho, I., Quan, F., Choe, N., Lee, J. (2012). Ascorbic acid ameliorates oxidative damage induced by maternal low-level lead exposure in the hippocampus of rat pups during gestation and lactation. *Food and Chemical Toxicology, 50*(2), 104-108.

Chao, S. L., Moss, J. M., & Harry, G. J. (2007). Lead-induced alterations of apoptosis and neurotrophic factor mRNA in the developing rat cortex, hippocampus, and cerebellum. *Journal of Biochemical and Molecular Toxicology, 21*(5), 265-272.

Chrivia, J. C., Kwok, R. P. S., Lamb, N., Hagiwara, M., Montminy, M. R., & Goodman, R. H. (1993). Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature, 365*(6449), 855-859.

Collombet, J., Béracochéa, D., Liscia, P., Piérard, C., Lallement, G., & Filliat, P. (2011). Long-term effects of cytokine treatment on cognitive behavioral recovery and neuronal regeneration in soman-poisoned mice. *Behavioural Brain Research, 221*(1), 261-270.

Cookman, G. R., & Regan, C. M. (1991). Studies of the antiproliferative action of inorganic lead in relation to the phases of the rat glial cell cycle. *Toxicology in Vitro, 5*(2), 127-132.

Counter, S. A., Buchanan, L. H., & Ortega, F. (2008). Zinc protoporphyrin levels, blood lead levels and neurocognitive deficits in andean children with chronic lead exposure. *Clinical Biochemistry, 41*(1-2), 41-47.

Cowan, N. (2001). The magical number 4 in short-term memory: A reconsideration of mental storage capacity. *Behavioral and Brain Sciences, 24*(1), 87-114.

Dale, R. (1986). Spatial and temporal response patterns on the 8-arm radial maze. *Physiology & Behavior, 36*(4), 787-790. doi:10.1016/0031-9384(86)90370-7

Deisseroth, K., Bito, H., & Tsien, R. W. (1996). Signaling from synapse to nucleus: Postsynaptic CREB phosphorylation during multiple forms of hippocampal synaptic plasticity. *Neuron, 16*(1), 89-101.

Dennis, N. A., Cabeza, R., Need, A. C., Waters-Metenier, S., Goldstein, D. B., & Labar, K. S. (2011). Brain-derived neurotrophic factor val66met polymorphism and hippocampal activation during episodic encoding and retrieval tasks. *Hippocampus*. *21*(9). 980-989.

Dent, E. W., Merriam, E. B., & Hu, X. (2011). The dynamic cytoskeleton: Backbone of dendritic spine plasticity. *Current Opinion in Neurobiology, 21*(1), 175-181.

Dragunow, M., & Faull, R. (1989). The use of c-fos as a metabolic marker in neuronal pathway tracing. *Journal of Neuroscience Methods. 29.* 261-265

Dribben, W. H., Creeley, C. E., & Farber, N. (2011). Low-level lead exposure triggers neuronal apoptosis in the developing mouse brain. *Neurotoxicology and Teratology, 33*(4), 473-480.

Duffy, S. N., Craddock, K. J., Abel, T., & Nguyen, P. V. (2001). Environmental enrichment modifies the PKA-dependence of hippocampal LTP and improves hippocampus-dependent memory. *Learning and Memory, 8*(1), 26-34.

Eichenbaum, H. (1999). The hippocampus and mechanisms of declarative memory. *Behavioural Brain Research, 103*(2), 123-133.

Fan, Y., Liu, Z., Weinstein, P. R., Fike, J. R., & Liu, J. (2007). Environmental enrichment enhances neurogenesis and improves functional outcome after cranial irradiation. *European Journal of Neuroscience, 25*(1), 38-46.

Farr, S. A., Flood, J. F., & Morley, J. E. (2000). The effect of cholinergic, GABAergic, serotonergic, and glutamatergic receptor modulation on posttrial memory processing in the hippocampus. *Neurobiology of Learning and Memory, 73*(2), 150-167.

Felix, R., & Levin, E. D. (1997). Nicotinic antagonist administration into the ventral hippocampus and spatial working memory in rats. *Neuroscience, 81*(4), 1009-1017.

Finkelstein, Y., Markowitz, M. E., & Rosen, J. F. (1998). Low-level lead-induced neurotoxicity in children: An update on central nervous system effects. *Brain Research Reviews, 27*(2), 168-176.

Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M., & Tsai, L. (2007). *Recovery of learning and memory is associated with chromatin remodelling*

Fordyce, D. E., & Farrar, R. P. (1991). Physical activity effects on hippocampal and parietal cortical cholinergic function and spatial learning in F344 rats. *Behavioural Brain Research, 43*(2), 115-123.

Fujita, H., Nishitani, C., & Ogawa, K. (2002). Lead, chemical porphyria, and heme as a biological mediator. *Tohoku Journal of Experimental Medicine, 196*(2), 53-64.

Giap, B. T., Jong, C. N., Ricker, J. H., Cullen, N. K., & Zafonte, R. D. (2000). The hippocampus: Anatomy, pathophysiology, and regenerative capacity. *Journal of Head Trauma Rehabilitation, 15*(3), 875-894.

Glassman, R. B., Leniek, K. M., & Haegerich, T. M. (1998). Human working memory capacity is 7 ± 2 in a radial maze with distracting interruption: Possible implication for neural mechanisms of declarative and implicit long-term memory. *Brain Research Bulletin, 47*(3), 249-256.

Goldstein, G. W. (1990). Lead poisoning and brain cell function. *Environmental Health Perspectives, 89*, 91-94.

Gu, H., Wei, X., Monnot, A. D., Fontanilla, C. V., Behl, M., Farlow, M. R., Du, Y. (2011). Lead exposure increases levels of β-amyloid in the brain and CSF and inhibits LRP1 expression in APP transgenic mice. *Neuroscience Letters, 490*(1), 16-20.

Guilarte, T. R., & McGlothan, J. L. (1998). Hippocampal NMDA receptor MRNA undergoes subunit specific changes during developmental lead exposure. *Brain Research, 790*(1-2), 98-107.

Guilarte, T. R., Toscano, C. D., McGlothan, J. L., & Weaver, S. A. (2003). Environmental enrichment reverses cognitive and molecular deficits induced by developmental lead exposure. *Annals of Neurology, 53*(1), 50-56.

Han, X., Wang, W., Xue, X., Shao, F., & Li, N. (2011). Brief social isolation in early adolescence affects reversal learning and forebrain BDNF expression in adult rats. *Brain Research Bulletin, 86*(3-4), 173-178.

Harris, K. M., & Teyler, T. J. (1984). Developmental onset of long-term potentiation in area CA1 of the rat hippocampus. *Journal of Physiology, VOL. 346*, 27-48.

Hattori, S., Hashimoto, R., Miyakawa, T., Yamanaka, H., Maeno, H., Wada, K., & Kunugi, H. (2007). Enriched environments influence depression-related behavior in adult mice and the survival of newborn cells in their hippocampi. *Behavioural Brain Research, 180*(1), 69-76.

Heidinger, V., Manzerra, P., Wang, X. Q., Strasser, U., Yu, S., Choi, D. W., & Behrens, M. M. (2002). Metabotropic glutamate receptor 1-induced upregulation of NMDA receptor current: Mediation through the Pyk2/Src-family kinase pathway in cortical neurons. *Journal of Neuroscience, 22*(13), 5452-5461.

Hensch, T. K. (2004). Critical period regulation. *Annu. Rev. Neurosci. 27,* 549–79.

Ho, N., Liauw, J. A., Blaeser, F., Wei, F., Hanissian, S., Muglia, L. M., Chatila, T. A. (2000). Impaired synaptic plasticity and cAMP response element-binding protein activation in Ca2+/calmodulin-dependent protein kinase type IV/Gr-deficient mice. *Journal of Neuroscience, 20*(17), 6459-6472.

Hodges, H. (1996). Maze procedures: The radial-arm and water maze compared. *Cognitive Brain Research, 3*(3-4), 167-181.

Holdstock, J. S., Mayes, A. R., Cezayirli, E., Isaac, C. L., Aggleton, J. P., & Roberts, N. (2000). A comparison of egocentric and allocentric spatial memory in a patient with selective hippocampal damage. *Neuropsychologia, 38*(4), 410-425.

Hu, F., Sun, W. W., Zhao, X. T., Cui, Z. J., & Yang, W. X. (2008). TRPV1 mediates cell death in rat synovial fibroblasts through calcium entry-dependent ROS production and mitochondrial depolarization. *Biochemical and Biophysical Research Communications, 369*(4), 989-993.

Huang, H., Bihaqi, S. W., Cui, L., & Zawia, N. H. (2011). In vitro pb exposure disturbs the balance between Aβ production and elimination: The role of AβPP and neprilysin. *Neurotoxicology, 32*(3), 300-306.

Izquierdo, I., Barros, D. M., E Souza, T. M., De Souza, M. M., & Izquierdo, L. A. (1998). Mechanisms for memory types differ [8]. *Nature, 393*(6686), 635-636.

Izquierdo, I., & Medina, J. H. (1997). Memory formation: The sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiology of Learning and Memory, 68*(3), 285-316.

Jarrard, L. E. (1983). Selective hippocampal lesions and behavior: Effects of kainic acid lesions on performance of place and cue tasks. *Behavioral Neuroscience. 97*(6). 873-889.

Jett, D. A., & Guilarte, T. R. (1995). Developmental lead exposure alters N-methyl-D-aspartate and muscarinic cholinergic receptors in the rat hippocampus: An autoradiographic study. *Neurotoxicology, 16*(1), 7-18.

Jett, D. A., Kuhlmann, A. C., Farmer, S. J., & Guilarte, T. R. (1997). Age-dependent effects of developmental lead exposure on performance in the morris water maze. *Pharmacology Biochemistry and Behavior, 57*(1-2), 271-279.

Jiang, Y., Long, L., Zhu, X., Zheng, H., Fu, X., Ou, S., Zheng, W. (2008). Evidence for altered hippocampal volume and brain metabolites in workers occupationally exposed to lead: A study by magnetic resonance imaging and 1H magnetic resonance spectroscopy. *Toxicology Letters, 181*(2), 118-125.

Jones, C. B., Auer, D. F., Fone, K. (2011). The mGluR2/3 agonist LY379268 reverses post-weaning social isolation-induced recognition memory deficits in the rat. *Psychopharmacology, 214*(1), 269-283. doi:10.1007/s00213-010-1931-7

Juberg, D. R., Kleiman, C. F., & Kwon, S. C. (1997). Position paper of the american council on science and health: Lead and human health. *Ecotoxicology and Environmental Safety, 38*(3), 162-180.

Klann, E., Chen, S., & Sweatt, J. D. (1993). Mechanism of protein kinase C activation during the induction and maintenance of long-term potentiation probed using a selective peptide substrate. *Proceedings of the National Academy of Sciences of the United States of America, 90*(18), 8337-8341.

Kolata, W., & Kolata, S. (2009). A model of working memory capacity in the radial-arm maze task. *Journal of Mathematical Psychology, 53*(4), 242-252.

Koller, K., Brown, T., Spurgeon, A., & Levy, L. (2004). Recent developments in low-level lead exposure and intellectual impairment in children. *Environmental Health Perspectives, 112*(9), 987-994.

Krapivinsky, G., Krapivinsky, L., Manasian, Y., Ivanov, A., Tyzio, R., Pellegrino, C., Medina, I. (2003). The NMDA receptor is coupled to the ERK pathway by a direct interaction between NR2B and RasGRF1. *Neuron, 40*(4), 775-784.

Kudo, K., Wati, H., Qiao, C., Arita, J., & Kanba, S. (2005). Age-related disturbance of memory and CREB phosphorylation in CA1 area of hippocampus of rats. *Brain Research, 1054*(1), 30-37.

Kuhlmann, A. C., McGlothan, J. L., & Guilarte, T. R. (1997). Developmental lead exposure causes spatial learning deficits in adult rats. *Neuroscience Letters, 233*(2-3), 101-104.

Kumaran, D., & Maguire, E. A. (2005). The human hippocampus: Cognitive maps or relational memory? *Journal of Neuroscience, 25*(31), 7254-7259.

Laird, F. M., Cai, H., Savonenko, A. V., Farah, M. H., He, K., Melnikova, T., Wong, P. C. (2005). BACE1, a major determinant of selective vulnerability of the brain to amyloid-β amyloidogenesis, is essential for cognitive, emotional, and synaptic functions. *Journal of Neuroscience, 25*(50), 11693-11709.

Langdon, K. D., & Corbett, D. (2012). Improved working memory following novel combinations of physical and cognitive activity. *Neurorehabilitation and Neural Repair, 26*(5), 523-532.

Lau, G. C., Saha, S., Faris, R., & Russek, S. J. (2004). Up-regulation of NMDAR1 subunit gene expression in cortical neurons via a PKA-dependent pathway. *Journal of Neurochemistry, 88*(3), 564-575.

Lee, I., & Kesner, R. P. (2003). Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory. *Journal of Neuroscience, 23*(4), 1517-1523.

Leggio, M. G., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., & Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behavioural Brain Research, 163*(1), 78-90.

Levin, E. D., Bettegowda, C., Weaver, T., & Christopher, N. C. (1998). Nicotine-dizocilpine interactions and working and reference memory performance of rats in the radial-arm maze. *Pharmacology Biochemistry and Behavior, 61*(3), 335-340.

Levin, E. D., Bradley, A., Addy, N., & Sigurani, N. (2002). Hippocampal α7 and α4β2 nicotinic receptors and working memory. *Neuroscience, 109*(4), 757-765.

Levin, E. D., Christopher, N. C., Weaver, T., Moore, J., & Brucato, F. (1999). Ventral hippocampal ibotenic acid lesions block chronic nicotine- induced spatial working memory improvement in rats. *Cognitive Brain Research, 7*(3), 405-410.

Levine, E. S., & Kolb, J. E. (2000). Brain-derived neurotrophic factor increases activity of NR2B-containing N-methyl-D-aspartate receptors in excised patches from hippocampal neurons. *Journal of Neuroscience Research, 62*(3), 357-362.

Lewis, M. W., & Pitts, D. K. (2004). Inorganic lead exposure in the rat activates striatal cFOS expression at lower blood levels and inhibits amphetamine-induced cFOS expression at higher blood levels. *Journal of Pharmacology and Experimental Therapeutics, 310*(2), 815-820.

Li, N., Yu, Z. L., Wang, L., Zheng, Y. T., Jia, J. X., Wang, Q., . . . Li, W. J. (2009). Early-life lead exposure affects the activity of TNF-a and expression of SNARE complex in hippocampus of mouse pups. *Biological Trace Element Research, 132*(1-3), 227-238.

Lidsky, T. I., & Schneider, J. S. (2006). Adverse effects of childhood lead poisoning: The clinical neuropsychological perspective. *Environmental Research, 100*(2), 284-293.

Llorens-Martín, M. V., Rueda, N., Martínez-Cué, C., Torres-Alemán, I., Flórez, J., & Trejo, J. L. (2007). Both increases in immature dentate neuron number and decreases of immobility time in the forced swim test occurred in parallel after environmental enrichment of mice. *Neuroscience, 147*(3), 631-638.

Long, G. J., Rosen, J. F., & Schanne, F. A. X. (1994). Lead activation of protein kinase C from rat brain: Determination of free calcium, lead, and zinc by 19F NMR. *Journal of Biological Chemistry, 269*(2), 834-837.

Lukkes, J. L., Mokin, M. V., Scholl, J. L., & Forster, G. L. (2009). Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. *Hormones and Behavior, 55*(1), 248-256.

Luo, G., Niu, R., Sun, Z., Zhang, J., Wang, J., Wang, C., & Wang, J. (2011). Reduction of CaMKII expression in the hippocampus of rats from ingestion of fluoride and/or lead. *Fluoride, 44*(2), 63-69.

Marsden, C. A., King, M. V., & Fone, K. C. F. (2011). Influence of social isolation in the rat on serotonergic function and memory - relevance to models of schizophrenia and the role of 5-HT 6 receptors. *Neuropharmacology, 61*(3), 400-407.

Matsumori, Y., Hong, S. M., Fan, Y., Kayama, T., Hsu, C. Y., Weinstein, P. R., & Liu, J. (2006). Enriched environment and spatial learning enhance hippocampal neurogenesis and salvages ischemic penumbra after focal cerebral ischemia. *Neurobiology of Disease, 22*(1), 187-198.

Mattson, M. P. (1992). Calcium as sculptor and destroyer of neural circuitry. *Experimental Gerontology, 27*(1), 29-49.

Miachon, S., Rochet, T., Mathian, B., Barbagli, B., Claustrat, B., 1993. Long-term isolation of Wistar rats alters brain monoamine turnover, blood corticosterone, and ACTH. *Brain Res. Bull*. *32*. 611–614.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., & Nabeshima, T. (2000). Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *Journal of Neuroscience, 20*(18), 7116-7121.

Monje, P., Hernández-Losa, J., Lyons, R. J., Castellone, M. D., & Gutkind, J. S. (2005). Regulation of the transcriptional activity of c-fos by ERK: A novel role for the prolyl isomerase Pin1. *The Journal of Biological Chemistry. 280*(42), 35081-35084.

Moser, M. & Moser, E. (1998). Distributed encoding and retrieval of spatial memory in the hippocampus. *Journal of Neuroscience, 18*(18), 7535-7542.

Mullen, R. J., Buck, C. R., & Smith, A. M. (1992). NeuN, a neuronal specific nuclear protein in vertebrates. *Development, 116*(1), 201-211.

Munoz, C., Garbe, K., Lilienthal, H., & Winneke, G. (1988). Significance of hippocampal dysfunction in low level lead exposure of rats. *Neurotoxicology and Teratology, 10*(3), 245-253.

Nakajo, Y., Miyamoto, S., Nakano, Y., Xue, J., Hori, T., & Yanamoto, H. (2008). Genetic increase in brain-derived neurotrophic factor levels enhances learning and memory. *Brain Research, 1241*, 103-109.

Nathanson, J. A., & Bloom, F. E. (1975). Lead induced inhibition of brain adenyl cyclase. *Nature, 255*(5507), 419-420.

Nihei, M. K., & Guilarte, T. R. (1999). NNMDAR-2A subunit protein expression is reduced in the hippocampus of rats exposed to Pb2+ during development. *Molecular Brain Research, 66*(1-2), 42-49.

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews Neuroscience, 7*(9), 697-709.

Olson, I. R., Moore, K. S., Stark, M., & Chatterjee, A. (2006). Visual working memory is impaired when the medial temporal lobe is damaged. *Journal of Cognitive Neuroscience, 18*(7), 1087-1097.

Olton, D. S., Becker, J. T., & Handelmann, G. E. (1980). Hippocampal function: Working memory or cognitive mapping? *Physiological Psychology, 8*(2), 239-246.

Olton, D. S., & Samuelson, R. J. (1976). Remembrance of places passed: Spatial memory in rats. *Journal of Experimental Psychology: Animal Behavior Processes, 2*(2), 97-116.

Patil, A. J., Bhagwat, V. R., Patil, J. A., Dongre, N. N., Ambekar, J. G., Jailkhani, R., & Das, K. K. (2006). Effect of lead (pb) exposure on the activity of superoxide dismutase and catalase in battery manufacturing workers (BMW) of western maharashtra (india) with reference to heme biosynthesis. *International Journal of Environmental Research and Public Health, 3*(4), 329-337.

Piazza, F. V., Pinto, G. V., Trott, G., Marcuzzo, S., Gomez, R., & Fernandes, M. D. C. (2011). Enriched environment prevents memory deficits in type 1 diabetic rats. *Behavioural Brain Research, 217*(1), 16-20.

Pourmotabbed, A., Motamedi, F., Fathollahi, Y., Mansouri, F. A., & Semnanian, S. (1998). Involvement of NMDA receptors and voltage-dependent calcium channels on augmentation of long-term potentiation in hippocampal CA1 area of morphine dependent rats. *Brain Research, 804*(1), 125-134.

Powers, B. E., Lin, T., Vanka, A., Peterson, R. E., Juraska, J. M., & Schantz, S. L. (2005). Tetrachlorodibenzo-p-dioxin exposure alters radial arm maze performance and hippocampal morphology in female AhR +/- mice. *Genes, Brain and Behavior, 4*(1), 51-59.

Prybylowski, K., Fu, Z., Losi, G., Hawkins, L. M., Luo, J., Chang, K., . . . Vicini, S. (2002). Relationship between availability of NMDA receptor subunits and their expression at the synapse. *Journal of Neuroscience, 22*(20), 8902-8910.

Rabito, F. A., Shorter, C., & White, L. E. (2003). Lead levels among children who live in public housing. *Epidemiology, 14*(3), 263-268.

Ramesh, G. T., Manna, S. K., Aggarwal, B. B., & Jadhav, A. L. (2001). Lead exposure activates nuclear factor kappa B, activator protein-1, c-jun N-terminal kinase and caspases in the rat brain. *Toxicology Letters, 123*(2-3), 195-207.

Richter-Schmidinger, T., Alexopoulos, P., Horn, M., Maus, S., Reichel, M., Rhein, C., Kornhuber, J. (2011). Influence of brain-derived neurotrophic-factor and apolipoprotein E genetic variants on hippocampal volume and memory performance in healthy young adults. *Journal of Neural Transmission, 118*(2), 249-257.

Riedel, G., Wetzel, W., & Reymann, K. G. (1996). Comparing the role of metabotropic glutamate receptors in long-term potentiation and in learning and memory. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 20*(5), 761-789.

Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., . . . Caleo, M. (2006). Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *European Journal of Neuroscience, 24*(7), 1850-1856.

Ruff, H. A., Markowitz, M. E., Bijur, P. E., & Rosen, J. F. (1996). Relationships among blood lead levels, iron deficiency, and cognitive development in two-year-old children. *Environmental Health Perspectives, 104*(2), 180-185.

Russell, C. L., Semerdjieva, S., Empson, R. M., Austen, B. M., Beesley, P. W., & Alifragis, P. (2012). Amyloid-β acts as a regulator of neurotransmitter release disrupting the interaction between synaptophysin and VAMP2. *Plos One, 7*(8)

Sato, K., Akaishi, T., Matsuki, N., Ohno, Y., & Nakazawa, K. (2007). β-Estradiol induces synaptogenesis in the hippocampus by enhancing brain-derived neurotrophic factor release from dentate gyrus granule cells. *Brain Research, 1150*(1), 108-120.

Scharfman, H., Goodman, J., Macleod, A., Phani, S., Antonelli, C., & Croll, S. (2005). Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Experimental Neurology, 192*(2), 348-356.

Schneider, J. S., Anderson, D. W., Sonnenahalli, H., & Vadigepalli, R. (2011). Sex-based differences in gene expression in hippocampus following postnatal lead exposure. *Toxicology and Applied Pharmacology, 256*(2), 179-190.

Schneider, J. S., Lee, M. H., Anderson, D. W., Zuck, L., & Lidsky, T. I. (2001). Enriched environment during development is protective against lead-induced neurotoxicity. *Brain Research, 896*(1-2), 48-55.

Schrijver, N. C. A., Bahr, N. I., Weiss, I. C., & Würbel, H. (2002). Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. *Pharmacology Biochemistry and Behavior, 73*(1), 209-224.

Schrott, L. M., Franklin, L. M., & Serrano, P. A. (2008). Prenatal opiate exposure impairs radial arm maze performance and reduces levels of BDNF precursor following training. *Brain Research, 1198*, 132-140.

Stackman, R. W., & Walsh, T. J. (1995). Distinct profile of working memory errors following acute or chronic disruption of the cholinergic septohippocampal pathway. *Neurobiology of Learning and Memory, 64*(3), 226-236.

Stéphan, A., Laroche, S., & Davis, S. (2001). Generation of aggregated β-amyloid in the rat hippocampus impairs synaptic transmission and plasticity and causes memory deficits. *Journal of Neuroscience, 21*(15), 5703-5714.

Struyska, L., Bubko, I., Walski, M., & Rafalowska, U. (2001). Astroglial reaction during the early phase of acute lead toxicity in the adult rat brain. *Toxicology, 165*(2-3), 121-131.

Tees, R. C. (1999). The influences of rearing environment and neonatal choline dietary supplementation on spatial learning and memory in adult rats. *Behavioural Brain Research, 105*(2), 173-188.

Thomas, G. M., & Huganir, R. L. (2004). MAPK cascade signalling and synaptic plasticity. *Nature Reviews Neuroscience, 5*(3), 173-183.

Toscano, C. D., & Guilarte, T. R. (2005). Lead neurotoxicity: From exposure to molecular effects. *Brain Research Reviews, 49*(3), 529-554.

Valtorta, F., Pozzi, D., Benfenati, F., & Fornasiero, E. F. (2011). The synapsins: Multitask modulators of neuronal development. *Seminars in Cell and Developmental Biology, 22*(4), 378-386.

Van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nature Reviews Neuroscience, 1*(3), 191-198.

Veena, J., Srikumar, B. N., Raju, T. R., & Shankaranarayana Rao, B. S. (2009). Exposure to enriched environment restores the survival and differentiation of new born cells in

the hippocampus and ameliorates depressive symptoms in chronically stressed rats. *Neuroscience Letters, 455*(3), 178-182.

Wang, C, Lai, M., Lui, C., Yang, S., Tiao, M., Hsieh, C., Huang, L. (2007). An enriched environment improves cognitive performance after early-life status epilepticus accompanied by an increase in phosphorylation of extracellular signal-regulated kinase 2. *Epilepsy and Behavior, 11*(3), 303-309.

Watson, J. B., Sutcliffe, J. G., & Fisher, R. S. (1992). Localization of the protein kinase C phosphorylation/calmodulin-binding substrate RC3 in dendritic spines of neostriatal neurons. *Proceedings of the National Academy of Sciences of the United States of America, 89*(18), 8581-8585.

Weisskopf, M. G., Hu, H., Mulkern, R. V., White, R., Aro, A., Oliveira, S., & Wright, R. O. (2004). Cognitive deficits and magnetic resonance spectroscopy in adult monozygotic twins with lead poisoning. *Environmental Health Perspectives, 112*(5), 620-625.

Weisskopf, M. G., Hu, H., Sparrow, D., Lenkinski, R. E., & Wright, R. O. (2007). Proton magnetic resonance spectroscopic evidence of glial effects of cumulative lead exposure in the adult human hippocampus. *Environmental Health Perspectives, 115*(4), 519-523.

White, R. F., Diamond, R., Proctor, S., Morey, C., & Hu, H. (1993). Residual cognitive deficits 50 years after lead poisoning during childhood. *British Journal of Industrial Medicine, 50*(7), 613-622.

Woronowicz, A., Koshimizu, H., Chang, S., Cawley, N. X., Hill, J. M., Rodriguiz, R. M., Loh, Y. P. (2008). Absence of carboxypeptidase E leads to adult hippocampal neuronal degeneration and memory deficits. *Hippocampus, 18*(10), 1051-1063.

Wu, J., Basha, M. R., Brock, B., Cox, D. P., Cardozo-Pelaez, F., McPherson, C. A., Zawia, N. H. (2008). Alzheimer's disease (AD)-like pathology in aged monkeys after infantile exposure to environmental metal lead (pb): Evidence for a developmental origin and environmental link for AD. *Journal of Neuroscience, 28*(1), 3-9.

Xu, J., Yan, C., Yang, B., Xie, H., Zou, X., Zhong, L., Shen, X. (2009). The role of metabotropic glutamate receptor 5 in developmental lead neurotoxicity. *Toxicology Letters, 191*(2-3), 223-230.

Yang, J., Hou, C., Ma, N., Liu, J., Zhang, Y., Zhou, J., Li, L. (2007). Enriched environment treatment restores impaired hippocampal synaptic plasticity and cognitive deficits induced by prenatal chronic stress. *Neurobiology of Learning and Memory, 87*(2), 257-263.

Yang, S., Wu, J., Liu, D., & Tung, C. (1998). Metabotropic glutamate receptors are involved in calcium-induced LTP of AMPA and NMDA receptor-mediated responses in the rat hippocampus. *Brain Research Bulletin, 46*(6), 505-512.

Zhang, W., Hao, J., Liu, R., Zhang, Z., Lei, G., Su, C., Li, Z. (2011). Soluble Aβ levels correlate with cognitive deficits in the 12-month-old APPswe/PS1dE9 mouse model of alzheimer's disease. *Behavioural Brain Research, 222*(2), 342-350.

Zhu, Q., Hou, W., Zhao, J., Yang, Y., Zhang, Q., Guo, L.,Wu, C. (2010). N-methyl-D-aspartate receptor subunit expression in memory-related brain areas of lead-exposed rats. *Neural Regeneration Research, 5*(23), 1787-1794.

Zilka, N., Filipcik, P., Koson, P., Fialova, L., Skrabana, R., Zilkova, M., Novak, M. (2006). Truncated tau from sporadic alzheimer's disease suffices to drive neurofibrillary degeneration in vivo. *FEBS Letters, 580*(15), 3582-3588.