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Inorganic lead (Pb) exposure in the rat activates striatal cFOS expression at lower blood levels and inhibits amphetamine-induced cFOS expression at higher blood levels.

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Running title:
Pb activates cFOS & inhibits amphetamine-induced expression

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References: 49
Abstract, words: 237
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Non-Standard Abbreviations:
AMPH – Amphetamine
cFOS-IR – cFOS immunoreactivity
CaM - Calmodulin
Con – Control
CRE – cAMP response element
CREB – cAMP response element binding protein
IEG – immediate early gene
LTP – long-term potentiation
PKC – Protein kinase C
Veh - Vehicle

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Abstract

The impact of inorganic lead (Pb)-exposure on dopamine (DA) neurotransmission in the basal ganglia was examined. Amphetamine (AMPH)-induced cFOS immunoreactivity (cFOS-IR) in the striatum was determined following 3 weeks exposure to Pb acetate (0, 50, or 250 ppm). On the 21st day of Pb-exposure, rats were challenged with AMPH (4mg/kg, i.p.) or saline vehicle (Veh) and were assayed for presence of cFOS-IR. In the untreated control (Con) group, AMPH challenge (Con/AMPH) increased cFOS-IR expression by approximately 35 fold over Veh challenge (Con/Veh) ($P<0.01$). In the Pb50/Veh group, cFOS-IR expression was approximately 7 fold greater than in the Con/Veh group ($P<0.05$). Given that there was negligible cFOS-IR expression in the Con/Veh group, this indicates that the Pb50 exposure induced cFOS expression. The increase in cFOS-IR in the Pb50/AMPH was also significant ($P<0.01$), but was not different from the Con/AMPH ($P>0.20$). Neither the Pb250/Veh group nor the Pb250/AMPH group had a significant increase in cFOS IR relative to Con/Veh ($P>0.20$). These results indicate that chronic 50 ppm Pb-exposure induced a low, but statistically significantly level of cFOS gene activation, and did not affect the AMPH-induced cFOS activation. However, chronic 250 ppm Pb-exposure inhibited AMPH-induced activation of cFOS in the striatum by about 89%. Therefore, Pb is capable of both activating cFOS expression at low levels of exposure (mean blood Pb level: 21.6±1.9 µg/dl), and inhibiting AMPH-induced cFOS expression at higher levels of exposure (mean blood Pb level: 47.4±2.6 µg/dl).

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Introduction

The toxic effects of lead (Pb)-exposure on humans have been known for centuries, but have only been systematically studied in the last half of the 20th century. Low level Pb-exposure is associated with impaired learning and cognitive function, and higher distractibility and impulsiveness (Needleman, 1993; Rice, 1993). The Center for Disease Control (CDC) has designated the blood Pb level of 10 µg/dl as the threshold concentration for concern in humans (1991). Recently, however, blood levels below the CDC threshold have been reported to impair cognition in children (Canfield et al., 2003a,b).

Numerous studies have shown that Pb exposure alters the turn-over or release of many classical neurotransmitters including glutamate, acetylcholine, GABA, and DA (Braga et al., 1999; Cory-Slechta, 1995; Kala and Jadhav, 1995a,b; Lasley and Gilbert, 2002; Minnema et al., 1986; Shao and Suszkiw, 1991). The present study focuses on the effects of *in vivo* Pb exposure on the midbrain DA system. It has been well established that the midbrain DA system is affected by Pb exposure at clinically relevant blood Pb levels in the rat (e.g., see Cory-Slechta, 1995;1997; Kala and Jadhav, 1995a, b; Tavakoli-Nezhad et al., 2001).

The midbrain DA system is involved in cognition, attention, reward mechanisms and motor function, and has been shown to be an important participant in many neurological and psychiatric disorders. Abnormalities in the midbrain DA system are highly suspect in the etiology of attention-deficit hyperactivity disorder (DiMaio et al., 2003; Krause et al., 2003; Sagvolden and Sergeant, 1998; Solanto, 2002). Although

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the etiology of attention-deficit hyperactivity disorder (ADHD) is likely multifactorial (e.g., see Davids et al., 2003; DiMaio et al., 2003), evidence suggests that Pb-exposure is a risk factor for the development of ADHD in an exposed subpopulation of ADHD patients (Canfield et al., 2003b; Minder et al., 1994; Tuthill, 1996). However, the precise nature of the effects of Pb on attention mechanisms, and the degree to which Pb-induced attention deficits contribute to impaired learning, is a matter still under investigation (e.g., see Cory-Slechta, 2003).

Kala and Jadhav (1995a) have reported that exposure to 50 ppm Pb for 90 days resulted in a decrease in basal and K^+-stimulated release of DA and its metabolites in the nucleus accumbens (i.e., ventral striatum). Jadhav and Ramesh (1997) have also reported that exposure to 50 ppm Pb for 30 days resulted in a 43% decrease in tyrosine hydroxylase (TH) activity in the nucleus accumbens. These results suggest that the effects of drugs which elicit DA release may be altered by Pb exposure.

Amphetamine has been used as a pharmacological tool for probing catecholamine release mechanisms. Amphetamine is an indirect DA agonist, and it competitively inhibits DA transporters and elicits catecholamine release via transporter-mediated exchange, thereby increasing synaptic DA levels (see Leviel, 2001). Devoto et al. (2001) showed that, in rats exposed to Pb *in vivo*, the amphetamine-induced DA release in the nucleus accumbens was significantly lower in the Pb-exposed group (Devoto et al., 2001). It should also be noted that amphetamine and amphetamine-like drugs, such as methylphenidate, have been shown to be effective agents in the control of ADHD symptoms. Imaging studies of ADHD patients show forebrain abnormalities, particularly in striatal regions, and single photon emission computed tomography

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(SPECT) has shown an elevation in striatal dopamine transporter density in these patients (Krause et al., 2003).

Amphetamine has been shown to induce expression of cFOS in both the dorsal and ventral striatum (Graybiel et al., 1990). The amphetamine-induced expression of cFOS-IR has been shown to be dependent on both D1 and NMDA receptor activation (Berretta et al., 1992; Robertson et al., 1991;Young et al., 1991). D_1 receptor activation increases cAMP resulting in the phosphorylation of the cAMP response element binding protein (CREB) and the induction of cFOS in striatal neurons (Das et al., 1997). Recently Pb exposure has been shown to induce c-FOS in the rat striatum (Ramesh et al., 2001). Since Pb exposure has been shown to affect the midbrain DA system in animal Pb exposure models, alter basal forebrain DA release and amphetamine-elicited forebrain DA release, and also induce cFOS expression in the forebrain, we sought to determine if chronic Pb exposure would alter the amphetamine-induced expression of the cFOS gene in the striatum. This study examines the effects of chronic Pb on the trans-synaptic DA modulation of gene expression in the rat striatum.

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Methods

Animals and drug treatments

Animals were housed in facilities operated by the Department of Laboratory Animal Resources at Wayne State University and maintained according to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. Male Sprague Dawley male rats (Charles Rivers, USA) were 20 days old on the day of arrival. Animals received water and rat chow (Lab Diet 5001, PMI Nutrition International, LCC, Brentwood, MO) ad libitum. The day after arrival, the rats were weighed (mean body weight: 45±0.6 g, n=62) and randomly assigned to treatment groups. The control group received purified water (controls), and the lead (Pb) treated-groups received purified water containing either 50 or 250 part per million (ppm) of Pb acetate. Twenty-one day-old rats were maintained on the Pb-treated water regimen for 3 weeks and weight gain was monitored. Exposure to these two concentrations of Pb acetate induces clinically relevant blood Pb concentrations (Cory-Slechta, 1995, 1997; Kala and Jadhav,1995). This post-weaning protocol may best model pica behavior and oral ingestion of Pb by young children (e.g., see Bayer et al., 1993; Greene et al., 1992). Tavakoli-Nezhad et al. (2001) have reported that significant Pb effects on the midbrain DA system can be observed at clinically relevant blood Pb levels using this Pb exposure protocol.

Drug Challenge

Data from our preliminary dose-response studies and from literature indicate that the effect of AMPH on cFOS activation in the striatum is dose-dependent and that

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4mg/kg is an intermediate dose. The 4 mg/kg dose of AMPH was chosen so that Pb-induced increases or decreases in cFOS gene activation would be detectable. Control and Pb-treated rats were randomly assigned to receive an i.p. injection of either vehicle (normal saline) or d-amphetamine sulfate in normal saline (AMPH; 4mg/kg i.p.). Prior to injections, rat cages were transferred from the animal quarters to a quiet laboratory, where the injections took place. Following i.p. injection, all rats were returned to their home cage and maintained in the same location until sacrificed.

Tissue preparation

Two hours after injections, rats were deeply anesthetized with chloral hydrate (400mg/kg) and prepared for transcardial perfusion. Perfusion was initiated with ice cold phosphate buffered saline (0.1M; PBS) containing heparin (5 USP units/ml) and maintained until the effusate ran clear, then the rat was fixed with 4% paraformaldehyde in 0.1M phosphate buffer (fixative). The brain tissue was post-fixed for 2 hours before transfer to 30% sucrose in fixative. The brains were stored at 4^{o}C until they sunk to the bottom of the sucrose-fixative. Brains were blocked using Tissue-tek OCT compound (Sakura; Torrance CA, USA) and mounted on a freezing cryostat stage. Coronal sections (40 µm thick) were serially cut using a cryostat (Reichert-Jung Histoslide 2000) at -27°C. Brain sections were stored in cryoprotectant (see Drugs and solutions) at -20^{o}C until processed for detection of the cFOS protein with the anti-cFOS rabbit polyclonal IgG, sc-52 (Santa Cruz Biotechnology).

Every third forebrain section containing the striatum was selected for immunohistological examination. Floating brain slices in 1:5000 cFOS antiserum were

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maintained on orbital rocker overnight in a cold room at 4^{o}C. Biotinylated goat anti-rabbit antibody (Vector Laboratories, Berlingame, CA, USA) was utilized at 1:1000. Nuclear cFOS protein was visualized using the Vector Elite ABC reagents followed by DAB-nickel chromogen that stained the cFOS-labeled nuclei grey-black. The antibody specificity was tested in some sections by the deletion of the primary antibody. Under these circumstances, nuclear labeling was not observed.

Micrographs were taken with a Nikon Eclipse E600 with CoolSNAP Pro digital camera and transferred to Adobe PhotoShop for digital photo processing. Digital photos were analyzed with Image-Pro Plus software (Media Cybernetics, Silver Springs, MD, USA).

Blood and Brain Pb determinations

The blood Pb levels were measured in samples obtained by cardiac puncture in anesthitized rats. The electrolytic voltametric stripping method was used for all blood lead determinations (LeadCare® Blood Lead Test Kit; ESA, Inc. Chelmsford, MA, USA). The blood Pb level was determined from three levels of exposure, 0, 50 and 250 ppm. Brain Pb levels were determined from 100 to 300 mg brain samples from a coronal section through forebrain tissue at approximately the mid level of the striatum. The tissue was then digested in concentrated ultrapure nitric acid at 150^{o}C overnight. Digests were analyzed in a Zeeman 5100 atomic absorption spectrometer (Perkins-Elmer) and compared to a Pb-standard curve that ranged from 0-100 µg/ml (Pb atomic absorption standard: Aldrich, Milwaukee, Wis., USA). The groups of animals used for

blood and brain Pb level determinations were not included cFOS-IR study, but was raised together in the same cages and, therefore, are representative of the latter group.

Analysis

To insure that regional differences in cFOS expression were taken into account, the entire rostral-caudal extent of the striatum was analyzed for AMPH-induce cFOS gene activation. From the rostral to the caudal extent of the caudate-putamen, every third brain slice was examined for cFOS-positive neurons. Brain sections were digitally photographed at 100X magnification and analyzed for the presence of cFOS-positive neurons. cFOS-positive neurons were “manually” counted bilaterally with the assistance of Image-Pro Plus Software. Each of the cFOS positive nuclei were evaluated by an observer and marked on a computer monitor with a computer mouse “click” that tagged them as counted. The observer was blinded to the treatment. The counts were reported as the mean ± SEM per section for each rat.

Six groups were compared as follows; rats maintained on water alone and injected with vehicle (Con/Veh), rats maintained on water alone and injected with AMPH (Con/AMPH), rats maintained on 50 ppm Pb and injected with vehicle (50Pb/Veh), rats maintained on 50 ppm Pb injected with AMPH (50Pb/AMPH), rats maintained on 250 ppm Pb and injected with vehicle (250Pb/Veh), and rats maintained on 250 ppm Pb injected with AMPH (250Pb/AMPH). Statistical analysis was performed with 3X2 factorial ANOVA with the treatment factor being Pb exposure (i.e., 0, 50 and 250 ppm) and the drug-challenge factor being AMPH or Veh administration. Due to significant heterogeneity of variance ($P>0.05$; Levene’s test), the factorial ANOVA was

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followed by a Kruskal-Wallace Test and then by individual Mann-Whitney U Test for planned comparison of groups. A $P<0.05$ was considered statistically significant.

Drugs and solutions

d-Amphetamine sulfate (AMPH) was dissolved in normal saline at 1mg/ml. Cryoprotectant consisted of 30% sucrose, 30% ethylene glycol, 1% polyvinylpyroldidone in PBS. Lead-treated drinking water was made by adding lead acetate to nitrogen bubbled and purified de-ionized water. Drinking water was replaced with fresh solutions every 2- to 3-days. All Drugs and chemicals were obtained from (Sigma, Saint Louis, MO).

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Results

The study examined a total of 62 treated rats. Thirty-one rats were used in immunohistochemistry experiments and an additional 31 rats were used to obtain blood and brain Pb concentration and brain weight. Although there was a trend towards reduced body and brain weight at 250 ppm, there was no significant difference found in the body or brain weight between the 3 groups. However, there was a significant exposure-level dependent difference in the blood and brain Pb levels. The mean values (±SEM) for body and brain weight, and blood and brain Pb concentrations are given in table 1.

In preliminary experiments, the expression of amphetamine (AMPH)-induced cFOS-IR was found to be dose-dependent. When the dose of AMPH was increased from 1 to 10 mg/kg, a corresponding increase in cFOS-IR was found. Saline challenge failed to increase the expression of cFOS-IR, as did AMPH in the absence of the primary cFOS antigen (data not shown). We found the 4mg/kg dose of AMPH to induce an intermediate level of c-FOS-IR. Expression of c-Fos-IR was found throughout the rostral to caudal extent of the striatum. We restricted our analysis to the dorsal striatum (caudate-putamen) in coronal brain sections corresponding to the coordinates from Bregma 0.02 to 2.0. The dose of AMPH used in this study (4mg/kg) activated neurons predominately in the medial half of the striatum.

The experiment examining a 4mg/kg challenge dose of AMPH following three weeks of Pb-treatment was analyzed using a two-way ANOVA. There was a significant Pb-treatment effect (Pb: 0,50,250 ppm; $P<0.005$), drug-challenge effect (Veh/AMPH; $P<0.001$), and Pb-treatment by drug-challenge interaction ($P<0.001$). However,

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Levene's Test indicated that there was significant heterogeneity of variance among the various groups (P<0.001). Therefore, a nonparametric approach was utilized for further analysis. A nonparametric Kruskal-Wallis test indicated that there was still a significant difference among all groups (P<0.001). Planned comparisons were made using the nonparametric Mann-Whitney U-Test. In control animals, saline challenge (Veh) did not cause a significant induction of cFOS (**Figures 1a & 2,** Con/Veh: 31±29 cells/section; n=6), However, the level of the AMPH-induced expression of the cFOS gene was considerable. In the untreated control group, AMPH administration significantly increased the number of cFOS-IR-positive neurons located in the striatum (**Figures 1b & 2**; Con/AMPH vs. Con/Veh: 1301±402 vs. 31±29 cells/section; P<0.01, Mann-Whitney U-Test; n=5 & 6, respectively). Following Pb50 treatment, there was a significant basal activation of the cFOS gene in the saline challenged group relative to control group (**Figures 1c and 2**; Pb50/Veh vs. Con/Veh: 223±67 vs. 31±29 cells/section; P<0.05, n=5 and 6 respectively). However, Pb50 treatment had no effect on the AMPH-induced cFOS gene activation, because AMPH-injection still significantly increased cFOS-IR relative to the Pb50/Veh group (**Figures 1d and 2**; Pb50/AMPH vs. Pb50/Veh: 1801±263 vs. 223±67 cells/section; P<0.01; n= 5 each), and this level of gene activation was not significantly different than that observed following AMPH challenge in the untreated control group (Con/AMPH vs. Pb50/AMPH, 1301±402 vs. 1801±263 cells/section; P>0.20, n= 5 each). In the Pb250 group, Pb-treatment did not activate the cFOS gene, because the count of cFOS-IR positive cells in vehicle-challenged animals were not significantly different from the untreated control (**Figures 1e and 2**; Pb250/Veh vs. Con/Veh: 6±5 vs. 31±29 cells/section; P>0.50, n=5 & 6, respectively). Further more,

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in the Pb250 treated animals AMPH did not cause a significant induction of cFOS relative to vehicle-challenged controls (Pb250/AMPH vs Pb250/Veh; 148±79 vs. 6±5; P>0.20, n=5 each). In contrast to the Pb50 treatment, the Pb250 treatment significantly reduced the AMPH-induced expression of cFOS-IR by approximately 89% relative to untreated control (**Figures 1f and 2;** Pb250/AMPH vs. Con/AMPH: 148±79 vs. 1301±402; p<0.005, n=5 each).

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Discussion

This study examines the impact of Pb exposure on DA modulated gene expression in the rat striatum. An approximate seven-fold increase in cFOS expression in rats treated with 50 ppm Pb and not challenged with amphetamine was found. This indicates that low-level Pb treatment can induce cFOS, and is consistent with the study by Ramesh et al. (2001), which found that three months of exposure to 50 ppm Pb caused an increase in striatal cFOS in rats. When rats treated with 50 ppm Pb were challenged with amphetamine, the degree of cFOS activation was not significantly different from that of the untreated control group challenged with amphetamine. Therefore, at this exposure level, Pb did not appear to impair D_1 receptor-mediated nigrostriatal DA neurotransmission.

In contrast to the results from 50 ppm Pb exposure, the 250 ppm level of Pb exposure had no significant effect on basal cFOS levels. The 250 ppm level of Pb exposure did, however, dramatically reduce the amphetamine-induced increase in cFOS-IR in the striatum relative to amphetamine-challenged untreated-controls by about 89%. This finding strongly suggests that the 250 ppm Pb exposure impaired DA neurotransmission. The precise site(s) for this Pb-induced impairment is/are not known, but could include an alteration in amphetamine-induced DA release or events 'down-stream' of the immediate drug action.

Pb has been shown to alter the turnover or release DA (Kala and Jadhav, 1995a; Minnema et al., 1986; see Cory-Slechta, 1995) and glutamate (Braga et al., 1999; Lasley and Gilbert, 2002), both of which are required for the amphetamine-induced activation of striatal cFOS (Berretta et al.,1992; Kiba and Jayaraman, 1994; Liu et al.,

1994). It has been reported that exposure to 50 ppm Pb for 90 days resulted in a decrease in DA release in the nucleus accumbens (Kala and Jadhav, 1995a). Since a Pb-induced decrease in the number of spontaneously active midbrain DA neurons, but not Pb-induced DA neuron cell death, has been reported at low levels of exposure (i.e., 250 and 500 ppm) by Tavakoli-Nezhad et al. (2001), these findings indicate that Pb alters the physiological function of DA neurons in a manner consistent with attenuated DA neurotransmission at the presynaptic level. It should be noted that the apparent Pb-induced decrease in DA neurotransmission has been more clearly demonstrated in the nucleus accumbens (or ventral striatum) and not the dorsal striatum.

Kant et al. (1984) studied the effects of Pb exposure on amphetamine-stimulated DA release by using an extended period of exposure that included exposure of the dam prior to mating (1000 ppm Pb acetate 70 days prior to mating), the gestational period (dam continued on 1000 ppm Pb acetate) and the postnatal period (1000 ppm to the lactating dam and then the weanlings) up until the age of about 60 days-old. Their study showed that there was no Pb-based difference in amphetamine-stimulated DA release in striatal tissue minces (Kant et al., 1984). The protocol used by Kant et al. was, however, quite different from that of the present study. More recently Devoto et al. (2001) showed that the amphetamine-induced DA release in the nucleus accumbens was significantly lower in the Pb-exposed (350 ppm Pb acetate *in utero* until the day of testing) group relative to controls in rats. In the present study, it is tempting to speculate that the 250 ppm Pb exposure may have decreased the amphetamine-induced release of DA in the striatum. However, given the very large magnitude of the impact of Pb on cFOS in the present study, the apparently smaller magnitude of the effect of Pb on DA

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release in the nucleus accumbens reported by Devoto et al. (2001) may not completely explain our findings. It should also be noted that not only was a different nucleus studied (i.e., nucleus accumbens), but the exposure protocol used by Devoto et al. is dissimilar to the one used in the present study. In particular, it involved a much more extensive developmental exposure that included both the prenatal and postnatal periods.

Pokora et al. (1996) used a post-weaning exposure protocol similar to the one employed in the present study to examine the time-course of the effects of Pb on DA D_1-like and D_2-like receptor binding in the striatum and nucleus accumbens. In the striatum, Pb exposure at the 150 ppm level caused only a small and transient decrease in D_1-like binding after 8 months of Pb treatment (no longer significant after 12 months), but no effect at the earlier 2 week time-point. The nucleus accumbens, however, showed a pronounced decrease in D_1-like binding after 8 months, but this effect was transient (absent at the 12 month time-point), and there was no effect at earlier the 2 week time-point. A pronounced decrease in D_2-like binding sites was also observed in the nucleus accumbens at the 150 ppm level of exposure for all time-points measured. Pokora et al. (1996) concluded in this study that the nucleus accumbens was preferentially affected by Pb relative to the striatum.

A microiontophoretic study by Pitts et al. (2002) examined the effects of 250 ppm post-weaning Pb exposure on type I nucleus accumbens neurons that are presumably medium spiny GABAergic neurons (Onn et al., 1994). After three weeks of Pb exposure, they found that the sensitivity of neurons from Pb-exposed animals to the DA D_1 agonist, SKF-38393, was significantly less that that of controls. This finding is

consistent with a Pb-induced decrease in DA D_1 receptor sensitivity in a population of medium spiny neurons.

There is a complex distribution of DA receptors in the dorsal and ventral striatum, with DA D_1-like and D_2-like receptors associated with both somatodendritic membranes and presynaptic varicosities (see Nicola et al., 2000). In addition, DA receptors are associated with the somatodendritic membranes and presynaptic varicosities of neurons utilizing a variety of classical neurotransmitters (see Nicola et al., 2000). These considerations make the interpretation of the receptor binding and microiontophoretic studies difficult. Although the effects of Pb on the pre- and post-synaptic components of the DA system are still incompletely understood, the evidence does indicate that Pb exposure alters the number of DA receptor binding sites and DA receptor responses within the basal ganglia.

It is possible that the 250 ppm Pb exposure used in the present study down-regulated the DA D_1 receptors in the striatum, which are known to modulate cFOS expression, and this attenuated the amphetamine-induced response. Pb exposure has also been shown to alter NMDA receptor subunit composition and reduce CREB phosphorylation in the cortex and hippocampus (Toscano et al., 2002). Since the striatal cFOS response to amphetamine involves both D1 and NMDA receptors, an alteration in glutamatergic neurotransmission can not be ruled out as a potential component of the Pb-induced decrease in cFOS expression.

The expression of the cFOS gene involves multiple second messengers (see Sheng and Greenberg, 1990 for review) and Pb could likely affect these pathways. First, Pb has been shown to directly inhibit adenylyl cyclase and decrease cAMP

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concentration (Nathanson and Bloom, 1975, Sandhir and Gill, 1994a,b). If the inhibition of amphetamine-induced cFOS expression by Pb-exposure were mediated by inhibition of adenylyl cyclase, our data would suggest that the threshold for effect was between the 50 and 250 ppm, because the inhibition of cFOS expression was not seen at 50 ppm but was significant at 250 ppm. Another possibility is that normal intracellular Ca^{+2} signaling could have been disrupted by Pb-exposure. Pb-exposure has been shown to activate calmodulin (Habermann et al., 1983). Through the mimicry of Ca^{+2}, and the activation of calmodulin, Pb-exposure might induce the phosphorylation CREB resulting in the expression of the cFOS gene. This scenario could possibly explain the effect of 50 ppm Pb to induce cFOS expression in the striatum in the absence of amphetamine, but would not explain the inhibition of amphetamine-induced cFOS expression by 250 ppm Pb. Finally, Pb-exposure has been shown to activate the second messenger, PKC (Goldstein, 1993; Habermann et al., 1983; Markovac and Goldstein, 1988). Pb could act via this pathway to activate the cFOS gene in the absence of the amphetamine challenge. This pathway is independent of the cAMP-mediated pathway, and is not considered to be the pathway involved in the amphetamine-induced D_1-mediated activation of the striatal cFOS gene (Das et al., 1997).

The results of this study indicate that Pb-exposure adversely affects DA regulated gene expression within the rat striatum at low-levels of Pb exposure, and indicates that the striatum is sensitive to Pb exposure at clinically relevant blood levels. These results also indicate that the exposure level is a critical determinant for the outcome for cFOS expression observed in the striatum. Further studies will be

necessary to ascertain the relative importance of pre- and post-synaptic mechanisms in the effects of Pb on cFOS expression within the striatum.

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Figure 1. Coronal forebrain sections are shown 2 hours after either saline (A, C and E) or amphetamine administration (B, D and F; 4mg/kg, i.p.) in control (A and B), Pb50 (C and D) and Pb250 (E and F) rats. Saline administration resulted in little or no cFOS-IR in the striatum of untreated control rats (A). In untreated control rats, amphetamine administration resulted in a significant increase in cFOS gene activation. Note the black nuclear cFos staining with DAB-Nickel (B). Saline administration in the Pb50 group resulted in a low level of cFOS gene activation, that was significant compared to the saline-challenged untreated-control (C). Amphetamine administration increased cFOS gene activation in the Pb50 group (D). Saline administration had no effect on cFOS-IR in the Pb250 group (E). Amphetamine administration failed to activate the cFOS gene in the Pb250 group (F). Scale bar, 250µm shown in figure A is the same throughout. Vent = ventricle.

Figure 2. A two-way ANOVA indicated that there was a significant Pb-treatment by amphetamine (AMPH) challenge interaction ($P<0.001$). A nonparametric approach was utilized for further analysis due to heterogeneity of variance. A Kruskal-Wallis test also indicated that there was a significant difference among groups ($P<0.001$). Mann-Whitney U-tests demonstrated significant AMPH-induced responses in untreated control (Con) and 50 ppm Pb-treated rats relative to vehicle (Veh) challenge (** $P<0.01$). A significant 50 ppm Pb-induced increase in cFOS was associated with the Veh challenge relative to Con/Veh (*$P<0.05$). The 250 ppm Pb-treated groups were <u>not</u> found to be significantly elevated relative to their respective controls. The cFOS induction associated with amphetamine challenge in 250 ppm Pb treated was significantly lower

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than respective controls by approximately 89% (***P<0.005). The numbers below the abscissa that are inside parentheses indicate the sample size (n).

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Table 1

Measures of growth and tissue Pb levels following 3 weeks Pb exposure

Tissue Weight or Pb Level	Lead Treatment Condition		
	0 ppm	50 ppm	250 ppm
Rat Weight (g)	205±9	209±2	195±8
Brain Weight (g)	1.70± 0.05	1.70 ± 0.01	1.62 ± 0.04
Blood Lead (µg/dl) *	2.8 (n=1) < 1.4, B.D.[a] (n=15)	21.6 ± 1.9	47.4 ± 2.6
Brain Lead (ng/g) *	B.D.	77 ± 7	440 ± 39
n =	16	3	12

*** Kruskal-Wallis Test, P<0.001**

[a] B.D. Below limits of detection

Figure 1

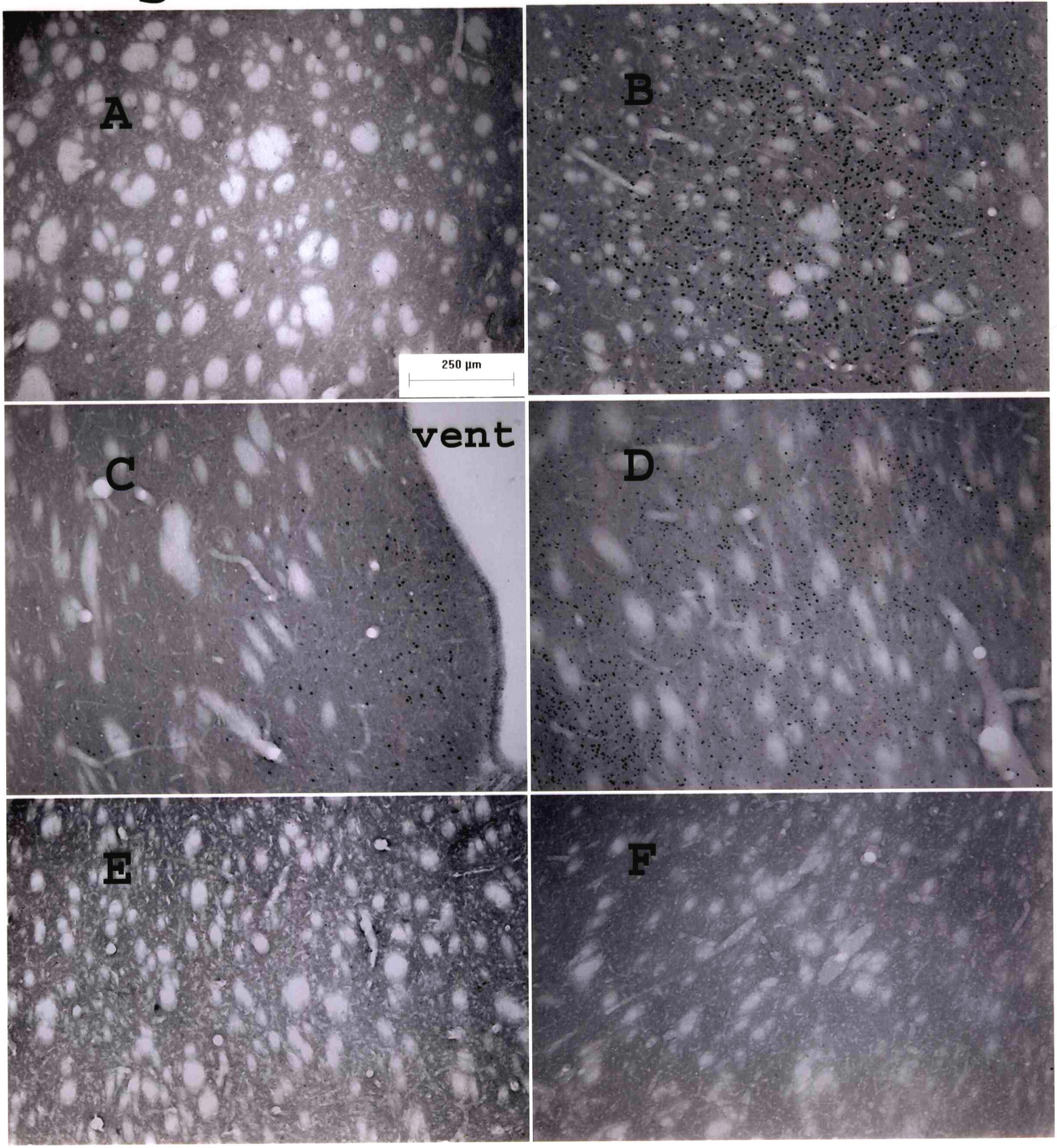
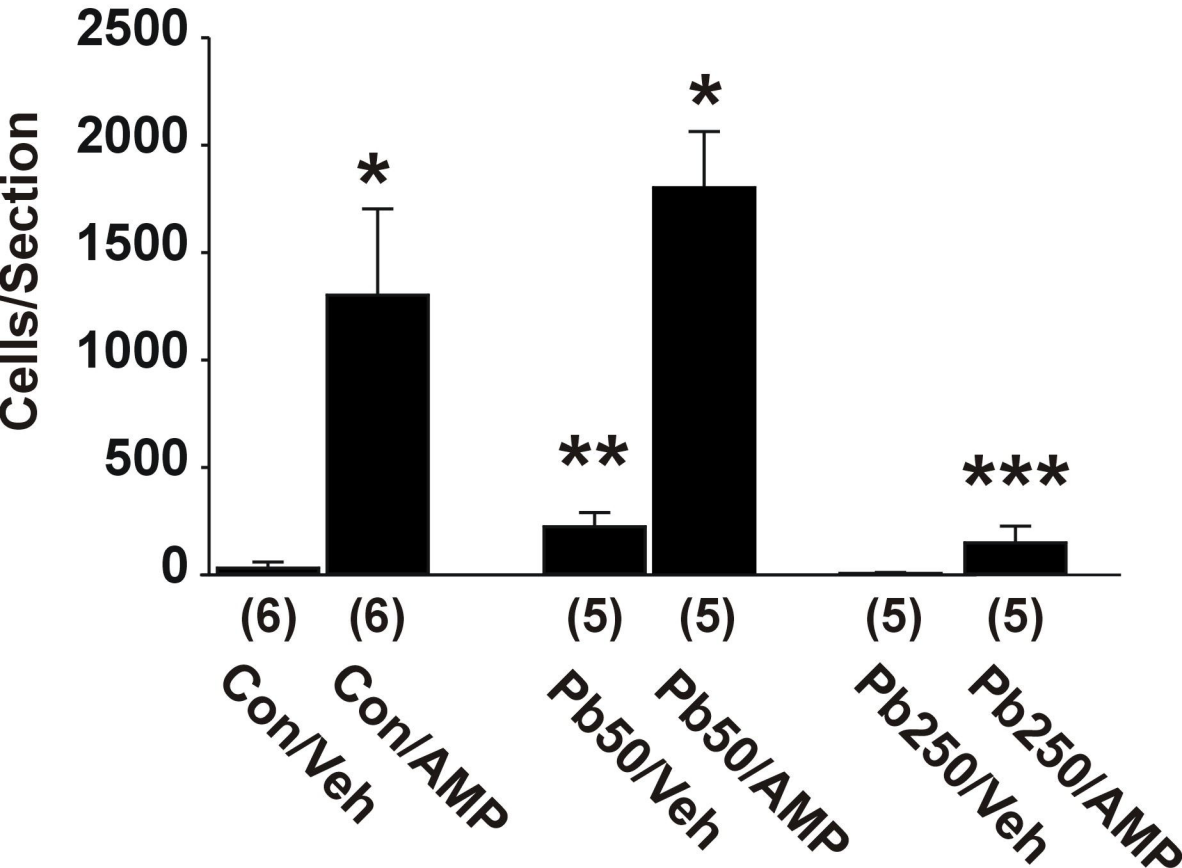


Figure 2


Cells/Section
2500
2000
1500
1000
500
0
*
*
**
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(6)
(6)
(5)
(5)
(5)
(5)
Con/Veh
Con/AMP
Pb50/Veh
Pb50/AMP
Pb250/Veh
Pb250/AMP