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Differential Effect of Postnatal Lead Exposure on Gene Expression in the Hippocampus and Frontal Cortex

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Abstract

Although developmental lead exposure is known to have detrimental effects on a variety of cognitive functions that depend on the integrity of the hippocampus and frontal cortex, little is known about how low levels of lead exposure affect expression of key families of genes in these structures. The present study examined the effects of exposure to environmentally-relevant levels of lead during the sensitive early post-weaning period in the rat on the expression profiles of a select number of neurobiologically relevant genes (i.e., genes for neurotrophic factors, NMDA receptors, metabotropic glutamate receptors, synaptic function/plasticity, cell signaling, and transcription/regulation) in the rat hippocampus and frontal cortex. Exposure to lead (180 and 375 ppm lead acetate in food for 30 days) significantly increased blood lead levels (5.8 to 10.3 $\mu\text{g/dl}$) and significantly affected expression of many of the genes examined. In many instances, lead exposure had different effects on the same gene depending on the brain region in which the expression of that gene was examined. Gene expression in the frontal cortex was often more sensitive to modification than gene expression in the hippocampus. These results suggest that even past infancy, exposures to low levels of lead can have significant effects on gene expression in frontal cortex and the hippocampus with the potential to exert long-term effects on behavior and cognition.

Keywords

Lead; gene expression; mRNA; hippocampus; frontal cortex

Introduction

Despite major initiatives to reduce environmental sources of exposure, lead poisoning remains an important public health problem. Although lead is potentially dangerous to people regardless of age, young children are particularly vulnerable to its toxic effects (Lidsky and Schneider, 2003). Lead poisoning in children is particularly insidious because the toxic threshold has not yet been definitively determined. Recent studies suggest that exposure to lead resulting in blood lead levels below the 10 $\mu\text{g/dL}$ level of concern adopted by the Centers for Disease Control in 1991 results in decreases in IQ as well as specific and persisting neuropsychological impairments (Bellinger, 1996; Canfield et al., 2003a; Canfield et al., 2004; Feldman and White, 1992; Lanphear et al., 2005; Stokes et al., 1998). Unfortunately, neurobehavioral deficits associated with childhood lead poisoning persist into adulthood (Winneke et al., 1996; Fergusson et al., 1997).

Despite wide variations in dosing, developmental period in which dosing occurred, duration of exposure, and methods of behavioral analyses, the animal experimental literature has described impairments in spatial learning as a consequence of lead exposure. In particular, deficits in acquisition of a water maze learning task in animals chronically exposed to lead as well as impairments in hippocampal long term potentiation and alterations in hippocampal NMDA receptor (NMDAR) subunit gene expression (Gilbert et al., 1996; Guilarte and McGlothan, 1998; Nihei et al., 2000; Toscano et al., 2002) have been reported, although the precise nature of the effects of lead on these receptors remains to be clarified.

In addition to affects on cognitive functions typically associated with hippocampal functioning, a number of frontal cortical-related behaviors (ex., attention, working memory, and various executive functions) are also affected by lead exposure in rats (Brockel and Cory-Slechta, 1999) and in humans (Canfield et al., 2003a; Canfield et al., 2003b; Nigg et al., 2008). Even rats with lead exposure confined to the post-weaning period displayed a variety of behavioral problems related to frontal lobe functioning including deficits in serial reversal learning, attention, and spatial memory as well impulsivity, disinhibition, and perseveration (e.g., (Canfield et al., 2003a; Chiodo et al., 2007; Koller et al., 2004). Lanphear et al. (2000) also suggested that cognitive domains frequently affected by childhood lead exposure — attention, executive function, visual-motor integration and social behavior—most likely reflect lead-induced damage to frontal cortices.

Although the most at-risk population for lead poisoning is children up to 2 years of age, exposure to lead and consequent damage to the nervous system of a child can occur throughout childhood and adolescence. Recent data suggest that blood lead concentrations taken at 5 to 7 years of age are more strongly associated with IQ than levels taken during early childhood (Hornung et al., 2009). Although recent research has provided an enhanced understanding of the adverse effects of childhood lead exposure, the age of greatest susceptibility is still in question and thus, it is important to evaluate the molecular and neurobiological effects of lead exposure at later as well as early developmental time points. However, almost nothing is known of the molecular consequences of such exposures on structures critical to the neurobehavioral functions most disrupted by lead exposure (i.e., frontal cortex and hippocampus).

The present study was conducted to examine the effects of exposure to environmentally-relevant levels of lead during the sensitive early post-weaning period in the rat on the expression of select neurobiologically relevant genes (i.e., genes for neurotrophic factors, NMDA receptors, metabotropic glutamate receptors, synaptic function/plasticity genes, cell signaling genes and genes related to gene transcription/regulation) in the hippocampus and frontal cortex. The goal of this study was to examine an important subset of genes, based on existing literature, either known or suspected to be altered by developmental lead exposure and to examine the extent to which these genes may be differentially modulated by low level lead exposures in the hippocampus and frontal cortex, key brain regions associated with cognitive and behavioral disturbances in lead-exposed individuals.

Materials and Methods

Animals and Treatments

At weaning (25 days of age), male Long-Evans rats, housed 4 per standard cage, were fed *ad libitum* diets containing lead acetate (180 ppm or 375 ppm in RMH 1000 chow) or control diet without added lead for the next 30 days. Other than their differences in diet, all animals were housed and handled in exactly the same manner during the study and were exposed to a 12h:12h light:dark cycle for the duration of the study. Animals were euthanized at the same time of day on the last day of lead exposure (i.e., postnatal day 55) and whole

hippocampi and frontal cortices (including medial, ventral and lateral subdivisions (Dalley et al., 2004) were rapidly removed, frozen on dry ice and stored at -70°C until processed. Blood was collected into lead-free tubes at the time of euthanasia and analyzed for lead levels by graphite furnace atomic absorption with Zeeman background correction (ESA Labs, MA). The use of animals was in compliance with NIH Guidelines for the Care and Use of Laboratory Animals and the study was approved by the institutional animal care and use committee at Thomas Jefferson University.

Tissue Handling, RNA Manipulation, and Quantitative RT-PCR

Approximately 60 μg of total RNA was extracted from each sample for subsequent qPCR analysis. Total RNA was homogenized and isolated using Qiagen miRNeasy kits according to the manufacturer's suggested protocol with the addition of DNase. Briefly, samples were first homogenized in RNase/DNase/Protease free tubes (Kimble-Kontes Inc.) and then placed in an automated Qiagen QiaCube, to provide highly reproducible extraction of RNA. Extraction was followed by a quality check (QA/QC) on a GE Nanovue spectrophotometer. Samples were accepted for further processing only if they had a 260nm/280nm ratio of >2.0 . mRNA was reverse transcribed using Omniscript RT kits according to the manufacturer's instructions. Real-time PCR was then performed using a LightCycler 480 (Roche Diagnostics, Ltd) according to the manufacturer's instructions with gene specific optimized primers generated as reverse phase HPLC purified oligonucleotides (Idaho Biotech, UT., and SAB Biosciences – See Table 1), LC480 SYBR Green I Master Mix (Roche Diagnostics, Ltd) and 50 ng of cDNA per reaction. A typical reaction took approximately 50 minutes to complete and included a 5 minute denaturation step at 95°C , followed by 45 cycles of 95°C for 5s (melting), 55°C for 5s (annealing) and 72°C for 5s (extension). To confirm specificity of amplification, the products were subjected to a melting curve analysis at the end of the final extension period. Standard curve amplification was performed using known amplicon dilutions ranging from 10^{-6} – 10^{-1} attomoles. The generated standard curve for each primer set was stored as a reference curve for use in future experiments and compared to standards in each run and against the unknown samples.

Data Analysis

Analysis of variance (ANOVA) was used to model difference in expression levels with respect to dose groups (Control, 180 ppm, 375 ppm). Separate models were fit for each gene within each brain region. Within each brain region, p-values for the test of any group differences from the ANOVA on each gene were adjusted for multiple testing using the method of Benjamini and Hochberg to control the false discovery rate at 5%. Differences in dose groups were considered significant if the adjusted p-value was <0.05 . Separately, again within tissue, p-values from all possible pairwise comparisons of dose groups (regardless of the significance of the overall F-test from the ANOVA) were adjusted to control the false discovery rate among all possible pairwise comparisons at 5%. Pairwise differences are reported only for genes with significant group differences with differences considered significant when the adjusted p-value was <0.05 .

Results

Animal Characteristics

There were no significant differences in body weight between lead exposed animals and control animals (Table 2). The mean blood lead level for control animals was $<1.0 \mu\text{g/dl}$. Mean blood lead levels for all lead-exposed groups were statistically significantly different from control and from each other ($F(2, 19) = 264.4$, $p<0.0001$). Blood lead levels are shown in Table 2.

Neurotrophic Factor Gene Expression

Gene expression for brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophic factor-3 (NT-3), and basic fibroblast growth factor (bFGF) were examined due to their localization in and functional importance to both the hippocampus and frontal cortex (Figure 1). bFGF gene expression was unchanged by lead in the hippocampus (Figure 1A) but significantly down-regulated in the frontal cortex ($p = 0.0014$) and the effect was dose dependent (Figure 1B). NT-3 gene expression was significantly up-regulated in the hippocampus ($p = 0.03$) at the lowest level of exposure while no significant effect on the expression of this gene was observed in the frontal cortex. (Figure 1C and D). Neither NGF nor BDNF gene expression was significantly affected by lead exposure in either brain region (Figure 1E and F; Figure 1G and H).

NMDA Receptor Subtype Gene Expression

Gene expression for the NMDA receptor subtypes NR1, NR2A, NR2B, NR3A, and NR3B were examined in view of the importance of these receptors to hippocampal and frontal cortical function and their sensitivity, at least in the hippocampus, to lead exposure. NR1 gene expression was not significantly affected in the hippocampus (Figure 2A) but was down-regulated in the frontal cortex ($p < 0.0016$) in the 375ppm exposure group (Figure 2B). NR2A gene expression was also down-regulated in the frontal cortex in the 375 ppm lead exposure group ($p = 0.0004$) and was unaffected in the hippocampus (Figure 2C and D). NR2B gene expression in the hippocampus was very sensitive to modulation by lead exposure ($p < 0.0001$) (Figure 2E). The expression of this gene was also significantly down-regulated in the frontal cortex ($p < 0.0001$) but only in the 375 ppm exposure group (Figure 2F). NR3A gene expression was not significantly affected in the frontal cortex or in the hippocampus (Figure 2G and H). NR3B gene expression was not significantly affected in the frontal cortex but was altered in the hippocampus ($p = 0.0019$), with expression up-regulated in the 375 ppm exposure group (Figure 2I).

Metabotropic Glutamate Receptor Subtype Gene Expression

Metabotropic glutamate receptors (mGluRs) play important roles modulating intracellular calcium release and may play a role in a variety of developmental and plastic processes in the frontal cortex and hippocampus (Corti et al., 2002). mGluR3 gene expression was insensitive to modulation by lead exposure (Figure 3A and B). mGluR4 gene expression in the hippocampus ($p < 0.0001$) was significantly down-regulated at both levels of exposure (Figure 3C) while the expression of this gene in the frontal cortex ($p = 0.024$) was significantly up-regulated at the lowest exposure level (Figure 3D). mGluR5 gene expression was also affected both structures: hippocampal mGluR5 gene expression ($p < 0.0001$) was significantly down-regulated in the lowest exposure group (Figure 3E) while expression the frontal cortex ($p < 0.0001$) was down-regulated in a dose-dependent manner (Figure 3F).

Expression Profiles of Genes Related to Synaptic Function/Plasticity

Expression levels of a number of genes important for signal transduction, synaptic function and plasticity were examined. CamKII α gene expression in the frontal cortex was very sensitive to lead exposure ($p < 0.0035$) (Figure 4B), while in the hippocampus, this gene was unaffected (Figure 4A). In contrast, CamKII gene expression in both structures was insensitive to lead exposure (Figure 4C and D). ATP1B2 (Amog), a gene that encodes Na⁺/K⁺-ATPase proteins involved in modulating plasma membrane Na⁺ and K⁺ ion fluxes, was up-regulated in the 375 ppm exposure group in the hippocampus ($p = 0.04$) but was unaffected in the frontal cortex (Figure 4E and F). In the frontal cortex, gene expression for synaptic proteins RIM1 ($p = 0.0008$) and spinophilin ($p < 0.0001$) were very sensitive to

lead exposure (Figure 4H and J). Hippocampal expression of RIM1 was unaffected by lead exposure (Figure 4G) while spinophilin gene expression ($p < 0.0001$) was significantly down-regulated (Figure 4I).

Expression Profiles of Genes Related to Transcription and Gene Regulation

Gene expression for DNA methyltransferase 1 (DNMT1), an enzyme responsible for maintenance of DNA methylation levels, was very sensitive to lead exposure in the frontal cortex ($p = 0.017$) (Figure 5B) but was not altered in the hippocampus (Figure 5A). Methyl CpG binding protein 2 (MeCP2) gene expression in the frontal cortex ($p = 0.0016$) was very sensitive to lead exposure (Figure 5D) while in the hippocampus ($p = 0.03$), was affected only at the higher exposure group and in the opposite direction to that observed in the cortex (Figure 5C). Gene expression for the DNA binding protein Zfp111 in the frontal cortex ($p < 0.0001$) was also very sensitive to lead exposure, with significant up-regulation in the lower exposure group (Figure 5F). In contrast, in the hippocampus, Zfp111 gene expression ($p = 0.0015$) was significantly down-regulated at the lowest level of exposure (Figure 5E).

Discussion

The results of this study suggest that exposure to low, environmentally relevant levels of lead during the post-weaning period can affect the expression of a variety of genes important for nervous system maturation and function. In many instances, lead exposure had different effects on the same gene depending on the brain region in which the expression of that gene was examined. Brain-region specific differential gene expression patterns are not unique to this study and have been described in other circumstances such as brain response to acute ethanol intoxication (Kerns et al., 2005). Such brain region-specific alterations in gene expression could reflect region-specific signaling events subsequent to a toxin exposure and may be related at least in part to basal differences in expression of specific genes in different brain structures (Kerns et al., 2005).

In a number of instances, gene expression in the frontal cortex was more sensitive to modification by lead exposure than expression of the same gene in the hippocampus. This may suggest a potentially greater susceptibility of the frontal cortex to the effects of lead than the hippocampus or may reflect the differences in maturation of the frontal cortex and hippocampus at the time point presently studied. The hippocampus matures relatively early during post-natal development (Jones et al., 2003) and many hippocampal-mediated functions in the rat are believed to attain adult levels of proficiency by 14 to 30 days of age (Rice and Barone, 2000). In contrast, there is a relatively late functional maturation of the prefrontal cortex (Kolb and Nonneman, 1976; Kolb and Nonneman, 1978) with many processes continuing to mature during the time period of the present study and out to at least 90 days of age (Van Eden and Uylings, 1985). This protracted maturation period is similar in the human in which the frontal lobes continue to structurally and functionally mature well into early adulthood (Rice and Barone, 2000).

Although the blood brain barrier is maturing in the post-natal period, lead continues to cross the blood brain barrier after 25 days of age, the age at which exposure commenced in the present study. Additionally, even at low concentrations, lead disrupts blood brain barrier function in weanling rats, resulting in increased brain lead levels as well as permeability to plasma proteins (e.g., (Wang et al., 2007). Previous studies by us (Schneider et al., 2001) and others (e.g., (Brockel and Cory-Slechta, 1998; Wang et al., 2007) in which post-weaning rats were exposed to lead showed considerable accumulation of lead in brain tissue, indicating that there is little difficulty in lead crossing the blood brain barrier and affecting the functional integrity of the brain during the post-weaning period.

The neurotrophic factors BDNF, NT-3, NGF and bFGF are all survival-promoting molecules that also play important roles in the growth, development, maintenance, and function of several cortical and subcortical neuronal systems (Hyman et al., 1991) and play important roles in synaptic plasticity and signal transduction (Hyman et al., 1991; Lom and Cohen-Cory, 1999; McAllister, 1999; Shimada et al., 1998; Yacoubian and Lo, 2000). Neurotrophic factors are also integrally involved in learning-induced synaptic modifications in frontal cortex and hippocampus as evidenced by learning-induced modifications of mRNA for neurotrophic factors in these structures. However, these effects may be behavior and brain region specific (Naimark et al., 2007). For example, the frontal lobes of rats trained to perform an olfactory discrimination learning task showed increased mRNA expression of several neurotrophic factors while no such changes were observed in the hippocampus (Naimark et al., 2007). Interestingly, in the current study, changes were seen in NT-3 mRNA expression in the hippocampus and not the frontal cortex whereas bFGF mRNA expression was affected only in the cortex. The potential functional significance of these effects of lead on neurotrophic factor gene expression remain to be elucidated. It will be interesting to know, for example, if in addition to any basal changes in neurotrophic factor mRNA expression induced by lead there are modifications in neurotrophic factor gene expression in response to learning in the frontal cortex or hippocampus in lead-exposed animals.

The effects of lead on NMDA receptor subtype expression have been reported previously (see (Toscano and Guilarte, 2005) for review). Most of this work examined effects of lead on NMDA receptor expression and function in the hippocampus and there is comparatively little known about how lead might affect NMDA receptor subtypes in the frontal cortex. Gene expression for NR1, NR2A, and NR2B subtypes in the frontal cortex were found to be sensitive to change by exposure to lead while only the NR2B subtype was sensitive in the hippocampus. The NR2A and NR2B subunits are most abundantly expressed in hippocampal and cortical tissue (Monyer et al., 1994) and type and number of NR2 subunits that co-aggregate with NR1 influences the physiological and pharmacological properties of the receptor. The relative lack of effect on NR1 and NR2A mRNA expression in the hippocampus is somewhat different than what has been described previously (Toscano and Guilarte, 2005) but may be due to differences in lead exposures (prolonged exposures during pre- and post-natal period vs. post-weaning), different sub-regional changes in the hippocampus which may have been obscured in the present study, and effects of lead exposure on different sub-type splice variants (not examined in the present study). The NR3B subunit in particular may play a significant role in learning and memory, in part due to influences on calcium permeability (Matsuda et al., 2002; Wee et al., 2008). Compared to NR3A, whose expression declines in adulthood (Ciabarra et al., 1995; Sucher et al., 1995), NR3B mRNA expression persists in adulthood (Matsuda et al., 2002). While NR3A gene expression was unaffected by lead in both structures, NR3B gene expression changed similarly in the hippocampus and frontal cortex, suggesting a potentially important influence of these changes on learning and memory processes in both regions.

Metabotropic glutamate receptors (mGluRs) are also involved in various aspects of synaptic plasticity and learning. In the present study, mRNA expression of mGluR4 and mGluR5 in both structures was particularly sensitive to the effects of lead. In the hippocampus, mGluR4 is thought to regulate GABAergic transmission and excitatory synaptic input to interneurons, thus playing an important role in presynaptic inhibitory processes (Corti et al., 2002). In the cortex, long-term potentiation (LTP) and long-term depression (LTD) are modulated by mGluRs and dopamine receptors (Otani et al., 1999). mGluR5 in particular is expressed on dendritic spines and shafts, as well as on the membrane between spines, of cortical pyramidal neurons (Romano et al., 1995). Colocalization of dopamine and mGluRs on the same spines/shafts suggest that these two types of receptors interact with each other

post-synaptically (Otani et al., 1999). Modification of prefrontal glutamatergic transmission by dopamine is important for normal cognitive function. LTD in prefrontal cortex is readily induced when glutamatergic synapses are stimulated simultaneously with dopamine receptors. The decreased expression of mGluR5 in the frontal cortex of lead-exposed rats, together with previously described lead-induced alterations of dopamine receptor function and expression (Ma et al., 1999) could play important roles in lead-induced cognitive deficits related to frontal cortical dysfunction.

Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) plays roles in neurotransmission, synaptic plasticity, learning and memory (Lucchesi et al., 2011). CamKII α gene expression in the frontal cortex was particularly sensitive to lead exposure. A significant decrease in CaMKII α mRNA in bipolar patients is associated with some of the affective and cognitive alterations linked to prefrontal cortical dysfunction in bipolar disorder (Xing et al., 2002) and is of interest considering the increased risk of major depressive disorders in lead-exposed individuals (Bouchard et al., 2009). In the current study, the α/β isoform ratio of CaMKII, which plays a key role in neuronal maturation during development, was markedly decreased in the frontal cortex, a finding similarly described in schizophrenia patients (Musazzi et al., 2010). Increased expression levels of the β isoform of CaMKII relative to the α CaMKII isoform could result in significant dysregulation of CaMKII activity and phosphorylation (Musazzi et al., 2010).

RIM1 mRNA expression in the frontal cortex was sensitive to lead exposure. RIM1 is a multi-domain protein that constitutes a central component of the synaptic membrane active zone and cortical down-regulation of RIM1 gene expression could affect frontal cortical neurotransmitter release. Spinophilin gene expression was significantly down-regulated in both cortex and hippocampus. Spinophilin is an important scaffolding protein found predominantly in dendritic spines, shafts and pre-terminal axons (Muly et al., 2004) that modulate glutamatergic synaptic transmission and dendritic morphology. Changes in the number, size, and shape of dendritic spines (which primarily receive excitatory inputs (Feng et al., 2000)) are associated with learning (Horn et al., 1985; Moser et al., 1994) and developmental changes (Boyer et al., 1998; Feng et al., 2000; Harris et al., 1992) and postnatal lead exposure has been reported to decrease spine density on hippocampal pyramidal neurons (Kiraly and Jones, 1982).

MeCP2 is a DNA binding protein that preferentially binds to methylated CpG dinucleotides and is involved in transcriptional regulation of a multitude of genes (Yasui et al., 2007). MeCP2 mediates the gene-silencing effect of DNA methylation by recruiting transcription co-repressors to methylated gene regulatory elements (Bienvenu and Chelly, 2006). Alterations in MeCP2 expression and/or methylation are associated with Rett syndrome (Amir et al., 1999) as well as a variety of cognitive and behavioral disorders including autism, mental retardation, attention deficit/hyperactivity disorder, and mild learning disabilities (Nagarajan et al., 2006), many of which have also been associated with childhood lead poisoning. MeCP2 mRNA expression was significantly down-regulated in cortex at all exposure levels and up-regulated in the hippocampus at the higher exposure level. Alterations in mRNA expression of DNA methyltransferase 1 (DNMT1), an enzyme responsible for maintenance of DNA methylation levels, were only observed in the frontal cortex and support the hypothesis that lead influences DNA methylation levels needed to maintain global gene expression patterns crucial for neuronal function and plasticity.

There are some limitations to the present study. This study examined only transcriptomic data and thus the potential functional significance of the present findings remains to be delineated. While mRNA and protein data do not always correlate, further analysis of both transcriptomic and proteomic data could help to better elucidate the complex biological

processes that may be affected by low level lead exposures. Although a central dogma of molecular biology suggests a strong correlation between gene and protein expression, past empirical studies in fact suggest only a modest correlation (Nie et al., 2007; Tan et al., 2009). It is difficult to find a simple one-to-one relationship between genes and proteins empirically since such relationships can be complicated by post-transcriptional mechanisms affecting mRNA stability and protein degradation, as well as timing differences between gene and protein expressions (Tan et al., 2009). mRNA levels and protein levels certainly do not always correlate (Chen et al., 2002) and down-regulation of mRNA with concurrent with up-regulation of protein expression may occur and vice versa (Chen et al., 2002). Ultimately, transcriptional, translational and post-translational mechanisms will need to be investigated to fully understand the biological impact of lead on the brain. However, without knowing when during the lead exposure period changes in gene expression actually occurred (for example, did the gene expression changes observed in this study occur immediately or very shortly after initiation of lead exposure or did they only become apparent after a significant exposure period and become evident shortly before the end of the exposure period) protein expression data may be difficult to interpret, depending on how quickly proteins in question are turned over or metabolically processed. Additionally, a potential limitation of the present work is that the entire hippocampus and frontal cortex were homogenized for analysis and thus potential sub-regional differences in gene expression in these structures may have been obscured and could have influenced the results, especially if gene expression patterns are differentially modified by lead exposure in different sub-regions of these structures.

In conclusion, low level post-weaning lead exposure produced significant changes in the expression of a number of genes important to the structure, maturation, and function of the hippocampus and frontal cortex. In a number of cases, transcription was affected at the lowest level of lead exposure, gene expression in the frontal cortex was more sensitive to alteration by lead exposure than in the hippocampus, and expression of the same gene was affected in opposite directions in different brain structures. Further work is necessary to determine the extent to which the changes in gene expression currently observed may correlate with protein regulation and how these changes may be related to functional cognitive/behavioral outputs.

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References

- Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet.* 1999; 23:185–188. [PubMed: 10508514]
- Bellinger, D. Learning and behavioral sequelae of lead poisoning. In: Pueschel, SM.; Linakis, JG.; Anderson, AC., editors. *Lead Poisoning in Childhood*. P.H. Brooks Publishing; Baltimore, MD: 1996. p. 97-115.
- Bienvenu T, Chelly J. Molecular genetics of Rett syndrome: when DNA methylation goes unrecognized. *Nat Rev Genet.* 2006; 7:415–426. [PubMed: 16708070]
- Bouchard MF, Bellinger DC, Weuve J, Matthews-Bellinger J, Gilman SE, Wright RO, Schwartz J, Weisskopf MG. Blood lead levels and major depressive disorder, panic disorder, and generalized anxiety disorder in US young adults. *Arch Gen Psychiatry.* 2009; 66:1313–1319. [PubMed: 19996036]
- Boyer C, Schikorski T, Stevens CF. Comparison of hippocampal dendritic spines in culture and in brain. *J Neurosci.* 1998; 18:5294–5300. [PubMed: 9651212]

- Brockel BJ, Cory-Slechta DA. Lead, attention, and impulsive behavior: changes in a fixed-ratio waiting-for-reward paradigm. *Pharmacol Biochem Behav.* 1998; 60:545–552. [PubMed: 9632239]
- Brockel BJ, Cory-Slechta DA. Lead-induced decrements in waiting behavior: involvement of D2-like dopamine receptors. *Pharmacol Biochem Behav.* 1999; 63:423–434. [PubMed: 10418784]
- Canfield RL, Henderson CR Jr, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. *N Engl J Med.* 2003a; 348:1517–1526. [PubMed: 12700371]
- Canfield RL, Kreher DA, Cornwell C, Henderson CR Jr. Low-level lead exposure, executive functioning, and learning in early childhood. *Child Neuropsychol.* 2003b; 9:35–53. [PubMed: 12815521]
- Canfield RL, Gendle MH, Cory-Slechta DA. Impaired neuropsychological functioning in lead-exposed children. *Dev Neuropsychol.* 2004; 26:513–540. [PubMed: 15276907]
- Chen G, Gharib TG, Huang CC, Taylor JM, Misek DE, Kardia SL, Giordano TJ, Iannettoni MD, Orringer MB, Hanash SM, Beer DG. Discordant protein and mRNA expression in lung adenocarcinomas. *Mol Cell Proteomics.* 2002; 1:304–313. [PubMed: 12096112]
- Chiodo LM, Covington C, Sokol RJ, Hannigan JH, Jannise J, Ager J, Greenwald M, Delaney-Black V. Blood lead levels and specific attention effects in young children. *Neurotoxicol Teratol.* 2007; 29:538–546. [PubMed: 17553667]
- Ciabarra AM, Sullivan JM, Gahn LG, Pecht G, Heinemann S, Sevarino KA. Cloning and characterization of chi-1: a developmentally regulated member of a novel class of the ionotropic glutamate receptor family. *J Neurosci.* 1995; 15:6498–6508. [PubMed: 7472412]
- Corti C, Aldegheri L, Somogyi P, Ferraguti F. Distribution and synaptic localisation of the metabotropic glutamate receptor 4 (mGluR4) in the rodent CNS. *Neuroscience.* 2002; 110:403–420. [PubMed: 11906782]
- Dalley JW, Cardinal RN, Robbins TW. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav Rev.* 2004; 28:771–784. [PubMed: 15555683]
- Feldman RG, White RF. Lead neurotoxicity and disorders of learning. *J Child Neurol.* 1992; 7:354–359. [PubMed: 1469241]
- Feng J, Yan Z, Ferreira A, Tomizawa K, Liauw JA, Zhuo M, Allen PB, Ouimet CC, Greengard P. Spinophilin regulates the formation and function of dendritic spines. *Proc Natl Acad Sci U S A.* 2000; 97:9287–9292. [PubMed: 10922077]
- Fergusson DM, Horwood LJ, Lynskey MT. Early dentine lead levels and educational outcomes at 18 years. *Journal of child psychology and psychiatry, and allied disciplines.* 1997; 38:471–478.
- Gilbert ME, Mack CM, Lasley SM. Chronic developmental lead exposure increases the threshold for long-term potentiation in rat dentate gyrus in vivo. *Brain Res.* 1996; 736:118–124. [PubMed: 8930316]
- Guilarte TR, McGlothan JL. Hippocampal NMDA receptor mRNA undergoes subunit specific changes during developmental lead exposure. *Brain Res.* 1998; 790:98–107. [PubMed: 9593842]
- Harris KM, Jensen FE, Tsao B. Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. *J Neurosci.* 1992; 12:2685–2705. [PubMed: 1613552]
- Horn G, Bradley P, McCabe BJ. Changes in the structure of synapses associated with learning. *J Neurosci.* 1985; 5:3161–3168. [PubMed: 4078621]
- Hornung RW, Lanphear BP, Dietrich KN. Age of greatest susceptibility to childhood lead exposure: a new statistical approach. *Environ Health Perspect.* 2009; 117:1309–1312. [PubMed: 19672413]
- Hyman C, Hofer M, Barde Y, Juhasz M, Yancopoulos GD, Squinto SP, Lindsay RM. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature.* 1991; 350:230–232. [PubMed: 2005978]
- Jones SP, Rahimi O, O'Boyle MP, Diaz DL, Claiborne BJ. Maturation of granule cell dendrites after mossy fiber arrival in hippocampal field CA3. *Hippocampus.* 2003; 13:413–427. [PubMed: 12722981]
- Kerns RT, Ravindranathan A, Hassan S, Cage MP, York T, Sikela JM, Williams RW, Miles MF. Ethanol-responsive brain region expression networks: implications for behavioral responses to

- acute ethanol in DBA/2J versus C57BL/6J mice. *J Neurosci*. 2005; 25:2255–2266. [PubMed: 15745951]
- Kolb B, Nonneman AJ. Functional development of prefrontal cortex in rats continues into adolescence. *Science*. 1976; 193:335–336. [PubMed: 935872]
- Kolb B, Nonneman AJ. Sparing of function in rats with early prefrontal cortex lesions. *Brain Res*. 1978; 151:135–148. [PubMed: 678999]
- Koller K, Brown T, Spurgeon A, Levy L. Recent developments in low-level lead exposure and intellectual impairment in children. *Environ Health Perspect*. 2004; 112:987–994. [PubMed: 15198918]
- Lanphear BP, Dietrich K, Auinger P, Cox C. Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. *Public Health Rep*. 2000; 115:521–529. [PubMed: 11354334]
- Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J, Roberts R. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect*. 2005; 113:894–899. [PubMed: 16002379]
- Lidsky TI, Schneider JS. Lead neurotoxicity in children: basic mechanisms and clinical correlates. *Brain*. 2003; 126:5–19. [PubMed: 12477693]
- Lom B, Cohen-Cory S. Brain-derived neurotrophic factor differentially regulates retinal ganglion cell dendritic and axonal arborization in vivo. *J Neurosci*. 1999; 19:9928–9938. [PubMed: 10559401]
- Lucchesi W, Mizuno K, Giese KP. Novel Insights into CaMKII function and regulation during memory formation. *Brain Res Bull*. 2011; 85:2–8. [PubMed: 21070840]
- Ma T, Chen HH, Ho IK. Effects of chronic lead (Pb) exposure on neurobehavioral function and dopaminergic neurotransmitter receptors in rats. *Toxicol Lett*. 1999; 105:111–121. [PubMed: 10221273]
- Matsuda K, Kamiya Y, Matsuda S, Yuzaki M. Cloning and characterization of a novel NMDA receptor subunit NR3B: a dominant subunit that reduces calcium permeability. *Mol Br Res*. 2002; 100:43–52.
- McAllister AK. Subplate neurons: a missing link among neurotrophins, activity, and ocular dominance plasticity? *Proc Natl Acad Sci U S A*. 1999; 96:13600–13602. [PubMed: 10570118]
- Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron*. 1994; 12:529–540. [PubMed: 7512349]
- Moser MB, Trommald M, Andersen P. An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc Natl Acad Sci U S A*. 1994; 91:12673–12675. [PubMed: 7809099]
- Muly EC, Smith Y, Allen P, Greengard P. Subcellular distribution of spinophilin immunolabeling in primate prefrontal cortex: localization to and within dendritic spines. *J Comp Neurol*. 2004; 469:185–197. [PubMed: 14694533]
- Musazzi L, Di Daniel E, Maycox P, Racagni G, Popoli M. Abnormalities in alpha/beta-CaMKII and related mechanisms suggest synaptic dysfunction in hippocampus of LPA1 receptor knockout mice. *Int J Neuropsychopharmacol*. 2010:1–13.
- Nagarajan RP, Hogart AR, Gwye Y, Martin MR, LaSalle JM. Reduced MeCP2 expression is frequent in autism frontal cortex and correlates with aberrant MECP2 promoter methylation. *Epigenetics*. 2006; 1:e1–11. [PubMed: 17486179]
- Naimark A, Barkai E, Matar MA, Kaplan Z, Kozlovsky N, Cohen H. Upregulation of neurotrophic factors selectively in frontal cortex in response to olfactory discrimination learning. *Neural Plas*. 2007; 2007:13427.
- Nie L, Wu G, Culley DE, Scholten JC, Zhang W. Integrative analysis of transcriptomic and proteomic data: challenges, solutions and applications. *Crit Rev Biotechnol*. 2007; 27:63–75. [PubMed: 17578703]
- Nigg JT, Knottnerus GM, Martel MM, Nikolas M, Cavanagh K, Karmaus W, Rappley MD. Low blood lead levels associated with clinically diagnosed attention-deficit/hyperactivity disorder and mediated by weak cognitive control. *Biol Psychiatry*. 2008; 63:325–331. [PubMed: 17868654]

- Nihei MK, Desmond NL, McGlothan JL, Kuhlmann AC, Guilarte TR. N-methyl-D-aspartate receptor subunit changes are associated with lead-induced deficits of long-term potentiation and spatial learning. *Neurosci.* 2000; 99:233–242.
- Otani S, Auclair N, Desce JM, Roisin MP, Crepel F. Dopamine receptors and groups I and II mGluRs cooperate for long-term depression induction in rat prefrontal cortex through converging postsynaptic activation of MAP kinases. *J Neurosci.* 1999; 19:9788–9802. [PubMed: 10559388]
- Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect.* 2000; 108(Suppl 3):511–533. [PubMed: 10852851]
- Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J Comp Neurol.* 1995; 355:455–469. [PubMed: 7636025]
- Schneider JS, Lee MH, Anderson DW, Zuck L, Lidsky TI. Enriched environment during development is protective against lead-induced neurotoxicity. *Brain Res.* 2001; 896:48–55. [PubMed: 11277972]
- Shimada A, Mason CA, Morrison ME. TrkB signaling modulates spine density and morphology independent of dendrite structure in cultured neonatal Purkinje cells. *J Neurosci.* 1998; 18:8559–8570. [PubMed: 9786964]
- Stokes L, Letz R, Gerr F, Kolczak M, McNeill FE, Chettle DR, Kaye WE. Neurotoxicity in young adults 20 years after childhood exposure to lead: the Bunker Hill experience. *Occup Environ Med.* 1998; 55:507–516. [PubMed: 9849536]
- Sucher NJ, Akbarian S, Chi CL, Leclerc CL, Awobuluyi M, Deitcher DL, Wu MK, Yuan JP, Jones EG, Lipton SA. Developmental and regional expression pattern of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. *J Neurosci.* 1995; 15:6509–6520. [PubMed: 7472413]
- Tan CS, Salim A, Ploner A, Lehtio J, Chia KS, Pawitan Y. Correlating gene and protein expression data using Correlated Factor Analysis. *BMC bioinformatics.* 2009; 10:272. [PubMed: 19723309]
- Toscano CD, Hashemzadeh-Gargari H, McGlothan JL, Guilarte TR. Developmental Pb²⁺ exposure alters NMDAR subtypes and reduces CREB phosphorylation in the rat brain. *Dev Br Res.* 2002; 139:217–226.
- Toscano CD, Guilarte TR. Lead neurotoxicity: from exposure to molecular effects. *Brain Res Rev.* 2005; 49:529–554. [PubMed: 16269318]
- Van Eden CG, Uylings HB. Cytoarchitectonic development of the prefrontal cortex in the rat. *J Comp Neurol.* 1985; 241:253–267. [PubMed: 2418068]
- Wang Q, Luo W, Zheng W, Liu Y, Xu H, Zheng G, Dai Z, Zhang W, Chen Y, Chen J. Iron supplement prevents lead-induced disruption of the blood-brain barrier during rat development. *Toxicol Appl Pharmacol.* 2007; 219:33–41. [PubMed: 17234227]
- Wee KS, Zhang Y, Khanna S, Low CM. Immunolocalization of NMDA receptor subunit NR3B in selected structures in the rat forebrain, cerebellum, and lumbar spinal cord. *J Comp Neurol.* 2008; 509:118–135. [PubMed: 18425811]
- Winneke G, Lilienthal H, Kramer U. The neurobehavioural toxicology and teratology of lead. *Arch Toxicol Suppl.* 1996; 18:57–70. [PubMed: 8678821]
- Xing G, Russell S, Hough C, O'Grady J, Zhang L, Yang S, Zhang LX, Post R. Decreased prefrontal CaMKII alpha mRNA in bipolar illness. *Neuroreport.* 2002; 13:501–505. [PubMed: 11930170]
- Yacoubian TA, Lo DC. Truncated and full-length TrkB receptors regulate distinct modes of dendritic growth. *Nat Neurosci.* 2000; 3:342–349. [PubMed: 10725923]
- Yasui DH, Peddada S, Bieda MC, Vallero RO, Hogart A, Nagarajan RP, Thatcher KN, Farnham PJ, LaSalle JM. Integrated epigenomic analyses of neuronal MeCP2 reveal a role for long-range interaction with active genes. *Proc Natl Acad Sci U S A.* 2007; 104:19416–19421. [PubMed: 18042715]

Figure 1.

Effects of post-weaning lead exposure on neurotrophic factor gene expression profiles in the hippocampus and frontal cortex. Quantitative PCR analysis of mRNA expression levels of basic fibroblast growth factor (bFGF; A, B), neurotrophic factor-3 (NT-3; C, D), nerve growth factor (NGF; E, F) and brain derived neurotrophic factor (BDNF; G, H) in hippocampus and frontal cortex of control and lead-exposed animals. Structure and dose specific patterns of response to lead were observed for NT3 and bFGF gene expression. Data are mean number of attomoles of mRNA/50 ng cDNA S.E.M. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Figure 2. Effects of post-weaning lead exposure on NMDA receptor subtype gene expression profiles in the hippocampus and frontal cortex. Quantitative PCR analysis of mRNA expression levels for five NMDA receptor subtypes: NR1 (A, B), NR2A (C, D), NR2B (E, F), NR3A (G, H) and NR3B (I, J) in the hippocampus and frontal cortex of control and lead-exposed animals. Significant structure and dose specific effects were observed. Data are mean number of attomoles of mRNA/50 ng cDNA S.E.M. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Figure 3.

Effects of post-weaning lead exposure on metabotropic glutamate receptor subtype gene expression profiles in the hippocampus and frontal cortex. Quantitative PCR analysis of mRNA expression levels for metabotropic glutamate receptors mGluR3 (A, B), mGluR4 (C, D) and mGluR5 (E, F) in the hippocampus and frontal cortex of control and lead-exposed animals. Significant effects were seen within a structure with different lead exposure levels. Data are mean number of attomoles of mRNA/50 ng cDNA S.E.M. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Figure 4.

Lead-related changes in expression of genes related to synaptic function and plasticity in the hippocampus and frontal cortex. Quantitative PCR analysis of mRNA expression levels for Ca²⁺/calmodulin-dependent protein kinase II alpha (CaMKII α ; A, B), Ca²⁺/calmodulin-dependent protein kinase II beta (CaMKII β ; C, D), ATP1B2 (Amog; E, F) regulating synaptic membrane exocytosis 1 (RIMS1; G, H) and spinophilin (I, J) was performed on hippocampal (A, C, E, G, I) and frontal cortex (B, D, F, H and J) extracts from control and lead-exposed animals. Significant effects were seen within a structure with different lead exposure levels. Data are mean number of attomoles of mRNA/50 ng cDNA S.E.M. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Figure 5.

Effects of lead exposure on mRNA expression of genes related to transcription and regulation in the hippocampus and frontal cortex. Quantitative PCR analysis of mRNA expression levels for DNA methyltransferase 1 (DNMT1; A, B), Methyl CpG binding protein 2 (MeCP2; C, D) and zinc finger protein 111 (Zfp111; E, F) was performed on extracts from the hippocampus (A, C, and E) and in the frontal cortex (B, D, and F) of control and lead-exposed animals. A number of structure and dose specific effects were observed. Data are mean number of attomoles of mRNA/50 ng cDNA S.E.M. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Table 1

Primer sequences and annealing temperatures for all primers

Primer Name	Forward Primer Sequence	Reverse Primer Sequence	Annealing Temp (°C)
<i>bFGF</i>	CGTCAAACCTACAGCTCCA	CAGTGCCACATACCAAC	55
<i>NT3</i>	GCCATTGACATTTCGGG	GCTCGGACGTAGTTT	55
<i>NGF</i>	AGCCACGGACATCAAG	CGTCTGTTGTCAACGC	55
<i>BDNF</i>	GCGTGTGTGACAGTATTAG	GGCATTGCGAGTTCCA	55
<i>NR1</i>	ACACAATTACGAGAGCG	CCGAACCCATGTCTTATC	55
<i>NR2A</i>	GGATTAACCGACAGCAC	CCCGATAACTAAGCGTTG	55
<i>NR2B</i>	AAGCCCGACTAATTCCA	AAGGGTTTTGCGTGAC	55
<i>NR3A</i>	CTACATACACAGCGAACT	TGGCACGTTGTACCTT	55
<i>NR3B</i>	GGGAAACCGAACAACAC	CCATACCTTGAAATGCCG	57
<i>mGluR3</i>	TCCTACGACAGCGTGA	AACCAGGGGTACGAT	55
<i>mGluR4</i>	TTTGTGCGCTACAACG	CGGTAAATGCGGTTGG	57
<i>mGluR5</i>	CAGTGAACCGTGTGAGA	GGAGTGTCGCGATAAATG	55
<i>CaMKIIα</i>	ACCCGATTACGGAAG	GGAGTCGGACGATATTGG	55
<i>CaMKIIβ</i>	TTTGGATTGCGGGAAC	GTAACGGTGTCCTACT	55
<i>RIM1</i>	CAACCACCACTGAACA	AGATTCCACACGTCGG	55
<i>Spinophilin</i>	AGGTACGCAAGATCAAAC	CCTCTCTAGTGTGCGC	55
<i>DNMT1</i>	CGTTAGCAACATTACAAGG	TGGTTATGACGCCATCT	57
<i>MeCP2</i>	GGGCAAACATGAACCAC	CGCGTCCAACCTTCAG	55
<i>Zfp111</i>	CACGATGTGATGCTGG	AGTAGTGCTGGAATGGT	55
Commercially Available Primer (SAB Biosciences)			Annealing Temp (°C)
<i>AMOG</i>	RefSeq Accession # NMO_12507.3	Ref Position: 1649	55

Table 2

Body weights and blood lead levels

	Control	180ppm Lead	375ppm Lead
Weight (g)	255.0 ± 6.2	271.9 ± 10.1	267.3 ± 9.0
Blood Lead Level (µg/dl)	<1.0(BDL)	5.8 ± 0.2 ¹	10.3 ± 0.6 ²

¹ p<0.05 versus Control,² p<0.001 versus Control;

BDL = below detectable limit