Modulation of Dopaminergic System Ontogeny by Low-Level Lead Exposure: A Potential Underlying Mechanism for the Onset of Drug Sensitization

Barbara Domingos Soares

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Abstract

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Lead (Pb^{2+}) is an environmental toxin that is known to cause lasting cognitive deficits following early life exposure. Previously, our laboratory demonstrated increased sensitivity to the psychostimulant effects of cocaine in animals with elevated blood Pb^{2+} levels (BLL). This effect was abolished following introduction of dopamine (DA) receptor antagonists, indicating that the dopaminergic (DAergic) system may be a target of Pb^{2+}'s toxic effects. However, the biological mechanisms through which Pb^{2+} increased sensitization to cocaine's psychostimulant effects have not been fully elucidated. There is some disagreement regarding the magnitude and direction of Pb^{2+}'s effects on the DAergic system. Furthermore, many studies to date have measured the effects of Pb^{2+} in only one sex (usually male), one exposure, and one or two time-points, making it difficult to determine any potential sex-, age-, and exposure-dependent effects.

In the present study, we used a well-validated animal model and Pb^{2+} exposure paradigm that uses chronic dietary exposure to 180ppm and 1500ppm Pb^{2+} acetate (PbAC) in the diet. These levels of Pb^{2+} in the diet resulted in low and moderate levels of BLLs that on average approximated 4.5 and 22.0µg/dl in young adult rats. These levels of Pb^{2+} exposure are relevant to contemporary levels of BLL in intoxicated children in many cities in the United States and in many parts of the world where Pb^{2+} exposure continues to be a major public health concern. It should be noted that at the low level of Pb^{2+} exposure, the resulting BLL of 4.5µg/dl is just below the current CDC level of action.

Using this well-defined rat model of chronic Pb^{2} exposure, in Aim 1, we measured DA concentration and turnover in the dorsal striatum (STR) of juvenile (PN14), adolescent (PN28), and young adult (PN50) male and female rats. Tyrosine hydroxylase (TH) protein, the rate-

limiting step in the synthesis of DA, and phosphorylation of TH at serine 40 (pser40TH) were assessed as an indirect measure of TH activity. Thus, we measured the ratio of pser40TH to total TH protein. We also measured vesicular monoamine transporter-type 2 (VMAT2) levels in the STR, nucleus accumbens (NAC), and olfactory tubercle (OT) since this protein is critical for the sequestration of DA in presynaptic vesicles and has been used as a biomarker for DA terminal integrity. In Aim 2, we examine the effect of chronic Pb^{2+} exposure on D1 and D2 dopamine receptor (D1R and D2R) in the OT, NAC, and STR. Analysis of D1R and D2R is important since the downstream effects of DA are dependent on the DA receptor subtype it activates.

In Aim 1, we observed significant increases in DA and its metabolites homovanillic acid (HVA) and 3,4-Dihydroxyphenylacetic acid (DOPAC) in the STR of adolescent and young adult male rats with BLL as low as 4.5µg/dl in the absence of phosphorylation at the serine 40 residue of TH or altered VMAT2 levels. In Aim 2, a significant increase in D2R was detected in the juvenile male rat STR. We also observed increases in D1R expression in adolescent male rats in the NAC, OT, STR, and in the OT of adolescent female rats. Together, these results demonstrate that chronic Pb^{2+} exposure alters DA receptor levels in a manner characteristic of a hyperactive DAergic state. The observations presented in this work suggest that a hyperactive DAergic system underlies the heightened sensitization to cocaine we previously observed in Pb^{2+}-exposed animals. This work builds upon the current understanding of how Pb^{2+} modulates the DAergic system and provides some elucidation of the mechanisms underlying increased drug sensitization our laboratory has previously observed in rats exposed to Pb^{2+}.

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List of Abbreviations

^{3}H	Tritium
AC	adenylyl cyclase
A-OT	anterior olfactory tubercle
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
BDNF	brain-derived neurotrophic factor
BLL	blood lead level
C	Celsius
cAMP	cyclic adenosine monophosphate
CDC	Centers for Disease Control and Prevention
CNS	central nervous system
CREB	cAMP response element-binding protein
C-STR	caudal dorsal striatum
D1R	D1-like dopamine receptor
D2R	D2-like dopamine receptor
D2RL	D2-like dopamine receptor, short isoform
D2RS	D2-like dopamine receptor, long isoform
DA	dopamine

DAergic	dopaminergic
DARPP-32	dopamine- and cAMP-regulated neuronal phosphoprotein
DAT	dopamine transporter
dL	deciliter
DOPAC	3,4-dihydroxyphenylacetic acid
DTBZ	dihydrotetrabenazine
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
EPA	United States Environmental Protection Agency
Fmol	femtomole
g	gram
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	high-performance liquid chromatography
HVA	homovanillic acid
IQ	intelligence quotient
Kg	kilogram
L	Liter
LTP	Long-term potentiation

M	Molar
mg	milligram
min	minute
ml	milliliter
mm	millimeter
M-STR	middle dorsal striatum
NAC	nucleus accumbens
NAC-C	nucleus accumbens core
NaCl	sodium chloride
NAC-S	nucleus accumbens shell
NHANES	National Health and Nutrition Examination Survey
NIOSH	The National Institute for Occupational Safety and Health
NMDAR	n-methyl-d-aspartate receptor
OSHA	Occupational Safety and Health Administration
OT	olfactory Tubercle
Pb^{2+}	lead
PbAC	lead acetate
PBS	phosphate buffered saline

PBST	phosphate buffered saline with Tween-20
PKA	protein kinase A
PKC	protein kinase C
PN	postnatal day
P-OT	posterior olfactory tubercle
PP1	protein Phosphatase 1
PPM	parts per million
Pser40TH	phosphorylated-serine(40)-tyrosine hydroxylase
RAC	raclopride
rpm	revolutions per minute
R-STR	rostral dorsal striatum
SCH	SCH 23390
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
STR	dorsal striatum
TH	tyrosine hydroxylase
Thr34	DARPP-32 threonine residue 34
TrkB	tropomyosin-related kinase B

VMAT2	vesicular monoamine transporter 2
WHO	World Health Organization
WIN	[N-methyl-^{3}H]- WIN, 35,428
δ -ALA	δ-Aminolevulinic acid
δ -ALAD	δ-Aminolevulinic acid dehydratase
µg	microgram
µm	micrometer

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"It's the job that's never started that takes longest to finish."-J. R. R. Tolkien

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Dedication

A.M.D.G

I dedicate this work to my parents Abilio and Maria Soares, who were raised in Portugal during a time when a post-primary education was reserved for a very small elite. Poverty and circumstance denied both of you access to education beyond the fourth grade and prevented either of you from ever realizing your academic dreams. You taught your three daughters to never take education for granted and now have 3 bachelor's degrees, 4 master's degrees, and a new doctorate degree to show for it. May this work be a testament to you and to others who were denied an education by poverty, war, corruption, racism, sexism, religious persecution, and other circumstances that continue to silence brilliant minds throughout the world.

In memory of

Antonio Matthew Santos

whose college dreams were cut short by Ewing's Sarcoma

2/22/97 – 7/15/15

"Beat the Beast"

Chapter 1: Introduction and Background

Introduction

Public health policies implemented in the 1970s to remove lead (Pb^{2+}) from paint and gasoline have gone a long way to reduce the number of children with Pb^{2+} intoxication. However, despite this public health policy success, the potential for children to be exposed to Pb^{2+} from paint in old homes and in low-rent public housing in many inner cities continues to be a major public health problem affecting an estimated 4 million households in the United States (Needleman 2009, CDC 2015). The widespread contamination of Pb^{2+} in the environment from man-made sources is best exemplified by the current crisis of elevated Pb^{2} levels in the drinking water in Flint, Michigan (Hanna-Attisha and Kuehn 2016, Hanna-Attisha, LaChance et al. 2016) and many other municipalities such as Newark, New Jersey and Detroit, Michigan, in which levels of Pb^{2+} in drinking water resulting from the corrosion of old pipes containing Pb^{2+} greatly exceed the United States Environmental Protection Agency (EPA) level of 15ppb (Toscano and Guilarte 2005, Nriagu, Burt et al. 2006, Nriagu, Senthamarai-Kannan et al. 2011, Brown and Margolis 2012, Triantafyllidou, Nguyen et al. 2013, AP 2016, Hanna-Attisha, LaChance et al. 2016, Santora 2016).

These tragic examples of thousands of children being exposed to Pb^{2+} levels known to produce serious health problems should remind public health practitioners, physicians, and state and federal government agencies that the scourge of childhood Pb^{2+} intoxication persist even today. The majority of current cases of Pb^{2+} exposure, as history can confirm, mainly occur in underserved African American and Hispanic communities living in inner city neighborhoods that have historically encountered a disproportionately higher risk for Pb^{2+} exposure (Fishbein, Todd et al. 2008, Nriagu, Senthamarai-Kannan et al. 2011, Pugh Smith and Nriagu 2011). These are the same communities that have a higher prevalence of stress, delinquency, and drug use and

abuse and lack the social and healthcare infrastructure than more socioeconomically affluent communities are able to enjoy (Dietrich, Ris et al. 2001, Needleman, McFarland et al. 2002, Cory-Slechta, Virgolini et al. 2004, Hubbs-Tait, Nation et al. 2005, Fishbein, Todd et al. 2008). There is a growing concern in the scientific and public health communities that chronic exposure to Pb^{2+} during early life may be associated with the emergence of a variety of maladaptive behaviors such as delinquency (Dietrich, Ris et al. 2001), drug use and abuse (Nation, Cardon et al. 2003, Nation, Smith et al. 2004, Fishbein, Todd et al. 2008), and mental health problems such as schizophrenia (Opler, Brown et al. 2004, Opler, Buka et al. 2008, Guilarte, Opler et al. 2012), anxiety (Moreira, Vassilieff et al. 2001, Bouchard, Bellinger et al. 2009, McFarlane, Searle et al. 2013), and depression (Sciarillo, Alexander et al. 1992, de Souza Lisboa, Goncalves et al. 2005, McFarlane, Searle et al. 2013) in adolescence and adulthood. It is remarkable that for many of these mental afflictions there is evidence that disruption of the brain's dopaminergic system may play an important role (Volkow and Li 2004, Jones and Miller 2008, Koob and Volkow 2010, Hong, Im et al. 2015, Volkow, Koob et al. 2016). There is previous evidence that chronic Pb^{2+} exposure alters dopaminergic (DAergic) signaling (Nation, Frye et al. 1989, White, Cory-Slechta et al. 2007, Stansfield, Ruby et al. 2015). However, the picture is far from complete. The aim of this work was to advance the current understanding of the neurotoxic effects of chronic early-life Pb^{2+} exposure on the DAergic system, in order to better understand its potential role in drug abuse and, to a broader extent, mental disease.

History of Environmental Pb^{2+} Pollution

Decades of clinical and experimental animal studies have shown that Pb^{2+} is an element that has no known biological function. On the other hand, its practical applications have resulted

in widespread dispersal of Pb^{2+} into the environment for the past 6000 years (Waldron 1973, Budd, Montgomery et al. 2004, Toscano and Guilarte 2005). Exposure to Pb^{2+} was minimal throughout most of human history as most naturally occurring Pb^{2+} was trapped beneath the earth's surface or compounded with other metals in ores (Boeckx 1986). However, anthropogenic activity such as mining and smelting began releasing it into the environment as early as 6000 to 8000 years ago (Waldron 1973, Needleman 1999, Budd, Montgomery et al. 2004, Papanikolaou, Hatzidaki et al. 2005). Because of its physical properties such as malleability and resistance to corrosion, Pb^{2+} was widely used in ancient world in glazes, plumbing, figurines, architecture, paints, food preservation, and cosmetics among many other uses (Waldron 1973).

Cupellation, a refinery process that extracts precious metals from ore, dramatically increased production of Pb^{2+} waste and resulted in widespread exposure since its discovery 5000 years ago (Boeckx 1986). It is estimated that 300 million tons of Pb^{2+} were released into the environment in the past 5000 years as a result of mining and metallurgy (Tong, von Schirnding et al. 2000). The pollution of water near mines where Pb^{2+} and other metals were extracted was described as early as the first century BC by Vitruvius (Waldron 1973). Once released into the environment, Pb^{2+} does not readily degrade, becoming a persistent source of exposure (Papanikolaou, Hatzidaki et al. 2005). Due to the amount of Pb^{2+} released into the environment by human activity and its persistence in the environment, the body Pb^{2+} burden of modern-day populations is much greater than those in the ancient world (Boeckx 1986, Tong, von Schirnding et al. 2000).

The use of Pb^{2+} as a sweetener and food preservative, together with the use of Pb^{2+} in pottery glazes and plumbing resulted in widespread Pb^{2+} poisoning epidemics throughout the

Roman Empire (Matte, Landrigan et al. 1992, Tong, von Schirnding et al. 2000). Although Pb^{2+} production decreased during the Middle Ages, Pb^{2+} continued to be used in medicinal preparations and as a wine sweetener, leading to sporadic Pb^{2+} poisoning epidemics in Europe and later in America until the 18th century (Boeckx 1986, Nriagu 1992, Hernberg 2000).

It is possible that childhood Pb^{2+} poisoning epidemics occurred throughout history but remained unrecognized (Hernberg 2000). However, the first reported epidemic of childhood Pb^{2+} poisoning resulting from direct environmental exposure occurred in 1892 among children in Brisbane, Australia who lived and played in town homes with deteriorating Pb^{2+} paint. These children exhibited a number of symptoms associated with acute Pb^{2+} poisoning following ingestion of Pb^{2+} paint such as vomiting, abdominal cramps, paralysis, and seizures (Turner 1909); the source of this epidemic was identified as Pb^{2+} paint 12 years later in 1904 (Needleman 2004).

History of Occupational Pb^{2+} exposure

The effects of acute Pb^{2+} exposure were long believed to be limited to occupational exposures, with the first report of occupational Pb^{2+} poisoning being reported in a metal-worker as early as 370BC (Boeckx 1986, Hernberg 2000, Needleman 2009). The toxicity of Pb^{2+} dust and fumes were well known in ancient times, having been associated with the prevalence of paralysis, seizures, and death in miners and smelters (Waldron 1973, Kazantzis 1989). What Paracelsus once described as "the miner's disease" would come to afflict plumbers, painters, potters, window-makers, ship builders, firearms makers, book printers, and many others as Pb^{2+} use increased across a variety of industries with the onset of the Industrial Revolution (Waldron 1973, Matte, Landrigan et al. 1992, Hernberg 2000, Tong, von Schirnding et al. 2000).

Epidemics of paralysis, lethargy, cachexia, sterility, encephalopathy, colic, and death among Pb^{2+} workers would eventually lead to the passing of The 1883 Factory and Workshop Act in the United Kingdom requiring Pb^{2+} factories to meet safety standards designed to protect the health of workers (Hernberg 2000, Tong, von Schirnding et al. 2000). During the Industrial Revolution, it was also observed that the children of exposed female Pb^{2+} workers and the wives of male Pb^{2+} workers suffered from intellectual disabilities as well as convulsions (Boeckx 1986). Even so, the recognition of children as victims of Pb^{2+} poisoning would not occur until the end of the 19th century (Needleman 2009).

Policy Achievements

The greatest source of environmental Pb^{2+} pollution in recent history was leaded gasoline, which resulted in the release of 4-5 million tons of Pb^{2+} into the atmosphere over the course of 50 years (Needleman 2000, Laidlaw and Filippelli 2008). During this time, child blood Pb^{2+} levels (BLL) increased drastically in the United States from the 1920s through the 1970s (Levin, Brown et al. 2008) as evidenced by the National Health and Nutrition Examination Survey (NHANES) 1976-1980 survey estimating a mean blood lead level (BLL) of 15µg/dl for children in the United States (CDC 2013).

Following the 1892 Pb^{2+} poisoning epidemic in Brisbane, Australia concerns of Pb^{2+} toxicity originating from paints spread resulting in decreased use or complete bans of Pb^{2+} in paint in Europe starting in the 1920s (Hernberg 2000, Needleman 2000). Pb^{2+} paint was banned in the United States in 1971 as a result of The Lead-Based Poisoning Prevention Act (Boeckx 1986). The passing of the Clean Air act in 1970 resulted in the gradual phase-out of tetraethyl Pb^{2+} in gasoline beginning in 1970 because it fouled the newly required catalytic converter, and

was completely removed by 1986 (Needleman 2000, Taylor, Schniering et al. 2011). The discontinuation of tetraethyl Pb^{2+} as a fuel additive resulted in a sharp decrease in BLL in children (Levin, Brown et al. 2008). The removal of Pb^{2+} solder from canned foods beginning with its voluntary removal from baby food containers in the early 1980s and ending with a complete ban of Pb^{2+} solder from canned foods by the U.S. Food and Drug Administration in 1995 also contributed to the decreases in BLL (Mielke 1999, Toscano and Guilarte 2005). As a result of these measures, the mean BLL for children aged 1-5 in the United States declined from 15 µg/dl (NHANES 1976-1980), to 3.6 µg/dl (NHANES 1988-1991), and to 1.3µg/dl (2007-2010) (CDC 2013).

A number of measures have also been put into effect to minimize Pb^{2+} use and occupational exposure across industries in developed nations (Matte, Landrigan et al. 1992). The National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit is an average of 50 micrograms of Pb^{2+} per cubic meter of air ($\mu g/m^3$) over a period of 8 hours (NIOSH 2013). The permissible exposure limit set by the Occupational Safety & Health Administration (OSHA) is also an average of 50 $\mu g/m^3$ of Pb^{2+} over a period of 8 hours. OSHA standards also set an action level of 30 $\mu g/m^3$ over an 8-hour period, requiring employers to begin specific compliance activities to ensure safety of employees (OSHA, accessed 2015). There is no effective treatment to remove Pb^{2+} in individuals with BLL below 30µg/dl and preventing exposure is the best public health and occupational approach (Needleman 2009).

Pb^{2+} Exposure Today

Sources of Pb^{2+} exposure vary across countries and populations. Worldwide, the primary sources of Pb^{2+} exposure are Pb^{2+} soldering, contaminated drinking water, the use of leaded

gasoline, Pb^{2+} glazes, Pb^{2+}-based paints, and folk remedies (Papanikolaou, Hatzidaki et al. 2005). In developing countries such as China and India, informal recycling of electronic waste (e-waste) is a growing source of environmental Pb^{2+} pollution (WHO 2010, Sthiannopkao and Wong 2013) and in African countries such as Nigeria, artisanal mining of gold has exposed children to fatal levels of Pb^{2+} exposure (Dooyema, Neri et al. 2012).

In the present-day United States, an estimated 535,000 children under the age of 5 have blood Pb^{2+} levels above 5μg/dl, the current Centers for Disease Control and Prevention (CDC) action level (CDC 2015). The contemporary sources of environmental Pb^{2+} exposure in the United States are Pb^{2+}-contaminated dust and deteriorating Pb^{2+} paint, which can be found in an estimated 24 million homes (Levin, Brown et al. 2008, Kordas 2010, CDC 2015). Other sources of exposure in the United States contributing to elevated BLL include folk remedies, consumption of wild game killed with lead-based ammunition, ceramics, food, and batteries (Levin, Brown et al. 2008, Olympio, Goncalves et al. 2009). Foods, electronics, jewelry, and toys imported from countries where Pb^{2+} is not effectively regulated may also contain large quantities of Pb^{2+} that can be ingested (Meyer, Brown et al. 2008). Plants grown in Pb^{2+}-contaminated soil can also serve as potential sources of exposure (Clark, Brabander et al. 2006, Pruvot, Douay et al. 2006). Furthermore, an estimated 804,000 workers in general industry and another 838,000 workers in construction are at risk for occupational Pb^{2+} exposure (OSHA, accessed 2015). These individuals may bring Pb^{2+} dust in their clothes and into their homes and thus, expose their families (CDC 2015).

Up to 81 million homes in the United States may still be at risk for Pb^{2+} contamination in water due to particulate Pb^{2+} in water as well as the presence of Pb^{2+} in plumbing as of 2013 (Triantafyllidou, Nguyen et al. 2013). However, the recent Pb^{2+} contamination episode in Flint,

Michigan and other municipalities has demonstrated that the dangers of Pb^{2+} contamination in water persist throughout the country. In 2014, the city of Flint, Michigan temporarily switched from Detroit-supplied Lake Huron water to the Flint River and discontinued corrosion-control treatments (Hanna-Attisha 2016). The corrosive properties of the Flint River water was further exacerbated by the addition of ferric chloride to reduce its trihalomethane content, making it 19 times more corrosive than the Lake Huron water that was previously delivered to the town (Bellinger 2016, Hanna-Attisha 2016). As a result, Pb^{2+} leached from Pb^{2+}-based plumbing into the tap water over time, increasing its Pb^{2+} content and increasing the incidence of elevated BLL in the children of Flint from 2.4% in 2013 to 4.9% in 2015 (Hanna-Attisha 2016). As evidenced by the recent shut-off of water fountains at 30 out of 67 schools in the Newark Public Schools district due to elevated Pb^{2+} levels, the devastating crisis in Flint, Michigan has reinforced the need for action to eliminate Pb^{2+} from drinking water and highlighted the heavy price of negligence on the part of local, state, and even federal officials (AP 2016, Santora 2016).

Biomarkers of Pb^{2+} Exposure

The use of a particular biomarker is dependent on the type of exposure to be characterized (Sanders, Liu et al. 2009). The half-life of Pb^{2+} in the body varies across tissues and fluids, making it possible to study both short-term and long-term exposures (Papanikolaou, Hatzidaki et al. 2005, Sanders, Liu et al. 2009). Blood, bone, tooth, hair, urine, nail, and fecal Pb^{2+} concentration can all be used as biomarkers for exposure. Blood Pb^{2+} concentration (in µg/dl) is the primary biomarker used for Pb^{2+} burden in exposure studies (Sanders, Liu et al. 2009). The half-life of Pb^{2+} in blood (27-36 days) makes blood Pb^{2+} concentration a suitable biomarker for recent exposure (Lidsky and Schneider 2003). Most of the Pb^{2+} in blood

accumulates in red blood cells, where it binds to δ-aminolevulinic acid dehydratase (δ-ALAD) and inhibits its function. Inhibition of δ-ALAD results in accumulation of δ-aminolevulinic acid (δ-ALA) in the blood and urine. δ-ALA in blood and urine can be used as a biomarker for Pb^{2+} exposure (Opler, Brown et al. 2004, Ahamed and Siddiqui 2007, Opler, Buka et al. 2008). Bone and tooth Pb^{2+} concentration are suitable biomarkers for cumulative exposure as Pb^{2+} has a half-life of 10-30 years in bone (Rabinowitz 1991, Sanders, Liu et al. 2009). More recently, advances in micro-spatial analytical methods have allowed for measurement of dentine Pb^{2+} levels at multiple sampling points within a tooth, allowing for the determination of prenatal and postnatal Pb^{2+} exposure over time (Arora, Austin et al. 2014)

Pb^{2+} Toxicokinetics

Absorption

Human Pb^{2+} exposure occurs through ingestion, inhalation, and percutaneous exposure and absorption varies by route of exposure (Olympio, Goncalves et al. 2009). Inhalation is the primary form of occupational exposure (Sakai 2000). Absorption of Pb^{2+} through the respiratory system is dependent on the size of the Pb^{2+} particles inhaled. Particles larger than 1-2 um are trapped in the upper respiratory tract and are eventually swallowed. Smaller particles, on the other hand, will be absorbed in the lower lung (Davidson CI 1992). Up to 50% of inhaled Pb^{2+} is absorbed into the bloodstream (Sakai 2000). Ingested Pb^{2+} is absorbed in the small intestine through divalent metal transporter 1, which is the primary transporter for iron (Kordas 2010). Percutaneous exposure of inorganic Pb^{2+} such as that found in leaded paint is negligible (Papanikolaou, Hatzidaki et al. 2005). However, organic Pb^{2+} compounds such as tetraethyl Pb^{2+}

are readily absorbed through skin into the bloodstream (Sakai 2000, Papanikolaou, Hatzidaki et al. 2005).

Children are more susceptible to Pb^{2+} exposure and Pb^{2+} toxicity than adults for numerous reasons. The intake of Pb^{2+} by children through ingestion and inhalation is higher than it is for adults since children ingest more food and water and breathe more air per unit of weight than adults (Tong, von Schirnding et al. 2000, WHO 2010). In children, blood Pb^{2+} levels peak between 15 and 24 months of age due to the fact that children are more likely to play and crawl on the floor and engage in hand-to-mouth activity which can lead to ingestion of Pb^{2+}-contaminated house dust (Sayre, Charney et al. 1974, Needleman 2004, Levin, Brown et al. 2008). Children with pica, the habit of eating non-nutritive substances, are also at risk Pb^{2+} intoxication due to ingestion of leaded paint or other Pb^{2+}-contaminated materials (Moncrieff, Koumides et al. 1964, Lin-Fu 1973, Lin-Fu 1973, WHO 2010).

Differences in Pb^{2+} toxicokinetics between children and adults also increase the vulnerability of children to Pb^{2+} exposure and Pb^{2+} toxicity (Davidson CI 1992, Lidsky and Schneider 2003). Though adults typically absorb up to 15% of the Pb^{2+} they ingest, absorption rates of Pb^{2+} are much higher in young children, with infants absorbing up to 50% of Pb^{2+} ingested (Davidson CI 1992, Sakai 2000, Lidsky and Schneider 2003, Papanikolaou, Hatzidaki et al. 2005). Pb^{2+} retention is also much higher in children (Kordas 2010). Furthermore, since the immature blood brain barrier is more permeable, a higher portion of Pb^{2+} is transported to the brains of children 5 and under compared to adults (Goyer 1990, Lidsky and Schneider 2003).

Distribution

Once absorbed, Pb^{2+} is distributed throughout the body by the bloodstream (Papanikolaou, Hatzidaki et al. 2005, Clark, Brabander et al. 2006). The transfer of Pb^{2+} from blood to soft tissue takes approximately 4-6 weeks (Papanikolaou, Hatzidaki et al. 2005). With regard to Pb^{2+} toxicity, Pb^{2+} crosses the blood brain barrier through transferrin or calcium-ATPase pumps (Lidsky and Schneider 2003, Kordas 2010). Blood brain barrier permeability can be enhanced by Pb^{2+}-induced damage to endothelial cells (Wang, Luo et al. 2007). Maternal Pb^{2+} readily crosses the placenta to the fetus (Goyer 1990, Marchetti 2003). Fetal uptake of Pb^{2+} from maternal stores begins at 12 weeks of gestation and continues until birth (Papanikolaou, Hatzidaki et al. 2005). Maternal blood and plasma Pb^{2+} levels follow a nonlinear U-shaped pattern throughout the course of the pregnancy, decreasing between weeks 12 and 20 of gestation and then increasing again between the twentieth week and birth in a manner corresponding to the transfer of maternal calcium to the fetus (Bellinger 2005). Maternal and fetal BLL are comparable as there is no effective barrier preventing the transfer of Pb^{2+} from a mother to the fetus (Goyer 1990, Graziano, Popovac et al. 1990).

Storage and Mobilization

The half-life of Pb^{2+} is less than an hour in plasma, 27-36 days in blood, 30-40 days in soft tissue, and 10-30 years in bone tissue (Sakai 2000, Papanikolaou, Hatzidaki et al. 2005). About 73% of the total Pb^{2+} body burden in children is found in bone (Papanikolaou, Hatzidaki et al. 2005). Bone turnover results in the mobilization and circulation of bone Pb^{2+} stores throughout the body (Needleman 2004). The demand for calcium during pregnancy and lactation results in the release of maternal bone Pb^{2+} stores to blood as bone metabolism increases (Bellinger 2005, White, Cory-Slechta et al. 2007). Bone resorption following menopause and

onset of osteoporosis also results in the release of Pb^{2+} into the bloodstream (Tsaih, Korrick et al. 2001, Needleman 2004).

Excretion

The main route of excretion for absorbed Pb^{2+} is through the urinary tract, with minute quantities of Pb^{2+} excreted through sweat, nails, and bile (Papanikolaou, Hatzidaki et al. 2005). Chelation therapy decreases body Pb^{2+} burdens by enhancing excretion of Pb^{2+} through the urinary tract (Cory-Slechta 1988, Papanikolaou, Hatzidaki et al. 2005). Ingested Pb^{2+} not absorbed in the gut is excreted in feces (Lidsky and Schneider 2003). During pregnancy, mobilized maternal bone Pb^{2+} stores may be transferred to the developing fetus through blood. Post-pregnancy, maternal Pb^{2+} can be transferred to the neonate through breast-milk (Bellinger 2005, White, Cory-Slechta et al. 2007, Levin, Brown et al. 2008).

Disparities in Exposed Populations

BLL in children continue to decline in the United States due to the removal of Pb^{2+} exposure sources, coupled with surveillance and prevention programs (Jones, Homa et al. 2009). It is estimated that BLL have decreased by up to 90% from the 1970s to 2000s in the United states following the ban of Pb^{2+} in paint in 1978, the ban of tetraethyl Pb^{2+} as a gasoline additive in 1995 and the ban of Pb^{2+} solder in canned foods in 1995 (Toscano and Guilarte 2005, Nevin 2007, Levin, Brown et al. 2008, Brown and Margolis 2012, Hu, Scheidell et al. 2014). Today, the majority of children living in the United States have BLL lower than 5µg/dl (CDC 2015).

The percentage of children with elevated BLL in the United States has dropped considerably in recent decades (Chandran and Cataldo 2010). Furthermore, the general mean

BLL for children aged 1-5 in the United States has dropped from 15µg/dl (1976-1980) to 1.3µg/dl (2007-2010) (CDC 2015). NHANES reports indicate that, although there has been a continued decline of mean BLL in children between 1 and 5 years of age, racial/ethnic and income level disparities persist. Historically, the percentage of minorities from low-income neighborhoods with elevated BLL has been disproportionately larger than the overall national average (Cory-Slechta, Virgolini et al. 2004, Muntner, Menke et al. 2005, Jusko, Henderson et al. 2008, Wright, Dietrich et al. 2008). In the 2007-2010 NHANES report, the general mean BLL for non-Hispanic black children aged 1-5 (1.8µg/dl) was significantly higher than that of either non-Hispanic white (1.3µg/dl) or Mexican American (1.3µg/dl) children. Furthermore, BLL were significantly higher for children from lower income households (1.6µg/dl) versus children from higher income households (1.2µg/dl) (CDC 2015).

Despite the fact that leaded gasoline has not been used in the United States since 1986, decades of heavy vehicle traffic resulted in higher concentrations of Pb^{2+} in urban soil versus soil from suburban and agricultural regions (Needleman 2000, Laidlaw and Filippelli 2008). Pb^{2+} that has accumulated in urban soil can be tracked into homes or, when suspended in air, penetrate through open windows and increase house dust Pb^{2+} load (Laidlaw and Filippelli 2008). As a result, residents of urban areas also have higher environmental Pb^{2+} burdens than the general population (Clark, Brabander et al. 2006). Housing condition can also play a role in determining how much external Pb^{2+} pollution enters a home. Loose-fitting windows in older homes and loss of structural integrity may result in increased penetration and accumulation of Pb^{2+} dust in homes, especially in urban areas where high levels of Pb^{2+} are still present in soil. (Laidlaw and Filippelli 2008).

The condition of Pb^{2+} paint in a household, for example, is strongly associated with elevated BLL. Deteriorating Pb^{2+} paint increases the Pb^{2+} load in dust, which can then be easily consumed by children through hand-to-mouth activity. In one study, 39% of children dwelling in houses with Pb^{2+} paint in poor condition had BLL greater than 10µg/dl versus 15.4% of children in houses with paint in good condition (Lanphear, Burgoon et al. 1998). In a 2002 study, children 24 months of age who never lived in rental housing had BLL of 5µg/dl, whereas children who sometimes lived in rental housing or always lived in rental housing had BLL of 8.8µg/dl and 8.1µg/dl, respectively. Furthermore, of children surveyed, 15% of children who never lived in rental housing had BLL at or above 10µg/dl versus 53.8% of children who sometimes lived in rental housing and 35.7% of children who always lived in rental housing. A significantly higher percentage of rental housing was found to be in poor condition versus owner-occupied housing (Lanphear, Hornung et al. 2002).

A number of nutritional factors are known to modulate the amount of Pb^{2+} absorbed in the gastrointestinal tract as well as the toxicity of Pb^{2+} absorbed. Total food intake and frequency of food intake, can alter the absorption of ingested Pb^{2+} in the gastrointestinal tract (Mahaffey 1990). A higher percentage of ingested Pb^{2+} is absorbed by fasting adults versus adults versus nonfasting adults (Davidson CI 1992). Low intake of essential metals like calcium, iron, zinc, and phosphorus may enhance absorption of ingested Pb^{2+} in the gut (Mahaffey 1990, Lanphear, Hornung et al. 2002). The amount of Pb^{2+} absorbed in the gut is higher in children with low dietary intake of calcium, phosphorous, zinc, or iron (Papanikolaou, Hatzidaki et al. 2005). Dietary iron intake has been inversely related to BLL in children (Lanphear, Hornung et al. 2002). Iron deficiency leads to increased expression of divalent metal transporter 1, which may contribute to increased absorption and bioavailability of ingested Pb^{2+} (Kordas 2010). Iron

deficiency has also been demonstrated to interact with Pb^{2+} exposure to increase risk for cognitive and behavioral deficits (Wasserman, Graziano et al. 1992, Levin, Brown et al. 2008). It is important to note that disadvantaged populations are at a particularly higher risk for iron deficiency (Kordas 2010).

Low calcium intake during pregnancy and lactation has been demonstrated to increase release of bone Pb^{2+} stores, resulting in higher blood and tissue Pb^{2+} levels in women (Mahaffey 1990, Bellinger 2005). This, in turn, may result in increased exposure of the fetus *in utero* as well as increased neonatal exposure through breast-milk during lactation (Mahaffey 1990, Levin, Brown et al. 2008). Increased calcium intake or calcium supplementation throughout pregnancy and lactation may decrease Pb^{2+} mobilization and subsequent transfer of maternal Pb^{2+} to the child (Bellinger 2005, White, Cory-Slechta et al. 2007). However, calcium intake may not be protective against elevated BLL in children (Lanphear, Hornung et al. 2002).

Childhood BLL are dependent on the environment of the child as well as the behaviors exhibited by the child at different ages (Lanphear, Hornung et al. 2002). Low socioeconomic status is associated with substandard housing, proximity to exposure sources, poorer nutrition status and other factors that may increase the risk for elevated BLL in children (Tong, von Schirnding et al. 2000). Economically disadvantaged populations are also more likely to live in urban areas, where there are increased rates of drug use (Miller, Nation et al. 2000).

Susceptibility of the Developing Brain to Pb^{2+} Toxicity

The developing brain is much more susceptible to Pb^{2+}'s neurotoxicity than the mature brain (Lidsky and Schneider 2003). Previous work by our laboratory and others has demonstrated that Pb^{2+} impairs the expression of long-term potentiation (LTP) (Nihei, Desmond

et al. 2000), a process by which synapses are strengthened or weakened based on signaling patterns between neurons. LTP is believed to underlie the formation of long-term memories and is dependent on n-methyl-d-aspartate receptor (NMDAR) activation. A seminal study by Morris et al (1986) first demonstrated *in vivo* that chronic administration of amino-5-phosphonovaleric acid, a competitive NMDAR inhibitor, impaired LTP and spatial memory (Morris, Anderson et al. 1986). As a noncompetitive NMDAR antagonist, Pb^{2+} inhibits NMDAR function, decreasing activity-dependent calcium influx, thereby inhibiting a number of calcium-responsive signaling processes necessary for transcription of genes that maintain LTP (Toscano, McGlothan et al. 2003).

Previous work by our laboratory and others has demonstrated that developmental Pb^{2+} exposure inhibits spatial memory and reduces the amplitude of LTP (Jett, Kuhlmann et al. 1997, Nihei, Desmond et al. 2000). Our laboratory has further demonstrated that developmental Pb^{2+} exposure alters NMDAR subunit expression, thereby altering NMDAR function and downstream signaling (Guilarte and McGlothan 1998, Nihei, Desmond et al. 2000, Guilarte, Toscano et al. 2003). Pb^{2+} exposure during synaptogenesis also results in decreased presynaptic neurotransmitter release, which may be due to decreased expression of synaptophysin and synaptobrevin (Neal, Stansfield et al. 2010). Further work by our laboratory demonstrated that Pb^{2+} exposure during synaptogenesis of hippocampal neurons resulted in dysregulation of brain-derived neurotrophic factor-tropomyosin-related kinase B (BDNF-TrkB) transsynaptic signaling. Altered synapse maturation and function resulting from Pb^{2+}-induced dysregulation of BDNF-TrkB signaling could, in turn, underlie cognitive and behavioral deficits associated with developmental Pb^{2+} exposure (Stansfield, Pilsner et al. 2012).

Neurological Effects of Childhood Pb^{2+} Intoxication

In the years preceding 1943, it was assumed that the toxic effects of Pb^{2+} poisoning were reversible following treatment and removal of the exposure (Olympio, Goncalves et al. 2009). However, a landmark study by Byers and Lord (1943) found that of 20 children that had been treated for Pb^{2+} poisoning in their infancy, 19 of these children experienced learning deficits as well as behavioral disorders such as aggression and antisocial behavior years later (Byers and Lord 1943). However, since all of the children in this study had presented clinical symptoms of Pb^{2+} intoxication, this study was unable to demonstrate the deleterious effects of low-level Pb^{2+} exposure on the cognitive function of otherwise asymptomatic children (Needleman 2009). As a result, the long-term effects of low-level Pb^{2+} exposure would remain unstudied for another three decades (Needleman 2009, Sanders, Liu et al. 2009).

In 1979, Needleman et al. (1979) demonstrated that cognitive deficits occurred in otherwise asymptomatic children that had been Pb^{2+} exposed (Needleman, Gunnoe et al. 1979). In a follow-up study assessing the academic performance of these children 11 years later, Needleman et al. (1990) found that elevated dentine Pb^{2+} levels in childhood were associated with a sevenfold increase in high school graduation failure eleven years later (Needleman, Schell et al. 1990). Elevated dentine Pb^{2+} levels were also associated with lower IQ scores in a sample of first-grade children in Denmark (Hansen, Trillingsgaard et al. 1989) and elevated cord blood Pb^{2+} levels corresponding with prenatal exposure were associated with cognitive impairments in children at 24 months of age (Bellinger, Leviton et al. 1990). As a result of these studies and others demonstrating the long-term impact of low-level childhood Pb^{2+} exposure on cognitive function, there has been a shift in the focus of Pb^{2+}'s toxic effects from high occupational exposure in adults to low exposures in children (Needleman 2004).

Early-life Pb^{2+} exposure has been associated with a number of behavioral and cognitive function deficits that may impair academic performance and increase the risk for school failure later in life (Needleman and Gatsonis 1990, Needleman, Schell et al. 1990, Miranda, Kim et al. 2007). A number of studies have reported IQ deficits in children following low-level Pb^{2+} exposure (Needleman and Gatsonis 1990, Tong, Baghurst et al. 1996, Factor-Litvak, Wasserman et al. 1999, Canfield, Henderson et al. 2003). In another study, children with BLL of 5-10µg/dl scored significantly lower IQ-adjusted reading, mathematics, reading comprehension, and listening comprehension scores than children with BLL of 1-2µg/dl (Surkan, Zhang et al. 2007). Childhood Pb^{2+} exposure has also been associated with poor attention span, delinquency, deficits in language processing, reading disabilities, decreased working memory, and impaired executive function (Needleman and Gatsonis 1990, Needleman, Schell et al. 1990, Needleman 2000, Surkan, Zhang et al. 2007, Fishbein, Todd et al. 2008, McFarlane, Searle et al. 2013).

Deficits in Cognitive Function

In 1960, the toxic threshold for Pb^{2+} exposure in children was 60µg/dl, the detection limit for BLL at the time, and a level at which clinical symptoms of Pb^{2+} poisoning manifest themselves (Olympio, Goncalves et al. 2009). In the decades that followed, however, it became evident that lower BLL were associated with cognitive deficits in otherwise asymptomatic children (Needleman 2004, Olympio, Goncalves et al. 2009).

As BLL in children decreased and research methodologies improved over time, it became possible to identify cognitive function deficits at lower thresholds, prompting the Centers for CDC to decrease the toxic threshold for Pb^{2+} several times (Cory-Slechta 1995, Needleman 2004). As of 2012, the CDC no longer uses the term "level of concern." Instead, the CDC now

employs a reference value of 5µg/dl based on the NHANES BLL distribution in children aged 1-5 to identify exposed children and limit future exposures (CDC 2012). Even so, the CDC continues to recommend chelation therapy to decrease body burden of Pb^{2+} in children with BLL of 45µg/dl and higher (CDC 2004, CDC 2012). It should be noted that although chelation therapy has been used successfully to decrease the body burden of Pb^{2+} in children with BLL of 45µg/dl and higher, it does not seem to reverse or ameliorate cognitive impairments resulting from Pb^{2+} exposure, making primary prevention a key course of action in the prevention of cognitive deficits resulting from low-level Pb^{2+} exposure (Rogan, Dietrich et al. 2001, Dietrich, Ware et al. 2004).

IQ deficits have been described in children with elevated blood or dentine Pb^{2+} levels since the 1970s (Needleman and Gatsonis 1990, Needleman 2004). A number of studies have concluded that there is no safe threshold for early-life Pb^{2+} exposure since lasting effects on cognitive function have been associated with even the lowest detectable exposures (Chiodo, Jacobson et al. 2004, Lanphear, Hornung et al. 2005, Sanders, Liu et al. 2009, Hong, Im et al. 2015). Recent studies suggest that the dose-response curve for childhood Pb^{2+} exposure is non-linear, with the greatest decrement in IQ points occurring with the first 10µg/dl increase in BLL (Canfield, Henderson et al. 2003, Lanphear, Hornung et al. 2005). For example, increases in BLL from 1µg/dl to 10µg/dl were associated with a loss of up to 7.4 IQ points, whereas an increase from 10µg/dl to 30µg/dl was associated with a 2.5-point decrement (Canfield, Henderson et al. 2003).

A pooled analysis of seven prospective Pb^{2+} cohorts also found a strong dose-response relationship between lower BLL in children (below 7.5µg/dl) and IQ points lost. In the pooled data, an increase in BLL from 1µg/dl to 10µg/dl was associated with a loss of 6.2 IQ points

whereas a 10-20µg/dl increase was associated with a 1.9-point decrement and a 20-30µg/dl increase was associated with a 1.1-point decrement (Lanphear, Hornung et al. 2005). A more recent study, using linear and semi-parametic models to estimate the association between lifetime average blood Pb^{2+} concentration and IQ, estimated a 0.15-point decrease in IQ per 1µg/dl increase in children with BLL ranging from 20-30µg/dl. A 0.32-point decrease in IQ was estimated per 1µg/dl increase in BLL for children with BLL ranging from 10-20µg/dl. Consistent with previous studies suggesting a non-linear dose-response curve for childhood Pb^{2+} exposure, a 1.2-point decrement in IQ points was estimated with children in the lowest BLL range of 2.1-10µg/dl (Jusko, Henderson et al. 2008). Thus, the greatest drop in IQ occurs in the very low BLL range.

Behavioral Effects

Prenatal and early-life Pb^{2+} exposure has been found to be associated with an increase in delinquency in early adulthood (Wright, Dietrich et al. 2008). A 2002 case-control study compared tibial bone Pb^{2+} concentrations of 12-18-year-old males charged with delinquent acts to those of age-adjusted males with no arrest records. Those charged with delinquent acts had significantly higher bone Pb^{2+} concentrations versus males with no arrest records (11ppm Pb^{2+} in cases versus 1.5ppm in controls), suggesting that low BLL is associated with increased risk for adjudicated delinquency (Needleman, McFarland et al. 2002). A prospective longitudinal birth cohort Dietrich (2001) found a significant relationship between prenatal Pb^{2+} exposure and antisocial and delinquent behavior in adolescence. Of note, this study also found a strong association between drug use and antisocial and delinquent behaviors in the adolescents (Dietrich, Ris et al. 2001).

In an ecological study, Mielke and Zahran (2012) found that aggravated assault rates in six U.S. cities were associated with air Pb^{2+} levels 22 years prior. Furthermore, every 1% increase in the tons of Pb^{2+} released into the atmosphere 22 years prior was associated with a .46% increase in the aggravated assault rate (Mielke and Zahran 2012). Using temporal changes in tetraethyl Pb^{2+} gasoline consumption and corresponding exposure rates as a proxy for temporal changes in BLL in the United States from 1941 to 1987, Nevin (2000) found that changes in unwed teenage pregnancy and violent crime rates corresponded with tetraethyl Pb^{2+} gasoline consumption years prior. This study suggested that increased tetraethyl Pb^{2+} gasoline consumption in 1960s and 1970s may have contributed to an epidemic of violent crimes and unwed teen pregnancies from the mid-1980s to mid-1990s (Nevin 2000). A similar follow-up analysis investigating the association between early-life BLL and crime trends in the United States and 8 other countries also found a significant association between preschool BLL and arrest rates later in life (Nevin 2007).

Low-level Pb^{2+} exposure may impair cognitive control, contributing to the onset of attention deficit hyperactive disorder (ADHD) (Nigg, Knottnerus et al. 2008, Roy, Bellinger et al. 2009). Braun (2006) described a significant dose-response relationship between environmental Pb^{2+} exposure and ADHD in children. Children with BLL higher than 2 μg/dL had a 4.1-fold increased risk for ADHD versus children with BLL less than 0.8μg/dL (Braun, Kahn et al. 2006). Postnatal Pb^{2+} exposure from diet of game hunted with Pb^{2+} pellets was associated with hyperactive-impulsive type ADHD in a population of Inuit children (Boucher, Jacobson et al. 2012). In another study, elevated BLL were associated with inattention-type ADHD in a population of children from Chennai, India. This study also found a strong association between elevated BLL and anxiety and poor sociability (Roy, Bellinger et al. 2009).

A cross-sectional epidemiological study has also found increased risk of panic and depressive disorders in young adults with elevated BLL. This increased risk persisted even when individuals with BLL ≥ 10 μg/dL were excluded from the analysis, indicating that even low-level Pb^{2+} exposure may contribute to the onset of these disorders (Bouchard, Bellinger et al. 2009). Elevated childhood BLL may increase the likelihood for impulsive behavior later in life (Reyes 2015). Childhood BLL have been associated with early sexual activity, teenage pregnancy, and increased risk for sexually transmitted infections (Nelson, Shacham et al. 2015, Reyes 2015). An association between elevated childhood BLL and teenage alcohol and marijuana use has also been described (Reyes 2015). Poor impulse control may underlie the higher rates of arrest for violent crimes, ADHD, and teenage pregnancy that have been associated with early-life Pb^{2+} exposure (Nevin 2000, Bellinger 2008).

Early life Pb^{2+} Exposure and Drug Sensitization

Addiction is characterized by compulsive and uncontrolled drug seeking and abuse behaviors (Baler and Volkow 2006, Ersche, Jones et al. 2012). Not all individuals exposed to drugs become addicted and one of the challenges in drug addiction research has been to determine the neurobiology underlying the vulnerability of some individuals to transition from drug use to drug addiction (Koob and Le Moal 1997, Volkow and Wise 2005). Many factors such as drug availability, genetics, history of drug use, stress, and life events contribute to the transition from drug use to drug addiction (Volkow and Li 2004). However, exposure to environmental toxins such as Pb^{2+} may also play a role in this transition (Jones and Miller 2008).

For example, Fishbein and colleagues (2008) found that tibial bone Pb^{2+} concentration in a sample of 26 female heroin users in Baltimore was 1.8 times higher per gram of bone than

those in age-matched unexposed females (14.5µg/g versus 8µg/g). Furthermore, frequency of heroin use in users was significantly associated with interactions between tibial Pb^{2+} and risky decision-making as well as tibial Pb^{2+} and cognitive inflexibility (Fishbein, Todd et al. 2008). The results of this study suggest that populations with higher body Pb^{2+} burdens are more at risk for drug use and abuse. Although no definitive link has been established between early-life Pb^{2+} exposure and drug use and abuse later in life in humans, it should be noted that the populations most at risk for Pb^{2+} exposure are also most at risk for use and abuse of drugs, especially cocaine (Ensminger, Anthony et al. 1997, Fishbein, Todd et al. 2008). Also, Pb^{2+} exposure has been demonstrated to increase DAergic system activity, which may enhance sensitivity to drugs of abuse (Nation, Miller et al. 2000, Nation, Cardon et al. 2003, Nation, Smith et al. 2004, Jones and Miller 2008).

The Dopaminergic System as a Target for Pb^{2+} Neurotoxicity

Dopamine (DA) is a catecholaminergic neurotransmitter that plays a critical role in a wide range of central nervous system processes such as working memory, locomotion, reward, motivation, learning, attention, and addiction (David, Clark et al. 1972, Dietrich, Ris et al. 2001, Canfield, Kreher et al. 2003, Tran, Tamura et al. 2005, Beaulieu and Gainetdinov 2011). DAergic system disruptions are associated with alterations in cognitive and behavioral outcomes associated with Pb^{2+} exposure, making the DAergic system a likely target for Pb^{2+} neurotoxicity (Pokora, Richfield et al. 1996, Bouchard, Bellinger et al. 2009). Previous studies have demonstrated that Pb^{2+} exposure affects a number of processes in the DAergic system such as DA synthesis, turnover, and reuptake, as well as the number and function of DA receptors (Cory-

Slechta 1995). Regional as well as temporal differences in the effects of Pb^{2+} on the DAergic system have also been described (Zuch, O'Mara et al. 1998, Gedeon, Ramesh et al. 2001).

DAergic System Overview

DA synthesis begins with the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase (TH). A carboxyl group is removed from L-DOPA by L-DOPA decarboxylase to form DA (Vallone, Picetti et al. 2000). Once DA is synthesized, it is packaged into vesicles via vesicular monoamine transporter-type 2 (VMAT2) to prevent autooxidation (Harsing Jr 2008, Jones and Miller 2008). DA that is not stored in vesicles can be metabolized by monoamine oxidase into 3,4-Dihydroxyphenylacetic acid (DOPAC) which, in turn, can be further metabolized into homovanillic acid (HVA) catechol-o-methyl transferase (COMT). The metabolites HVA and DOPAC have been used as biomarkers of DA turnover as increased levels of these metabolites indicate increased release of DA and vice versa (Mignot and Laude 1985).

The work performed here focused on brain regions associated with the mesolimbic and mesostriatal pathways, that is, the dorsal striatum (STR), nucleus accumbens (NAC), and olfactory tubercle (OT). The mesolimbic pathway is comprised of DAergic projections from the lateral portion of the ventral tegmental area and retrorubral area to the limbic regions of the STR (anteromedial and ventral regions), NAC, OT, and central region of the amygdala; this pathway is strongly indicated in drug addiction (Zhou, Wilson et al. 2003, Bjorklund and Dunnett 2007, Chen, Hopf et al. 2010, Volkow, Wang et al. 2011). The sensorimotor region of the STR (dorsolateral region) is innervated by DAergic neurons of the mesostriatal pathway, which project from the dorsal and ventral tiers of the substantia nigra pars compacta (Bjorklund and

Dunnett 2007). This pathway also contributes to drug reward and addiction (Volkow, Wang et al. 2011).

DA receptors belong to the 7 transmembrane domain G-protein coupled receptor family (Vallone, Picetti et al. 2000). There are 5 known DA receptors that are divided into two groups, the D1-like receptors (D1R) and the D2-like receptors (D2R) (Lindgren, Usiello et al. 2003). DA can act as either a stimulatory or inhibitory neurotransmitter, depending on the DA receptor type it activates. Activation of D1R, comprised of D1 and D5 DA receptors, will result in a stimulatory signaling cascade in postsynaptic neurons through activation of adenylyl cyclase (AC) and increased production of cyclic adenosine monophosphate (cAMP). On the other hand, activation of D2R, comprised of D2, D3, and D4 receptors inhibits AC and decreases production of cAMP in postsynaptic neurons (Harsing Jr 2008). The opposing signaling cascades activated by D1R and D2R play a significant role in the regulation of the direct and indirect signaling pathways underlying motor function, behavior, and cognition (Bjorklund and Dunnett 2007, Gruber and McDonald 2012).

DA synthesis, release, and reuptake are tightly regulated. The binding of DA to D2R autoreceptors in presynaptic neurons results in decreased AC activity. This, in turn, decreases TH activity with a subsequent decrease in DA synthesis (Lindgren, Usiello et al. 2003). DA transporter (DAT), also present on the presynaptic cell terminal, facilitates the reuptake of DA from the synapse. Once taken up into the presynaptic terminal, DA can be repackaged into vesicles via VMAT2 for future release (Harsing Jr 2008). Together, these systems work to prevent excessive DA in the synaptic cleft and in the cytosol, preventing auto-oxidation of DA and subsequent generation of reactive oxygen species (Harsing Jr 2008, Jones and Miller 2008).

Targets of Pb^{2+} in the DAergic System

Previous studies have shown that Pb^{2+} exposure targets DAergic transmission. However, there is considerable disagreement in the literature regarding the mechanism(s) through which Pb^{2+} affects DAergic system function (Cory-Slechta 1995, Verstraeten, Aimo et al. 2008). The magnitude and direction of effect(s) can vary depending on the level of exposure, duration and developmental stage of exposure, and the models used (Verstraeten, Aimo et al. 2008). Overall, these studies demonstrate that there are multiple DAergic system biomarkers that are vulnerable to Pb^{2+} neurotoxicity (Lasley 1992). It should also be noted that insults to different DAergic targets can occur simultaneously, increasing vulnerability of DA transmission to Pb^{2+} neurotoxicity (White, Cory-Slechta et al. 2007).

A number of studies have reported hyperlocomotor function in rats exposed to Pb^{2+} (Meredith, McIntosh et al. 1988, Ma, Chen et al. 1999, Moreira, Vassilieff et al. 2001). Locomotor activity is mediated by D1R and D2R signaling (Ma, Chen et al. 1999). Disruption in the function of these receptors has been implicated in altered DAergic system function in animals with BLL of 9.26-18.06µg/dl following chronic, post-weaning exposure to Pb^{2+} (Gedeon, Ramesh et al. 2001).

Regional differences in D2R levels were observed by Moresco et al (1988), as Pb^{2+} exposure resulted in increased D2R levels in the STR and decreased D2R in the NAC; in contrast, no changes in D1R expression were observed in this study in either STR or NAC (Moresco, Dall'Olio et al. 1988). On the other hand, Pokora (1996) observed a 21% decrease in D2R binding in the NAC of Pb^{2+}-exposed rats with BLL of 29µg/dl versus control rats with BLL of <5µg/dl at 8 months. At 12 months, D2R binding in the NAC was decreased by 16% and 28% in Pb^{2+}-exposed rats with BLL of 17- and 27µg/dl, respectively, versus control rats with BLL of

<5µg/dl. No change in D2R binding was observed in the STR of Pb^{2+}-exposed rats versus control at any time point. A 68% decrease in D1R sites were also observed in the NAC after 8 months of exposure in Pb^{2+}-exposed rats with BLL of 29 µg/dl versus control rats, though this decrease disappeared at 12 months of exposure (Pokora, Richfield et al. 1996). Decreases in D2R in the NAC were also reported by Gedeon (2001) in Pb^{2+}-exposed rats (with BLL ranging from 9.26-18.06µg/dl) versus control (with BLL ranging from 0.81-1.74µg/dl) at postnatal day (PN) 30, PN60, and PN90, followed by significant increases at PN150. Significant increases in D1R were detected in the NAC at PN90, PN120, and PN150 (Gedeon, Ramesh et al. 2001). Ma et al. (1999) observed no change in D2R expression in either NAC or STR and no change in D1R binding in Pb^{2+}-exposed rats with BLL of 40µg/dl versus control rats with undetectable BLL (Ma, Chen et al. 1999). These studies demonstrate that DA receptors are a target for Pb^{2+} toxicity even though the observed effects of Pb^{2+} exposure varied across studies.

Pb^{2+} exposure may also target DA levels through a number of mechanisms. Pb^{2+} may also target D2 autoreceptor function in presynaptic DAergic cell terminals, altering DA release patterns (Leret, Garcia-Uceda et al. 2002). Chronic Pb^{2+} exposure may impair D2 autoreceptor-regulated DA synthesis through downregulation or decreased function of D2 autoreceptors, changes in calcium influx through voltage-gated calcium channels, or interference with calcium-dependent enzymes (Lasley and Lane 1988, Marchetti 2003, NourEddine, Miloud et al. 2005). Nation et al. (1989) reported a significant decrease in DA in the NAC, but also observed a significant increase in the OT, and no change in STR in Pb^{2+}-exposed animals with BLL of 61µg/dl versus control rats with BLL of 3µg/dl. Furthermore, all three of these regions exhibited significant increases in DOPAC levels as well as DA turnover (Nation, Frye et al. 1989). More recently, our laboratory observed significant increases in DA, DOPAC, HVA, and DA turnover

in the STR of Pb^{2+}-exposed rats with BLL of 22µg/dl versus control rats with BLL of 0.6µg/dl (Stansfield, Ruby et al. 2015). Interestingly, Gedeon (2001) demonstrated a significant increase in DA in the NAC at PN60 followed by significant decreases at subsequent time points, raising the possibility of a time-dependent effect of Pb^{2+} on DA availability (Gedeon, Ramesh et al. 2001).

As Pb^{2+} has been demonstrated to alter DA levels, several studies investigated its effects on TH expression and activity. Ramesh and Jadhav (1998) demonstrated that Pb^{2+} decreased TH activity and expression in the NAC but not in the STR of Pb^{2+}-exposed rats with BLL of 18 µg/dl versus control rats with BLL of 4µg/dl (Ramesh and Jadhav 1998). In an *in* vitro study, Pb^{2+} exposure produced a transient increase in TH activity in PC12 cells (Tian, Sun et al. 2000). This transient increase corresponded with the activity of protein kinase C (PKC), a known target of Pb^{2+} toxicity that modulates TH expression and activity (Vyas, Faucon Biguet et al. 1990, Kumer and Vrana 1996, Sun, Tian et al. 1999, Tian, Sun et al. 2000). Picomolar concentrations of Pb^{2+} have been demonstrated to increase PKC activity whereas micromolar concentrations inhibit PKC function (Marchetti 2003). As exposure paradigms vary across studies in the available literature, the concentration-dependent effects of Pb^{2+} on PKC function and may underlie some of the differences in TH activity observed across studies.

Kala (1995) reported significant decreases in basal and stimulated release of DA in the NAC of rats exposed to Pb^{2+}-exposed rats with BLL of 18µg/dl versus control rats with BLL of 4µg/dl (Kala and Jadhav 1995). Pb^{2+} exposure has also been observed to increase potassium chloride-evoked DA release in the NAC by Zuch et al (1998), who reported faster clearance of DA in both the NAC and STR of Pb^{2+}-exposed animals with BLL as low as 16µg/dl versus control rats with BLL <5µg/dl (Zuch, O'Mara et al. 1998). Prior work by this group also

demonstrated that DAT levels decreased in the NAC and not the STR of Pb^{2+}-exposed rats with BLL of 29µg/dl versus control rats with BLL <5µg/dl. This effect was reversed following treatment with DA agonists (Pokora, Richfield et al. 1996). More recently, our laboratory reported no changes in DAT or VMAT2 levels in the STR or NAC in Pb^{2+}-exposed animals (Stansfield, Ruby et al. 2015).

Does DAergic System Dysregulation Underlie Drug Sensitization in Pb^{2+} Exposure Models?

Studies by Nation and colleagues (Miller, Nation et al. 2000, Nation, Miller et al. 2000, Miller, Nation et al. 2001, Nation, Cardon et al. 2003, Nation, Smith et al. 2004) have shown that early-life Pb^{2+} exposure can increase the risk for drug abuse at later stages of life. Significant increases in locomotor response to repeated administration of cocaine have been observed in adult male rats that had been perinatally exposed to Pb^{2+}, demonstrating increased sensitivity to the locomotor stimulatory effects of cocaine (Nevin 2000). In a subsequent study, significantly higher response rates to cocaine reinstatement were observed in exposed versus control rats, demonstrating an association between perinatal Pb^{2+} exposure and increased risk for drug relapse in later life (Nation, Cardon et al. 2003). Further work by this group demonstrated significantly higher rates of self-administration at a low dose cocaine infusion (.06mg/kg/infusion) in exposed rats whereas no effect was observed in control rats. Furthermore, Pb^{2+}-exposed animals self-administered higher doses of cocaine (0.5, 0.25, and 0.15 mg/kg/infusion) at significantly lower rates than control animals, suggesting that perinatal Pb^{2+} exposure increases the reinforcing properties of cocaine (Nation, Smith et al. 2004).

Limitations in Current Knowledge

Pb^{2+} has been demonstrated to adversely affect the DAergic system by a number of studies. It is evident that the effects of Pb^{2+} on the DAergic system are not only dependent on sex and the level of exposure, but on the developmental stage of the exposure (Jones and Miller 2008). As such, it is probably inappropriate to generalize findings across studies that have employed different exposure paradigms and single-sex exposure groups.

Today, the majority of children below the age of 5 residing in the United States have BLL below 5µg/dl, the current CDC level of action. Even so, a number of studies have determined that IQ deficits occur in children with BLL at or below this action level. As such, it is possible that the DAergic system may also be affected in children with BLL at or below 5µg/dl. A number of studies (Kala and Jadhav 1995, Jadhav and Ramesh 1997, Ramesh and Jadhav 1998) have employed control groups with BLL near 5µg/dl and, as a result, masked any effects occurring at these low BLL. Furthermore, there is a scarcity of animal studies employing exposure paradigms resulting in BLL at or near the current CDC level of action.

Experimental animal studies have investigated the impact of Pb^{2+} on DAergic transmission. However, it is often difficult to generalize findings across studies due to a number of factors. Single-sex studies, which employ either all-male or all-female animal models, make up the majority of studies investigating Pb^{2+} toxicity. As the number of single-sex studies employing male animals greatly outweighs the number of those employing female animals, it is possible that our current understanding of Pb^{2+} effects on DAergic system ontogeny may not be generalized across sexes (Beery and Zucker 2011). Sex-dependent differences in cognitive function have been reported in children prenatally exposed to Pb^{2+}, indicating increased susceptibility of males to Pb^{2+} neurotoxic effects (Jedrychowski, Perera et al. 2009). On the other hand, McFarlane (2003) found that females were more prone to drug/alcohol abuse, anxiety and

other behavioral outcomes following early-life Pb^{2+} exposure versus males (McFarlane, Searle et al. 2013). Therefore, there may be sex-dependent effects of Pb^{2+} exposure on the DAergic system that have yet to be elucidated.

The DAergic system is dynamic and changes as the brain matures. Consequently, the levels of DA, DA receptors, and other markers differs across ages. Most studies investigating the impact of Pb^{2+} on the DAergic system have limited their assessment to one time-point (Cory-Slechta 1995). Moreover, as studies typically employ one or two age groups, it is difficult to determine any temporal effects of Pb^{2+} neurotoxicity on any given DAergic system biomarker. The identification of temporal effects is further complicated when methodologies, exposure paradigms, and BLL differ across studies. Together, these limitations highlight the importance of conducting a study that not only incorporates both male and female, but also investigates the impact of Pb^{2+} on the DAergic system at multiple time points and at exposure levels similar to those detected in children today.

Summary, Hypothesis, and Specific Aims of Thesis

Despite the success of public health policy to eliminate the presence of Pb^{2+} in the environment, low-level Pb^{2+} exposure continues to be a significant public health problem, especially for low-income and minority populations in urban areas. These are the same populations that have a higher risk for drug addiction. It is increasingly evident that elevated BLL may be a risk factor for drug addiction in later stages of life. Even so, the neurobiology underlying this increased risk is not yet fully understood. Work by our laboratory has demonstrated that Pb^{2+} exposure increases sensitization to the psychostimulant effects of cocaine in a rodent model for Pb^{2+} intoxication. This effect was eliminated following administration of

DA receptor antagonists, primarily D1R, suggesting that the effect was mediated by the DAergic system (Stansfield, Ruby et al. 2015). However, the neurochemical modifications underlying the increased sensitization observed in Pb^{2+}-exposed animals have not yet been described in the same animal model.

The purpose of this dissertation was be to elucidate the neurobiology underlying the increased drug sensitization observed in Pb^{2+}-exposed animals. We also aimed to address several limitations in the current understanding of Pb^{2+}'s effects on the DAergic system. Our present knowledge of Pb^{2+} neurotoxicity relies heavily on single-sex studies and, as a result, may fail to recognize potential sex-dependent effects. To address this limitation, this thesis studied both male and female experimental animals. This thesis also enhanced current understanding of the effects of low-level Pb^{2+} exposure by employing exposure models with BLL close to the current CDC level of action (~5µg/dl) and models with BLL associated with moderate exposure (~ 22µg/dl) (Stansfield, Ruby et al. 2015). Furthermore, it investigated the effects of Pb^{2+} on the ontogeny of the DAergic system by assessing its effects at three stages of rat development; juvenile, adolescent, and young adult.

The overarching hypothesis is that developmental Pb^{2+} exposure alters DAergic system ontogeny in a manner that increases sensitization to the psychostimulant effects of cocaine. We further hypothesized that DAergic system function is enhanced through increased levels of DA and DA turnover in the STR. We also hypothesized that Pb^{2+} exposure may enhance DAergic signaling by altering DA receptor density in the STR, NAC, and the OT as these regions are sensitive to the psychostimulant effects of cocaine (Ikemoto 2002, Ikemoto 2003, Ikemoto and Witkin 2003, Ikemoto 2007). To test these hypotheses, we sought to determine if chronic exposure to environmentally relevant levels of Pb^{2+} (1) increases the DA levels and turnover in

the STR, and (2) affects D1R and D2R density in brain regions sensitive to the psychostimulant effects of cocaine (see Fig.1.1 for biomarkers to be analyzed).

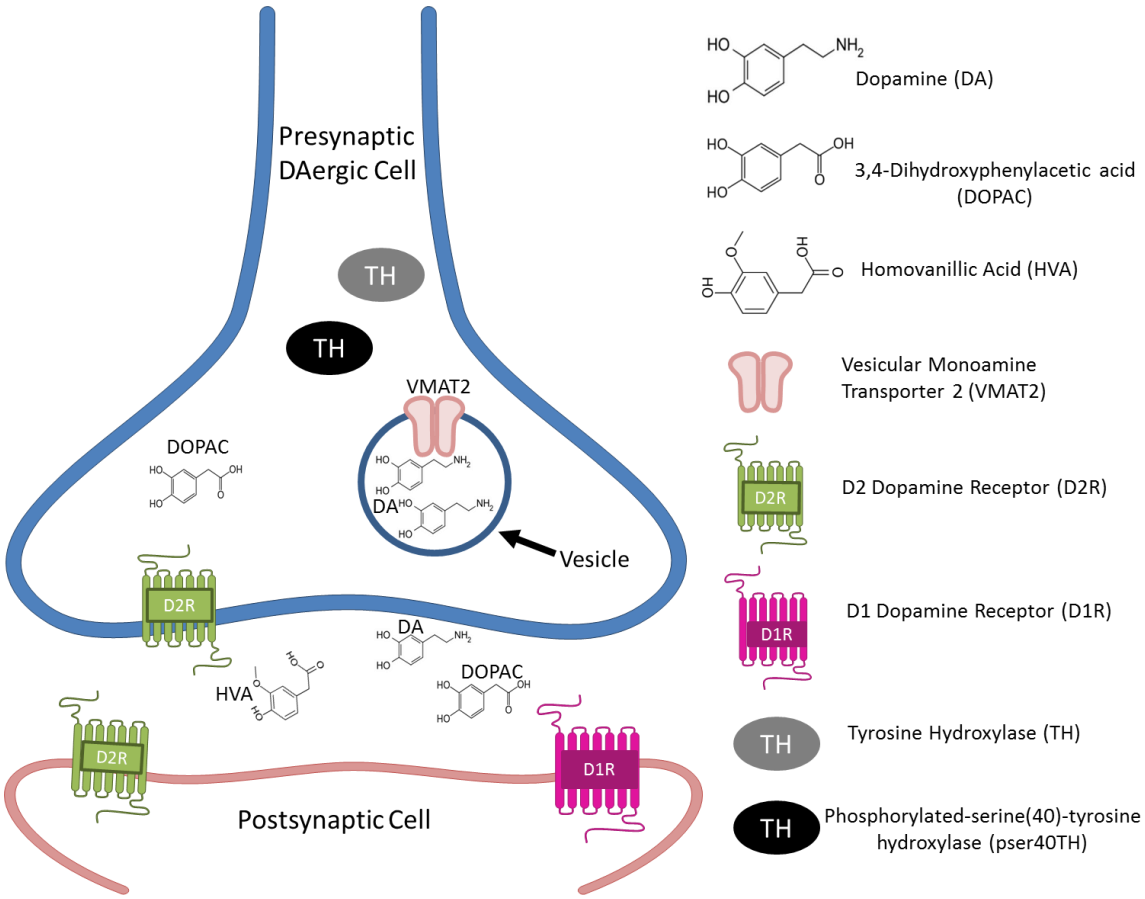


Figure 1.1: Dopaminergic system biomarkers examined in the current study

We measured DA and its metabolites DOPAC and HVA to determine DA availability and turnover in the STR. Additionally, we measured VMAT2 levels in the OT, NAC, and STR as this protein is essential in the transport of DA into vesicles in the presynaptic DAergic cell terminal. D1R and D2R levels were also measured in these regions. We measured TH levels as this enzyme is essential in the rate-limiting step of DA synthesis. We also measured pser40TH as a marker for TH activation as this enzyme is activated through phosphorylation.

References

Ahamed, M. and M. K. Siddiqui (2007). "Low level lead exposure and oxidative stress: current opinions." Clin Chim Acta **383**(1-2): 57-64.

AP, T. A. P. (2016). "Elevated Lead Levels Found in Newark Schools' Drinking Water." The New York Times.

Arora, M., C. Austin, B. Sarrafpour, M. Hernandez-Avila, H. Hu, R. O. Wright and M. M. Tellez-Rojo (2014). "Determining prenatal, early childhood and cumulative long-term lead exposure using micro-spatial deciduous dentine levels." PLoS One **9**(5): e97805.

Baler, R. D. and N. D. Volkow (2006). "Drug addiction: the neurobiology of disrupted self-control." Trends Mol Med **12**(12): 559-566.

Beaulieu, J. M. and R. R. Gainetdinov (2011). "The physiology, signaling, and pharmacology of dopamine receptors." Pharmacol Rev **63**(1): 182-217.

Beery, A. K. and I. Zucker (2011). "Sex bias in neuroscience and biomedical research." Neurosci Biobehav Rev **35**(3): 565-572.

Bellinger, D., A. Leviton and J. Sloman (1990). "Antecedents and correlates of improved cognitive performance in children exposed in utero to low levels of lead." Environ Health Perspect **89**: 5-11.

Bellinger, D. C. (2005). "Teratogen update: lead and pregnancy." Birth Defects Res A Clin Mol Teratol **73**(6): 409-420.

Bellinger, D. C. (2008). "Very low lead exposures and children's neurodevelopment." Curr Opin Pediatr **20**(2): 172-177.

Bjorklund, A. and S. B. Dunnett (2007). "Dopamine neuron systems in the brain: an update." Trends Neurosci **30**(5): 194-202.

Boeckx, R. L. (1986). "Lead poisoning in children." Anal Chem **58**(2): 274A-288A.

Bouchard, M. F., D. C. Bellinger, J. Weuve, J. Matthews-Bellinger, S. E. Gilman, R. O. Wright, J. Schwartz and M. G. Weisskopf (2009). "Blood lead levels and major depressive disorder, panic disorder, and generalized anxiety disorder in US young adults." Arch Gen Psychiatry **66**(12): 1313-1319.

Boucher, O., S. W. Jacobson, P. Plusquellec, E. Dewailly, P. Ayotte, N. Forget-Dubois, J. L. Jacobson and G. Muckle (2012). "Prenatal methylmercury, postnatal lead exposure, and evidence of attention deficit/hyperactivity disorder among Inuit children in Arctic Quebec." Environ Health Perspect **120**(10): 1456-1461.

Braun, J. M., R. S. Kahn, T. Froehlich, P. Auinger and B. P. Lanphear (2006). "Exposures to environmental toxicants and attention deficit hyperactivity disorder in U.S. children." Environ Health Perspect **114**(12): 1904-1909.

Brown, M. J. and S. Margolis (2012). "Lead in drinking water and human blood lead levels in the United States." MMWR Surveill Summ **61 Suppl**: 1-9.

Budd, P., J. Montgomery, J. Evans and M. Trickett (2004). "Human lead exposure in England from approximately 5500 BP to the 16th century AD." Sci Total Environ **318**(1-3): 45-58.

Byers, R. K. and E. E. Lord (1943). "Late effects of lead poisoning on mental development." American Journal of Diseases of Children **66**(5): 471-494.

Canfield, R. L., C. R. Henderson, Jr., D. A. Cory-Slechta, C. Cox, T. A. Jusko and B. P. Lanphear (2003). "Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter." N Engl J Med **348**(16): 1517-1526.

Canfield, R. L., D. A. Kreher, C. Cornwell and C. R. Henderson, Jr. (2003). "Low-level lead exposure, executive functioning, and learning in early childhood." Child Neuropsychol **9**(1): 35-53.

CDC, Centers for Disease Control and Prevention (2004). " Preventing Lead Exposure in Young Children: A Housing-Based Approach to Primary Prevention of Lead Poisoning.".

CDC, Centers for Disease Control and Prevention (2012, 7 Jun 2012). "CDC Response to Advisory Committee on Childhood Lead Poisoning Prevention Recommendations in "Low Level Lead Exposure Harms Children: A Renewed Call for Primary Prevention."." Retrieved 12/10/15, from http://www.cdc.gov/nceh/lead/ACCLPP/Final_Document_030712.pdf

CDC, Centers for Disease Control and Prevention (2013). "Blood Lead Levels in Children Aged 1–5 Years — United States, 1999–2010." Morbidity and Mortality Weekly Report **62**(13): 245-248.

CDC, Centers for Disease Control and Prevention (2015). "Childhood Blood Lead Levels — United States, 2007–2012." Morbidity and Mortality Weekly Report **62**(54): 76-80.

CDC, Centers for Disease Control and Prevention (2015, December 8, 2015). "Lead." Retrieved December 15, 2015, from http://www.cdc.gov/nceh/lead/.

Chandran, L. and R. Cataldo (2010). "Lead poisoning: basics and new developments." Pediatr Rev **31**(10): 399-405; quiz 406.

Chen, B. T., F. W. Hopf and A. Bonci (2010). "Synaptic plasticity in the mesolimbic system." Annals of the New York Academy of Sciences **1187**(1): 129-139.

Chiodo, L. M., S. W. Jacobson and J. L. Jacobson (2004). "Neurodevelopmental effects of postnatal lead exposure at very low levels." Neurotoxicol Teratol **26**(3): 359-371.

Clark, H. F., D. J. Brabander and R. M. Erdil (2006). "Sources, sinks, and exposure pathways of lead in urban garden soil." J Environ Qual **35**(6): 2066-2074.

Cory-Slechta, D. A. (1988). "Mobilization of lead over the course of DMSA chelation therapy and long-term efficacy." J Pharmacol Exp Ther **246**(1): 84-91.

Cory-Slechta, D. A. (1995). "Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter system functions." Annu Rev Pharmacol Toxicol **35**: 391-415.

Cory-Slechta, D. A., M. B. Virgolini, M. Thiruchelvam, D. D. Weston and M. R. Bauter (2004). "Maternal stress modulates the effects of developmental lead exposure." Environ Health Perspect **112**(6): 717-730.

David, O., J. Clark and K. Voeller (1972). "Lead and hyperactivity." Lancet **2**(7783): 900-903.

Davidson CI, R. M. (1992). Lead in the environment: from sources to human receptors. Human lead exposure. N. H. Boca Raton, FL, CRC Press.

de Souza Lisboa, S. F., G. Goncalves, F. Komatsu, C. A. Queiroz, A. A. Almeida and E. G. Moreira (2005). "Developmental lead exposure induces depressive-like behavior in female rats." Drug Chem Toxicol **28**(1): 67-77.

Dietrich, K. N., M. D. Ris, P. A. Succop, O. G. Berger and R. L. Bornschein (2001). "Early exposure to lead and juvenile delinquency." Neurotoxicol Teratol **23**(6): 511-518.

Dietrich, K. N., J. H. Ware, M. Salganik, J. Radcliffe, W. J. Rogan, G. G. Rhoads, M. E. Fay, C. T. Davoli, M. B. Denckla, R. L. Bornschein, D. Schwarz, D. W. Dockery, S. Adubato, R. L. Jones and G. Treatment of Lead-Exposed Children Clinical Trial (2004). "Effect of chelation therapy on the neuropsychological and behavioral development of lead-exposed children after school entry." Pediatrics **114**(1): 19-26.

Dooyema, C. A., A. Neri, Y. C. Lo, J. Durant, P. I. Dargan, T. Swarthout, O. Biya, S. O. Gidado, S. Haladu, N. Sani-Gwarzo, P. M. Nguku, H. Akpan, S. Idris, A. M. Bashir and M. J. Brown (2012). "Outbreak of fatal childhood lead poisoning related to artisanal gold mining in northwestern Nigeria, 2010." Environ Health Perspect **120**(4): 601-607.

Ensminger, M. E., J. C. Anthony and J. McCord (1997). "The inner city and drug use: initial findings from an epidemiological study." Drug Alcohol Depend **48**(3): 175-184.

Ersche, K. D., P. S. Jones, G. B. Williams, A. J. Turton, T. W. Robbins and E. T. Bullmore (2012). "Abnormal brain structure implicated in stimulant drug addiction." Science **335**(6068): 601-604.

Factor-Litvak, P., G. Wasserman, J. K. Kline and J. Graziano (1999). "The Yugoslavia Prospective Study of environmental lead exposure." Environ Health Perspect **107**(1): 9-15.

Fishbein, D. H., A. C. Todd, E. P. Ricketts and R. D. Semba (2008). "Relationship between lead exposure, cognitive function, and drug addiction: pilot study and research agenda." Environ Res **108**(3): 315-319.

Gedeon, Y., G. T. Ramesh, P. J. Wellman and A. L. Jadhav (2001). "Changes in mesocorticolimbic dopamine and D1/D2 receptor levels after low level lead exposure: a time course study." Toxicol Lett **123**(2-3): 217-226.

Goyer, R. A. (1990). "Transplacental transport of lead." Environ Health Perspect **89**: 101-105.

Graziano, J. H., D. Popovac, P. Factor-Litvak, P. Shrout, J. Kline, M. J. Murphy, Y. H. Zhao, A. Mehmeti, X. Ahmedi, B. Rajovic and et al. (1990). "Determinants of elevated blood lead during pregnancy in a population surrounding a lead smelter in Kosovo, Yugoslavia." Environ Health Perspect **89**: 95-100.

Gruber, A. J. and R. J. McDonald (2012). "Context, emotion, and the strategic pursuit of goals: interactions among multiple brain systems controlling motivated behavior." Front Behav Neurosci **6**: 50.

Guilarte, T. R. and J. L. McGlothan (1998). "Hippocampal NMDA receptor mRNA undergoes subunit specific changes during developmental lead exposure." Brain Res **790**(1-2): 98-107.

Guilarte, T. R., M. Opler and M. Pletnikov (2012). "Is lead exposure in early life an environmental risk factor for Schizophrenia? Neurobiological connections and testable hypotheses." Neurotoxicology **33**(3): 560-574.

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

Hanna-Attisha, M. and B. M. Kuehn (2016). "Pediatrician Sees Long Road Ahead for Flint After Lead Poisoning Crisis." JAMA **315**(10): 967-969.

Hanna-Attisha, M., J. LaChance, R. C. Sadler and A. Champney Schnepp (2016). "Elevated Blood Lead Levels in Children Associated With the Flint Drinking Water Crisis: A Spatial Analysis of Risk and Public Health Response." Am J Public Health **106**(2): 283-290.

Hansen, O. N., A. Trillingsgaard, I. Beese, T. Lyngbye and P. Grandjean (1989). "A neuropsychological study of children with elevated dentine lead level: assessment of the effect of lead in different socio-economic groups." Neurotoxicol Teratol **11**(3): 205-213.

Harsing Jr, L. (2008). Dopamine and the dopaminergic systems of the brain. Handbook of Neurochemistry and Molecular Neurobiology, Springer**:** 149-170.

Hernberg, S. (2000). "Lead poisoning in a historical perspective." Am J Ind Med **38**(3): 244-254.

Hong, S. B., M. H. Im, J. W. Kim, E. J. Park, M. S. Shin, B. N. Kim, H. J. Yoo, I. H. Cho, S. Y. Bhang, Y. C. Hong and S. C. Cho (2015). "Environmental lead exposure and attention

deficit/hyperactivity disorder symptom domains in a community sample of South korean school-age children." Environ Health Perspect **123**(3): 271-276.

Hu, H., J. Scheidell, X. Xu, A. M. Coatsworth and M. R. Khan (2014). "Associations between blood lead level and substance use and sexually transmitted infection risk among adults in the United States." Environ Res **135**: 21-30.

Hubbs-Tait, L., J. R. Nation, N. F. Krebs and D. C. Bellinger (2005). "Neurotoxicants, Micronutrients, and Social Environments: Individual and Combined Effects on Children's Development." Psychol Sci Public Interest **6**(3): 57-121.

Ikemoto, S. (2002). "Ventral striatal anatomy of locomotor activity induced by cocaine, D-amphetamine, dopamine and D1/D2 agonists." Neuroscience **113**(4): 939-955.

Ikemoto, S. (2003). "Involvement of the olfactory tubercle in cocaine reward: intracranial self-administration studies." J Neurosci **23**(28): 9305-9311.

Ikemoto, S. (2007). "Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex." Brain Res Rev **56**(1): 27-78.

Ikemoto, S. and B. M. Witkin (2003). "Locomotor inhibition induced by procaine injections into the nucleus accumbens core, but not the medial ventral striatum: implication for cocaine-induced locomotion." Synapse **47**(2): 117-122.

Jadhav, A. L. and G. T. Ramesh (1997). "Pb-induced alterations in tyrosine hydroxylase activity in rat brain." Mol Cell Biochem **175**(1-2): 137-141.

Jedrychowski, W., F. Perera, J. Jankowski, D. Mrozek-Budzyn, E. Mroz, E. Flak, S. Edwards, A. Skarupa and I. Lisowska-Miszczyk (2009). "Gender specific differences in neurodevelopmental effects of prenatal exposure to very low-lead levels: the prospective cohort study in three-year olds." Early Hum Dev **85**(8): 503-510.

Jett, D. A., A. C. Kuhlmann, S. J. Farmer and T. R. Guilarte (1997). "Age-dependent effects of developmental lead exposure on performance in the Morris water maze." Pharmacol Biochem Behav **57**(1-2): 271-279.

Jones, D. C. and G. W. Miller (2008). "The effects of environmental neurotoxicants on the dopaminergic system: A possible role in drug addiction." Biochem Pharmacol **76**(5): 569-581.

Jones, R. L., D. M. Homa, P. A. Meyer, D. J. Brody, K. L. Caldwell, J. L. Pirkle and M. J. Brown (2009). "Trends in blood lead levels and blood lead testing among US children aged 1 to 5 years, 1988-2004." Pediatrics **123**(3): e376-385.

Jusko, T. A., C. R. Henderson, B. P. Lanphear, D. A. Cory-Slechta, P. J. Parsons and R. L. Canfield (2008). "Blood lead concentrations < 10 microg/dL and child intelligence at 6 years of age." Environ Health Perspect **116**(2): 243-248.

Kala, S. V. and A. L. Jadhav (1995). "Low level lead exposure decreases in vivo release of dopamine in the rat nucleus accumbens: a microdialysis study." J Neurochem **65**(4): 1631-1635.

Kazantzis, G. (1989). Lead: Ancient Metal — Modern Menace? Lead Exposure and Child Development. M. A. Smith, L. D. Grant and A. I. Sors, Springer Netherlands**:** 119-128.

Koob, G. F. and M. Le Moal (1997). "Drug abuse: hedonic homeostatic dysregulation." Science **278**(5335): 52-58.

Koob, G. F. and N. D. Volkow (2010). "Neurocircuitry of addiction." Neuropsychopharmacology **35**(1): 217-238.

Kordas, K. (2010). "Iron, Lead, and Children's Behavior and Cognition." Annu Rev Nutr **30**: 123-148.

Kumer, S. C. and K. E. Vrana (1996). "Intricate regulation of tyrosine hydroxylase activity and gene expression." J Neurochem **67**(2): 443-462.

Laidlaw, M. A. S. and G. M. Filippelli (2008). "Resuspension of urban soils as a persistent source of lead poisoning in children: A review and new directions." Applied Geochemistry **23**(8): 2021-2039.

Lanphear, B. P., D. A. Burgoon, S. W. Rust, S. Eberly and W. Galke (1998). "Environmental exposures to lead and urban children's blood lead levels." Environ Res **76**(2): 120-130.

Lanphear, B. P., R. Hornung, M. Ho, C. R. Howard, S. Eberly and K. Knauf (2002). "Environmental lead exposure during early childhood." J Pediatr **140**(1): 40-47.

Lanphear, B. P., R. Hornung, J. Khoury, K. Yolton, P. Baghurst, D. C. Bellinger, R. L. Canfield, K. N. Dietrich, R. Bornschein, T. Greene, S. J. Rothenberg, H. L. Needleman, L. Schnaas, G. Wasserman, J. Graziano and R. Roberts (2005). "Low-level environmental lead exposure and children's intellectual function: an international pooled analysis." Environ Health Perspect **113**(7): 894-899.

Lasley, S. M. (1992). "Regulation of dopaminergic activity, but not tyrosine hydroxylase, is diminished after chronic inorganic lead exposure." Neurotoxicology **13**(3): 625-635.

Lasley, S. M. and J. D. Lane (1988). "Diminished regulation of mesolimbic dopaminergic activity in rat after chronic inorganic lead exposure." Toxicol Appl Pharmacol **95**(3): 474-483.

Leret, M. L., F. Garcia-Uceda and M. T. Antonio (2002). "Effects of maternal lead administration on monoaminergic, GABAergic and glutamatergic systems." Brain Res Bull **58**(5): 469-473.

Levin, R., M. J. Brown, M. E. Kashtock, D. E. Jacobs, E. A. Whelan, J. Rodman, M. R. Schock, A. Padilla and T. Sinks (2008). "Lead exposures in U.S. Children, 2008: implications for prevention." Environ Health Perspect **116**(10): 1285-1293.

Lidsky, T. I. and J. S. Schneider (2003). "Lead neurotoxicity in children: basic mechanisms and clinical correlates." Brain **126**(Pt 1): 5-19.

Lin-Fu, J. S. (1973). "Vulnerability of children to lead exposure and toxicity (first of two parts)." N Engl J Med **289**(23): 1229-1233.

Lin-Fu, J. S. (1973). "Vulnerability of children to lead exposure and toxicity (second of two parts)." N Engl J Med **289**(24): 1289-1293.

Lindgren, N., A. Usiello, M. Goiny, J. Haycock, E. Erbs, P. Greengard, T. Hokfelt, E. Borrelli and G. Fisone (2003). "Distinct roles of dopamine D2L and D2S receptor isoforms in the regulation of protein phosphorylation at presynaptic and postsynaptic sites." Proc Natl Acad Sci U S A **100**(7): 4305-4309.

Ma, T., H. H. Chen and I. K. Ho (1999). "Effects of chronic lead (Pb) exposure on neurobehavioral function and dopaminergic neurotransmitter receptors in rats." Toxicol Lett **105**(2): 111-121.

Mahaffey, K. R. (1990). "Environmental lead toxicity: nutrition as a component of intervention." Environ Health Perspect **89**: 75-78.

Marchetti, C. (2003). "Molecular targets of lead in brain neurotoxicity." Neurotox Res **5**(3): 221-236.

Matte, T. D., P. J. Landrigan, E. L. Baker and H. Needleman (1992). "Occupational lead exposure." Human lead exposure: 155-168.

McFarlane, A. C., A. K. Searle, M. Van Hooff, P. A. Baghurst, M. G. Sawyer, C. Galletly, M. R. Sim and L. S. Clark (2013). "Prospective associations between childhood low-level lead exposure and adult mental health problems: the Port Pirie cohort study." Neurotoxicology **39**: 11-17.

Meredith, P. A., M. J. McIntosh, M. A. Petty and J. L. Reid (1988). "Effects of lead exposure on rat brain catecholaminergic neurochemistry." Comp Biochem Physiol C **89**(2): 215-219.

Meyer, P. A., M. J. Brown and H. Falk (2008). "Global approach to reducing lead exposure and poisoning." Mutat Res **659**(1-2): 166-175.

Mielke, H. (1999). "Lead in the Inner Cities Policies to reduce children's exposure to lead may be overlooking a major source of lead in the environment." American scientist **87**(1): 62-73.

Mielke, H. W. and S. Zahran (2012). "The urban rise and fall of air lead (Pb) and the latent surge and retreat of societal violence." Environ Int **43**: 48-55.

Mignot, E. and D. Laude (1985). "Study of dopamine turnover by monitoring the decline of dopamine metabolites in rat CSF after alpha-methyl-p-tyrosine." J Neurochem **45**(5): 1527-1533.

Miller, D. K., J. R. Nation and G. R. Bratton (2000). "Perinatal exposure to lead attenuates the conditioned reinforcing properties of cocaine in male rats." Pharmacol Biochem Behav **67**(1): 111-119.

Miller, D. K., J. R. Nation and G. R. Bratton (2001). "The effects of perinatal exposure to lead on the discriminative stimulus properties of cocaine and related drugs in rats." Psychopharmacology (Berl) **158**(2): 165-174.

Miranda, M. L., D. Kim, M. A. Galeano, C. J. Paul, A. P. Hull and S. P. Morgan (2007). "The relationship between early childhood blood lead levels and performance on end-of-grade tests." Environ Health Perspect **115**(8): 1242-1247.

Moncrieff, A. A., O. P. Koumides, B. E. Clayton, A. D. Patrick, A. G. Renwick and G. E. Roberts (1964). "Lead Poisoning in Children." Arch Dis Child **39**: 1-13.

Moreira, E. G., I. Vassilieff and V. S. Vassilieff (2001). "Developmental lead exposure: behavioral alterations in the short and long term." Neurotoxicol Teratol **23**(5): 489-495.

Moresco, R. M., R. Dall'Olio, O. Gandolfi, S. Govoni, S. Di Giovine and M. Trabucchi (1988). "Lead neurotoxicity: a role for dopamine receptors." Toxicology **53**(2-3): 315-322.

Morris, R. G., E. Anderson, G. S. Lynch and M. Baudry (1986). "Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5." Nature **319**(6056): 774-776.

Muntner, P., A. Menke, K. B. DeSalvo, F. A. Rabito and V. Batuman (2005). "Continued decline in blood lead levels among adults in the United States: the National Health and Nutrition Examination Surveys." Arch Intern Med **165**(18): 2155-2161.

Nation, J. R., A. L. Cardon, H. M. Heard, R. Valles and G. R. Bratton (2003). "Perinatal lead exposure and relapse to drug-seeking behavior in the rat: a cocaine reinstatement study." Psychopharmacology (Berl) **168**(1-2): 236-243.

Nation, J. R., G. D. Frye, J. Von Stultz and G. R. Bratton (1989). "Effects of combined lead and cadmium exposure: changes in schedule-controlled responding and in dopamine, serotonin, and their metabolites." Behav Neurosci **103**(5): 1108-1114.

Nation, J. R., D. K. Miller and G. R. Bratton (2000). "Developmental lead exposure alters the stimulatory properties of cocaine at PND 30 and PND 90 in the rat." Neuropsychopharmacology **23**(4): 444-454.

Nation, J. R., K. R. Smith and G. R. Bratton (2004). "Early developmental lead exposure increases sensitivity to cocaine in a self-administration paradigm." Pharmacol Biochem Behav **77**(1): 127-135.

Neal, A. P., K. H. Stansfield, P. F. Worley, R. E. Thompson and T. R. Guilarte (2010). "Lead exposure during synaptogenesis alters vesicular proteins and impairs vesicular release: potential role of NMDA receptor-dependent BDNF signaling." Toxicol Sci **116**(1): 249-263.

Needleman, H. (2004). "Lead poisoning." Annu Rev Med **55**: 209-222.

Needleman, H. (2009). "Low level lead exposure: history and discovery." Ann Epidemiol **19**(4): 235-238.

Needleman, H. L. (1999). History of lead poisoning in the world. International Conference on lead Poisoning Prevention and Treatment, Bangalore.

Needleman, H. L. (2000). "The removal of lead from gasoline: historical and personal reflections." Environ Res **84**(1): 20-35.

Needleman, H. L. and C. A. Gatsonis (1990). "Low-level lead exposure and the IQ of children. A meta-analysis of modern studies." JAMA **263**(5): 673-678.

Needleman, H. L., C. Gunnoe, A. Leviton, R. Reed, H. Peresie, C. Maher and P. Barrett (1979). "Deficits in psychologic and classroom performance of children with elevated dentine lead levels." N Engl J Med **300**(13): 689-695.

Needleman, H. L., C. McFarland, R. B. Ness, S. E. Fienberg and M. J. Tobin (2002). "Bone lead levels in adjudicated delinquents. A case control study." Neurotoxicol Teratol **24**(6): 711-717.

Needleman, H. L., A. Schell, D. Bellinger, A. Leviton and E. N. Allred (1990). "The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report." N Engl J Med **322**(2): 83-88.

Nelson, E. J., E. Shacham, B. B. Boutwell, R. Rosenfeld, M. Schootman, M. Vaughn and R. Lewis (2015). "Childhood lead exposure and sexually transmitted infections: New evidence." Environ Res **143**(Pt A): 131-137.

Nevin, R. (2000). "How lead exposure relates to temporal changes in IQ, violent crime, and unwed pregnancy." Environ Res **83**(1): 1-22.

Nevin, R. (2007). "Understanding international crime trends: the legacy of preschool lead exposure." Environ Res **104**(3): 315-336.

Nigg, J. T., G. M. Knottnerus, M. M. Martel, M. Nikolas, K. Cavanagh, W. Karmaus and M. D. Rappley (2008). "Low blood lead levels associated with clinically diagnosed attention-deficit/hyperactivity disorder and mediated by weak cognitive control." Biol Psychiatry **63**(3): 325-331.

Nihei, M. K., N. L. Desmond, J. L. McGlothan, A. C. Kuhlmann and T. R. Guilarte (2000). "N-methyl-D-aspartate receptor subunit changes are associated with lead-induced deficits of long-term potentiation and spatial learning." Neuroscience **99**(2): 233-242.

NIOSH, T. N. I. f. O. S. a. H. (2013, September 30, 2013). "Lead Exposure Limits." Retrieved December 15, 2015, from http://www.cdc.gov/niosh/topics/lead/limits.html.

NourEddine, D., S. Miloud and A. Abdelkader (2005). "Effect of lead exposure on dopaminergic transmission in the rat brain." Toxicology **207**(3): 363-368.

Nriagu, J., B. Burt, A. Linder, A. Ismail and W. Sohn (2006). "Lead levels in blood and saliva in a low-income population of Detroit, Michigan." Int J Hyg Environ Health **209**(2): 109-121.

Nriagu, J., R. Senthamarai-Kannan, H. Jamil, M. Fakhori and S. Korponic (2011). "Lead poisoning among Arab American and African American children in the Detroit metropolitan area, Michigan." Bull Environ Contam Toxicol **87**(3): 238-244.

Nriagu, J. O. (1992). Saturnine drugs and medicinal exposure to lead: an historical outline. Human Lead Exposure H. L. Needleman. Boca Raton, Florida, CRC Press**:** 1- 21.

Olympio, K. P., C. Goncalves, W. M. Gunther and E. J. Bechara (2009). "Neurotoxicity and aggressiveness triggered by low-level lead in children: a review." Rev Panam Salud Publica **26**(3): 266-275.

Opler, M. G., A. S. Brown, J. Graziano, M. Desai, W. Zheng, C. Schaefer, P. Factor-Litvak and E. S. Susser (2004). "Prenatal lead exposure, delta-aminolevulinic acid, and schizophrenia." Environ Health Perspect **112**(5): 548-552.

Opler, M. G., S. L. Buka, J. Groeger, I. McKeague, C. Wei, P. Factor-Litvak, M. Bresnahan, J. Graziano, J. M. Goldstein, L. J. Seidman, A. S. Brown and E. S. Susser (2008). "Prenatal exposure to lead, delta-aminolevulinic acid, and schizophrenia: further evidence." Environ Health Perspect **116**(11): 1586-1590.

OSHA, O. S. H. A. (, accessed 2015). Safety and Health Topics: Lead.

Papanikolaou, N. C., E. G. Hatzidaki, S. Belivanis, G. N. Tzanakakis and A. M. Tsatsakis (2005). "Lead toxicity update. A brief review." Med Sci Monit **11**(10): RA329-336.

Pokora, M. J., E. K. Richfield and D. A. Cory-Slechta (1996). "Preferential vulnerability of nucleus accumbens dopamine binding sites to low-level lead exposure: time course of effects and interactions with chronic dopamine agonist treatments." J Neurochem **67**(4): 1540-1550.

Pruvot, C., F. Douay, F. Hervé and C. Waterlot (2006). "Heavy Metals in Soil, Crops and Grass as a Source of Human Exposure in the Former Mining Areas (6 pp)." Journal of Soils and Sediments **6**(4): 215-220.

Pugh Smith, P. and J. O. Nriagu (2011). "Lead poisoning and asthma among low-income and African American children in Saginaw, Michigan." Environ Res **111**(1): 81-86.

Rabinowitz, M. B. (1991). "Toxicokinetics of bone lead." Environ Health Perspect **91**: 33-37.

Ramesh, G. T. and A. L. Jadhav (1998). "Region-specific alterations in tyrosine hydroxylase activity in rats exposed to lead." Mol Cell Biochem **189**(1-2): 19-24.

Reyes, J. W. (2015). "Lead exposure and behavior: Effects on antisocial and risky behavior among children and adolescents." Economic Inquiry **53**(3): 1580-1605.

Rogan, W. J., K. N. Dietrich, J. H. Ware, D. W. Dockery, M. Salganik, J. Radcliffe, R. L. Jones, N. B. Ragan, J. J. Chisolm, Jr., G. G. Rhoads and G. Treatment of Lead-Exposed Children Trial (2001). "The effect of chelation therapy with succimer on neuropsychological development in children exposed to lead." N Engl J Med **344**(19): 1421-1426.

Roy, A., D. Bellinger, H. Hu, J. Schwartz, A. S. Ettinger, R. O. Wright, M. Bouchard, K. Palaniappan and K. Balakrishnan (2009). "Lead exposure and behavior among young children in Chennai, India." Environ Health Perspect **117**(10): 1607-1611.

Sakai, T. (2000). "Biomarkers of lead exposure." Ind Health **38**(2): 127-142.

Sanders, T., Y. Liu, V. Buchner and P. B. Tchounwou (2009). "Neurotoxic effects and biomarkers of lead exposure: a review." Rev Environ Health **24**(1): 15-45.

Santora, M. a. D. W. C. (2016). "Newark School Officials Knew of Lead Risks, 2014 Memo Shows." The New York Times.

Sayre, J. W., E. Charney, J. Vostal and I. B. Pless (1974). "House and hand dust as a potential source of childhood lead exposure." Am J Dis Child **127**(2): 167-170.

Sciarillo, W. G., G. Alexander and K. P. Farrell (1992). "Lead exposure and child behavior." Am J Public Health **82**(10): 1356-1360.

Stansfield, K. H., J. R. Pilsner, Q. Lu, R. O. Wright and T. R. Guilarte (2012). "Dysregulation of BDNF-TrkB signaling in developing hippocampal neurons by Pb(2+): implications for an environmental basis of neurodevelopmental disorders." Toxicol Sci **127**(1): 277-295.

Stansfield, K. H., K. N. Ruby, B. D. Soares, J. L. McGlothan, X. Liu and T. R. Guilarte (2015). "Early-life lead exposure recapitulates the selective loss of parvalbumin-positive GABAergic interneurons and subcortical dopamine system hyperactivity present in schizophrenia." Transl Psychiatry **5**: e522.

Sthiannopkao, S. and M. H. Wong (2013). "Handling e-waste in developed and developing countries: initiatives, practices, and consequences." Sci Total Environ **463-464**: 1147-1153.

Sun, X., X. Tian, J. L. Tomsig and J. B. Suszkiw (1999). "Analysis of differential effects of Pb 2+ on protein kinase C isozymes." Toxicology and applied pharmacology **156**(1): 40-45.

Surkan, P. J., A. Zhang, F. Trachtenberg, D. B. Daniel, S. McKinlay and D. C. Bellinger (2007). "Neuropsychological function in children with blood lead levels <10 microg/dL." Neurotoxicology **28**(6): 1170-1177.

Taylor, M. P., C. A. Schniering, B. P. Lanphear and A. L. Jones (2011). "Lessons learned on lead poisoning in children: one-hundred years on from Turner's declaration." J Paediatr Child Health **47**(12): 849-856.

Tian, X., X. Sun and J. B. Suszkiw (2000). "Upregulation of tyrosine hydroxylase and downregulation of choline acetyltransferase in lead-exposed PC12 cells: the role of PKC activation." Toxicol Appl Pharmacol **167**(3): 246-252.

Tong, S., P. Baghurst, A. McMichael, M. Sawyer and J. Mudge (1996). "Lifetime exposure to environmental lead and children's intelligence at 11-13 years: the Port Pirie cohort study." BMJ **312**(7046): 1569-1575.

Tong, S., Y. E. von Schirnding and T. Prapamontol (2000). "Environmental lead exposure: a public health problem of global dimensions." Bull World Health Organ **78**(9): 1068-1077.

Toscano, C. D. and T. R. Guilarte (2005). "Lead neurotoxicity: from exposure to molecular effects." Brain Res Brain Res Rev **49**(3): 529-554.

Toscano, C. D., J. L. McGlothan and T. R. Guilarte (2003). "Lead exposure alters cyclic-AMP response element binding protein phosphorylation and binding activity in the developing rat brain." Brain Res Dev Brain Res **145**(2): 219-228.

Tran, A. H., R. Tamura, T. Uwano, T. Kobayashi, M. Katsuki and T. Ono (2005). "Dopamine D1 receptors involved in locomotor activity and accumbens neural responses to prediction of reward associated with place." Proc Natl Acad Sci U S A **102**(6): 2117-2122.

Triantafyllidou, S., C. K. Nguyen, Y. Zhang and M. A. Edwards (2013). "Lead (Pb) quantification in potable water samples: implications for regulatory compliance and assessment of human exposure." Environ Monit Assess **185**(2): 1355-1365.

Tsaih, S. W., S. Korrick, J. Schwartz, M. L. Lee, C. Amarasiriwardena, A. Aro, D. Sparrow and H. Hu (2001). "Influence of bone resorption on the mobilization of lead from bone among middle-aged and elderly men: the Normative Aging Study." Environ Health Perspect **109**(10): 995-999.

Turner, A. J. (1909). "On lead poisoning in childhood." British medical journal **1**(2519): 895.

Vallone, D., R. Picetti and E. Borrelli (2000). "Structure and function of dopamine receptors." Neurosci Biobehav Rev **24**(1): 125-132.

Verstraeten, S. V., L. Aimo and P. I. Oteiza (2008). "Aluminium and lead: molecular mechanisms of brain toxicity." Arch Toxicol **82**(11): 789-802.

Volkow, N. D., G. F. Koob and A. T. McLellan (2016). "Neurobiologic Advances from the Brain Disease Model of Addiction." N Engl J Med **374**(4): 363-371.

Volkow, N. D. and T. K. Li (2004). "Drug addiction: the neurobiology of behaviour gone awry." Nat Rev Neurosci **5**(12): 963-970.

Volkow, N. D., G. J. Wang, J. S. Fowler, D. Tomasi and F. Telang (2011). "Addiction: beyond dopamine reward circuitry." Proc Natl Acad Sci U S A **108**(37): 15037-15042.

Volkow, N. D. and R. A. Wise (2005). "How can drug addiction help us understand obesity?" Nat Neurosci **8**(5): 555-560.

Vyas, S., N. Faucon Biguet and J. Mallet (1990). "Transcriptional and post-transcriptional regulation of tyrosine hydroxylase gene by protein kinase C." EMBO J **9**(11): 3707-3712.

Waldron, H. A. (1973). "Lead poisoning in the ancient world." Med Hist **17**(4): 391-399.

Wang, Q., W. Luo, W. Zheng, Y. Liu, H. Xu, G. Zheng, Z. Dai, W. Zhang, Y. Chen and J. Chen (2007). "Iron supplement prevents lead-induced disruption of the blood-brain barrier during rat development." Toxicol Appl Pharmacol **219**(1): 33-41.

Wasserman, G., J. H. Graziano, P. Factor-Litvak, D. Popovac, N. Morina, A. Musabegovic, N. Vrenezi, S. Capuni-Paracka, V. Lekic, E. Preteni-Redjepi and et al. (1992). "Independent effects of lead exposure and iron deficiency anemia on developmental outcome at age 2 years." J Pediatr **121**(5 Pt 1): 695-703.

White, L. D., D. A. Cory-Slechta, M. E. Gilbert, E. Tiffany-Castiglioni, N. H. Zawia, M. Virgolini, A. Rossi-George, S. M. Lasley, Y. C. Qian and M. R. Basha (2007). "New and evolving concepts in the neurotoxicology of lead." Toxicol Appl Pharmacol **225**(1): 1-27.

WHO, W. H. O. (2010). "Childhood lead poisoning."

Wright, J. P., K. N. Dietrich, M. D. Ris, R. W. Hornung, S. D. Wessel, B. P. Lanphear, M. Ho and M. N. Rae (2008). "Association of prenatal and childhood blood lead concentrations with criminal arrests in early adulthood." PLoS Med **5**(5): e101.

Zhou, F.-M., C. Wilson and J. A. Dani (2003). "Muscarinic and nicotinic cholinergic mechanisms in the mesostriatal dopamine systems." The Neuroscientist **9**(1): 23-36.

Zuch, C. L., D. J. O'Mara and D. A. Cory-Slechta (1998). "Low-level lead exposure selectively enhances dopamine overflow in nucleus accumbens: an in vivo electrochemistry time course assessment." Toxicol Appl Pharmacol **150**(1): 174-185.

CHAPTER 2: Chronic Pb^{2+} Exposure Alters Dopamine Levels and Turnover in the Dorsal Striatum

Abstract

Previous studies have demonstrated that lead (Pb^{2+}) exposure alters striatal dopamine (DA) levels and turnover (Nation, Frye et al. 1989, Cory-Slechta 1995). However, there is disagreement regarding the magnitude and direction of Pb^{2+} effects on DA levels and turnover (Gedeon, Ramesh et al. 2001). Also, age and dose have been reported to impact the effects of Pb^{2+} on dopaminergic (DAergic) transmission (Devi, Reddy et al. 2005, Jones and Miller 2008). Most studies have measured the effects of Pb^{2+} in only one sex (typically male), one exposure (usually high level exposure), and one or two time-points, making it difficult to determine any potential sex-, age-, and exposure-dependent effects. In the present study, we measured DA levels and turnover in the dorsal striatum (STR) of juvenile (PN14), adolescent (PN28), and young adult (PN50) male and female rats following chronic exposure to diets containing either 180ppm Pb^{2+} acetate (PbAC) diet or 1500ppm PbAc diet. As tyrosine hydroxylase (TH) is the rate-limiting enzyme in DA synthesis, we measured TH and phosphorylated-serine (40)-tyrosine hydroxylase (pser40TH) levels to determine if altered levels of DA, DA metabolites, and DA turnover are due to Pb^{2+}-induced changes in TH protein expression level and/or activation. Vesicular monoamine transporter 2 (VMAT2) levels were also measured as this transporter is critical for the packaging of free intracellular DA into synaptic vesicles.

We observed that chronic Pb^{2+} exposure significantly increased DA, and the DA metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA); as well as DA turnover in Pb^{2+}-exposed male rats with BLL as low as 6.8µg/dl at PN28 and 4.4µg/dl at PN50, as compared to rats on control with BLL of 0.4µg/dl and 0.6µg/dl at PN28 and PN50, respectively. No changes in VMAT2, TH, or pser40TH were detected in these animals. No changes in DA, DA metabolites, or DA turnover were detected in the STR of exposed female

rats at any age. However, VMAT2 levels were significantly decreased in PN14 females on 180ppm PbAC diet and increased in PN28 females on 180ppm and 1500ppm PbAC diets. Significant increases in pser40TH levels were detected in PN50 females on 1500ppm diet. The present study demonstrates that chronic Pb^{2+} exposure results in a hyperactive DAergic state in males with blood Pb^{2+} levels as low as 4.4µg/dl, but not in females. Furthermore, our results suggest that Pb^{2+} alters the DAergic system in a time-, sex-, and exposure-dependent manner.

Introduction

In a recent study, our laboratory observed increased locomotor activity induced by cocaine administration in male rats with BLL of 22µg/dl versus unexposed control rats (Stansfield, Ruby et al. 2015). Administration of a D1R antagonist (SCH23390) prior to cocaine injection resulted in a block of cocaine-induced locomotor activity, whereas administration of a D2R antagonist (Raclopride) resulted in a partial block of this effect (Stansfield, Ruby et al. 2015). Further work by our laboratory found that male rats with BLLs as low as 4.5µg/dl and female rats with BLLs as low as 4.2µg/dl exhibited increased locomotor activity following a 5mg/kg (low dose) injection of cocaine. Importantly, no response was detected in control rats following injection of the low dose of cocaine, suggesting that Pb^{2+}-exposed rats at even this low level of exposure are sensitized to cocaine-induced locomotor activation. A 15mg/kg (high dose) cocaine injection, however, resulted in similar levels of cocaine-induced locomotor activity between animals with BLLs of 4.5µg/dl and control animals (Stansfield KH 2015). These results suggested a hyperactive DAergic state underlying the increased sensitization to cocaine's psychostimulant effects observed in rats with BLLs lower than the current CDC level of action (CDC 2012).

DAergic systems play a critical role in a number of central nervous system processes, including drug addiction (Volkow and Li 2005, Baler and Volkow 2006, Volkow, Wang et al. 2011, Volkow, Wang et al. 2011a, Volkow, Koob et al. 2016). There is evidence to suggest that environmental Pb^{2+} exposure plays a role in drug addiction by modulating DAergic system activity (Jones and Miller 2008). However, there is still disagreement regarding the magnitude and direction of the effects of Pb^{2+} on DAergic transmissions with studies variously reporting hyperactive (Nation, Frye et al. 1989, Szczerbak, Nowak et al. 2007), and hypoactive DAergic states (Lasley, Greenland et al. 1984, Pokora, Richfield et al. 1996, Antonio and Leret 2000), or no change (Leret, Garcia-Uceda et al. 2002, Nowak, Szczerbak et al. 2008). Region-, dose-, and age-dependent differences on DA levels and turnover have also been described (Antonio and Leret 2000, Gedeon, Ramesh et al. 2001, Leret, Garcia-Uceda et al. 2002, Devi, Reddy et al. 2005).

The conflicting findings in these studies may be due in part to differences in methodology, endpoints measured, and differences in exposure paradigms employed. The blood Pb^{2+} levels (BLL) of exposed animals vary across studies, with some studies assessing the impact of Pb^{2+} exposure on models with BLL of $\leq$10µg/dl (Gedeon, Ramesh et al. 2001, Nation, Cardon et al. 2003), 11-20µg/dl (Kala and Jadhav 1995, Ramesh and Jadhav 1998, Zuch, O'Mara et al. 1998, Nation, Smith et al. 2004), 21-30µg/dl (Zuch, O'Mara et al. 1998, Leret, Garcia-Uceda et al. 2002, Stansfield, Ruby et al. 2015), 31-40µg/dl (Ma, Chen et al. 1999, Nation, Cardon et al. 2003), 41-50µg/dl (Cory-Slechta, Virgolini et al. 2004), or even $\geq$51µg/dl (Nation, Baker et al. 1986, Nation, Frye et al. 1989). Furthermore, given the different endpoints measured, as well as methodologies used, it is difficult to generalize findings across studies. Lastly, most published studies investigating the impact of Pb^{2+} on the brain have employed only

male animals, leaving a huge gap in our knowledge of Pb^{2+} effects on the female brain (Beery and Zucker 2011).

The present study seeks to address these limitations by analyzing separately the effects of Pb^{2+} in juvenile (postnatal day 14, PN14), adolescent (PN28), and young adult (PN50) male and female rats following chronic exposure to 180ppm Pb^{2+}-acetate (PbAC) or 1500ppm PbAC. The exposure paradigm employed in this study resulted in BLL as low as 4.4µg/dl at PN50 after chronic exposure to 180ppm PbAC diet, just below the current CDC level of action of 5µg/dl, and as high as 36µg/dl at PN14 after chronic exposure to 1500ppm PbAC **(Table 2.1A-C)** (CDC 2012). These BLLs are relevant to those detected in children of the United States (CDC 2015, Stansfield, Ruby et al. 2015, CDC 2016). We measured DA levels, DA metabolite levels, and DA turnover in the dorsal striatum (STR) using HPLC as the STR is considered an ideal system for analysis of the DAergic transmission (Lindgren, Usiello et al. 2003). We also used Western blotting techniques to measure tyrosine hydroxylase (TH) levels and phosphorylated-serine (40)-tyrosine hydroxylase (pser40TH) in the STR to determine if Pb^{2+} alters expression and activation of the rate-limiting enzyme in DA synthesis (Lindgren, Usiello et al. 2003). VMAT2 levels were measured using quantitative autoradiography, as this transporter plays a critical role in the sequestration of DA into vesicles, thereby regulating intracellular DA levels (Duchemin, Zhang et al. 2009, Guillot and Miller 2009). VMAT2 is also resistant to changes in DA levels at the synapse and can be used as a marker for DAergic terminal integrity (Wilson and Kish 1996, Guilarte, Nihei et al. 2003).

Materials and Methods

Animals

All animal studies were approved by the Columbia University Medical Center Animal Care and Use Committee and were carried out in accordance with the Guide for Care and Use of Laboratory Animals of the U.S. National Institutes of Health. Long-Evans rats were purchased from Charles River, Inc. (Charles River, Bar Harbor, ME, USA) and fed 0-, 180-, or 1500ppm PbAC diet. PbAC diet was prepared by and purchased from Dyets (Dyets, Bethlehem, PA, USA). The diet was comprised of Purina RMH 1000 diet with PbAC incorporated into chow mix. Food and water were provided *ad libitum*. Dams were initiated on Pb^{2+} diet 10 days prior to mating with Long-Evans male rats, which were maintained on control diet at all times. Litters were culled to 10 pups per litter at postnatal day PN 1-2 and weaned on PN21. Upon weaning, rats were maintained on same diet as their respective mother. Rats were maintained on a 12-hour light-dark cycle until sacrificed at PN14, PN28, and PN50. For PN14 age group, all pups from a litter were euthanized on the same day.

Tissue Collection

For VMAT2 quantitative autoradiography, whole rat brains were harvested immediately after decapitation, snap frozen, and then stored at -80^{o}C until used. For HPLC and Western blots, rat brains were dissected immediately after decapitation. STR was dissected on ice and snap frozen. Brain tissue was then stored at -80^{o}C until used for analysis. No more than one male and one female experimental determination was used per litter for statistical purposes.

Blood Pb^{2+} levels

Rats were anesthetized with a 25mg/kg dose of pentobarbital. Blood was collected transcardially from rats for each age group. BLL were measured using a Magellan LeadCare analyzer using manufacturer's instructions (ESA Laboratories, Chelmsford, MA, USA). BLL were averaged between litters in each exposure group at each age and for each sex.

High Performance Liquid Chromatography

Fresh-frozen dissected STR tissue was processed and analyzed using methods similar to Sheleg et al. (2013) (Sheleg, Yochum et al. 2013). Briefly, striatal tissue was sonicated in 500ul of .1N perchloric acid. Sonicated samples were then centrifuged at 14,000rpm for 20 minutes at 4^{o}C. Pellets were dried overnight and supernatant was collected and filtered through 0.22um filters before injection into a high-performance liquid chromatography system with electrochemical detection (Waters, Milford, MA, USA) for neurochemical analysis of DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Components were separated on a CMD-150 cation exchange column (150 x 3.2mm, ESA Biosciences, Chelmsford, MA, USA) using isocratic mobile phase (MD-TM mobile phase, ESA Biosciences) containing 2.2mM NaCl pumped at a constant flow rate of 0.5mL/min. Compounds were quantified with electrochemical detection using a glassy carbon working electrode and 2.0mm diameter in situ silver reference electrode (Flow Cell, 2mm GC WE, ISAAC; Waters). Pellets were dried at 30^{o}C overnight and then dissolved in 100uL of 0.5N NaOH in a sonicating water bath until dissolved. 400uL of water was then added to samples, bringing the concentration of NaOH to 0.1N. Protein concentration was determined using a bicinchoninic acid assay reagent kit (Pierce, Rockford, IL, USA). Samples were read at 562nm using a SpectraMax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Protein Harvesting and Western Blot

STR from control and Pb^{2+}-exposed rats was lysed in radioimmunoprecipitation assay buffer containing 150mM NaCl, 20% SDS, 5mM EGTA, 50mM Tris, 1% Triton, and 5% deoxycholate. Two experiments were run per gel, each experiment containing the same set of samples run in duplicate. After protein transfer, the membrane was cut into two pieces between experiments and each experiment was probed with either TH or pser40TH. Membranes were incubated in the appropriate primary antibodies: 1:1000 TH (Millipore, AB318), 1:1000 pser40TH (Millipore, AB5935), and 1:1000 β-actin (Santa Cruz, SC-1616) diluted in blocking solution overnight at 4 °C. Corresponding fluorescent secondary antibodies were used (LI-COR, IRDye® 680RD cat#925-68073, IRDye® 680LT cat#926-68022, IRDye® 800CW cat#925-32214). Corresponding membrane halves were visualized simultaneously using the Odyssey imaging system (LI-COR). Integrated intensity of the protein of interest was normalized to β-actin levels from the same blot.

Quantitative Autoradiography

Fresh-frozen brains were sectioned at 20-micron thickness in the coronal plane on a freezing cryostat (Leica Biosystems) and thaw-mounted on poly-L-lysine-coated slides. Slides were stored at − 20 °C until used. For VMAT2 autoradiography, slides were pre-washed in 20mM HEPES-sucrose buffer at room temperature for 15 min. For total binding, slides were incubated in HEPES-sucrose buffer containing 6.9nM [3H]-dihydrotetrabenazine (DTBZ) for 1 hour. Nonspecific binding was determined by adding 2 μM unlabeled DTBZ to buffer. Slides were then washed thrice in Tris-HCl-sucrose buffer at room temperature for 5 minutes and then

dipped in dH_20 at 4 °C and dried overnight. On the following day, slides were apposed to Kodak Biomax MR film, MR-1 for 4 weeks. [3H]-Microscales (Amersham, Arlington Heights, IL, USA) were included with each film to allow for quantitative analysis of images. Images were captured and analyzed using MCID Imaging software (MCID, InterFocus Imaging, Cambridgeshire, UK). A rat brain atlas (Paxinos and Watson 1998) was used to define regions in OT, NAC, and STR to be analyzed. VMAT2 levels in OT were determined by averaging binding intensity measurements for anterior OT (A-OT) (at Bregma 1.60mm) and posterior OT (P-OT) (at Bregma 0.70mm) (**Fig. 2.9**). VMAT2 levels in NAC were determined by averaging binding intensity measurements for the NAC core (NAC-C) and NAC shell (NAC-S) (both at Bregma 1.60) (**Fig. 2.10**). VMAT2 levels in STR were determined by averaging binding intensity measurements for rostral STR (R-STR) (at Bregma 1.60mm), middle STR (M-STR) (at Bregma -0.26mm), and caudal STR (C-STR) (at Bregma -0.92mm) (**Fig. 2.11**).

Data and Statistical Analysis

Statistical analysis was performed using one-way ANOVA with post hoc Tukey's test (Graphpad Software, Inc.). A Bonferroni correction was used for statistical analysis of VMAT2 autoradiography data to account for multiple comparison across three brain regions (OT, NAC, and STR). For autoradiography analysis, values of $p \leq .017$ were considered statistically significant. For statistical analyses of immunoblot and HPLC data, values of $P \leq .05$ were considered statistically significant. DA, DOPAC, and HVA levels presented as percent of control. DA turnover is presented as a ratio. TH, pser40TH, and the ratio of pser40TH to TH (pser40TH/TH) levels are presented as percent of control. OT, NAC, and STR VMAT2 levels are presented as femtomoles per milligram tissue (fmol/mg tissue). Prior to statistical analysis,

data was analyzed for outlier values. To accomplish this, data for each group was divided into quartiles. Outliers were defined as any data point more than 1.5 interquartile ranges below the first quartile or above the third quartile. Outliers were removed prior to statistical analysis.

Results

BLLs in our rat model of Pb^{2+} exposure

The exposure paradigm utilized in this study resulted in BLLs that were within the range of those detected in children aged 1-5 in recent NHANES surveys irrespective of age, sex, and exposure of animal (CDC 2012, CDC 2016). At each age, BLLs were significantly different for animals exposed to 0ppm, 180ppm, and 1500ppm PbAc diet ($p<.0001$, $F_{2,33}= 207.6$ at PN14; $p<.0001$, $F_{2,56}= 173.3$ at PN28, $p<.0001$, $F_{2,191} = 426.7$ at PN50) (**Fig. 2.1 A-C**). At PN14, the resulting BLL in these animals was 0.4±0.0 µg/dl for control (n=14), 9.9±0.7µg/dl for rats on 180ppm PbAC diet (n=10), and 36.0 ± 3.9µg/dl for rats on 1500ppm PbAC diet (n=12). At PN28, the resulting BLL in these animals was 0.6±0.1 µg/dl for control (n=31), 7.0±0.3µg/dl for rats on 180ppm PbAC diet (n=12), and 19.9 ± 1.7µg/dl for rats on 1500ppm PbAC (n=6). At PN50, the resulting BLLs in these animals was 0.6±0.1 µg/dl for control (n=70), 4.4±0.2µg/dl for rats on 180ppm PbAC diet (n=40), and 22.0 ± 0.7µg/dl for rats on 1500ppm PbAC diet (n=70) (**Fig. 2.1A-C**).

Increased DA and DA turnover in Pb^{2+}-exposed adolescent and young adult male rat STR

To determine how Pb^{2+} exposure affects DAergic system ontogeny in our model, we measured DA, DOPAC, and HVA levels, as well as DA turnover in males and female rats at PN14, PN28, and PN50. The time between PN14 and PN28 corresponds to a period of rapid

brain growth and maturation of the DAergic system while at PN50, DA content in the STR begins to reach adult levels (Broening and William Jr 1998). No significant changes in DA ($P=0.53$, $F_{2,14}=0.66$), DOPAC ($p=0.68$, $F_{2,13}=0.40$), or HVA ($p=0.98$, $F_{2,14}=0.02$) levels were measured in male STR at PN14(**Fig. 2.2A**). Significant increases in DA ($P=0.0005$, $F_{2,11}=16.49$), DOPAC ($p=0.0002$, $F_{2,13}=18.51$), and HVA ($p=<0.0001$, $F_{2,14}=29.10$) levels were measured in the STR of Pb^{2+}-exposed versus control male STR at PN28 (**Fig. 2.2B**). Significant increases in DA ($P=0.0004$) levels were observed in the STR in Pb^{2+}-exposed versus control PN50 male rats. A dose-dependent effect was observed for DOPAC ($p<0.0001$, $F_{2,15}=30.82$) and HVA ($p=<0.0001$, $F_{2,10}=55.59$) in STR of Pb^{2+}-exposed PN50 male rats (**Fig. 2.2C**).

When not packaged into vesicles, DA can be metabolized by monoamine oxidase into DOPAC which, in turn, can be further metabolized into homovanillic acid (HVA) by catechol-o-methyl transferase (COMT) (Mignot and Laude 1985). Pharmacological and lesion analyses have demonstrated that the concentration of DOPAC and HVA present in DA-rich regions of the brain are dependent on DA transmission. Inhibition of DA transmission results in decreased DOPAC and HVA levels relative to DA levels, whereas increased DOPAC and HVA can be expected with increased release and subsequent metabolism of DA. Therefore, the ratio of these DA metabolites to DA can be implemented as an indirect measure of DA neurotransmission (Bacopoulos, Hattox et al. 1979). We measured changes in DA turnover by calculating the ratio of DOPAC to DA (DOPAC/DA) as well as HVA to DA (HVA/DA) as an index of DA turnover. As such, higher DOPAC/DA and HVA/DA levels indicate increased transmission DA (increased DA turnover), whereas lower DOPAC/DA and HVA/DA levels indicate decreased transmission of DA (decreased DA turnover).

No significant changes in DOPAC/DA ($p=0.42$, $F_{2, 14} = 0.92$) or HVA/DA ($p=0.71$) ratio were detected in PN14 male rat STR (**Fig. 2.3A and 2.3D**). Significant increases in DOPAC/DA ratio were detected in rats exposed to 1500ppm PbAC diet at PN28 ($p=0.04$, $F_{2, 12} = 4.13$) (**Fig. 2.3B**) and PN50 ($p=0.01$, $F_{2, 13} = 6.41$) (**Fig. 2.3C**). The HVA/DA ratio was significantly increased in the STR of PN28 male rats exposed to 180 and 1500ppm diet versus control animals ($p=0.001$, $F_{2, 11} = 13.78$) (**Fig. 2.3E**). The HVA/DA ratio was significantly increased in the STR of PN50 male rats exposed to 1500ppm diet versus control animals and animals exposed to 180ppm diet ($p=0.01$, $F_{2, 12} = 6.41$) (**Fig. 2.3F**). The significant increases in DOPAC/DA and HVA/DA ratios indicate significant increases in DA turnover in exposed versus control males at PN28 and PN50.

No significant differences were detected in the female STR for DA ($p=0.90$, $F_{2,13} =0.11$ at PN14; $p=0.30$, $F_{2,12} =1.32$ at PN28; $p=0.78$, $F_{2,13}=0.25$ at PN50), DOPAC ($p=0.054$, $F_{2,14} =3.62$ at PN14; $p=0.77$, $F_{2,13} =0.26$ at PN28; $p=0.48$, $F_{2,12} =0.77$ at PN50), or HVA ($p=0.059$, $F_{2,12}=3.63$ at PN14; $p=0.20$, $F_{2,11}= 1.88$ at PN28; $p=0.13$, $F_{2,11} =2.44$ at PN50) (**Fig. 2.4A-C**). No significant differences in DOPAC/DA ratio ($p=0.40$, $F_{2,12} =0.99$ at PN14; $p=0.57$, $F_{2,14} =0.58$ at PN28; $p=0.23$, $F_{2,13} =1.63$ at PN50) were detected in female rat STR (**Fig. 2.5A-C**). Furthermore, no significant differences in HVA/DA ratio ($p=0.24$, $F_{2,13} =1.60$ at PN14; $p=0.20$, $F_{2,11} =1.88$ at PN28; and $p=0.11$, $F_{2,12} =2.73$ at PN50) were detected in female rat STR (**Fig. 2.5D-F**). Altogether, no significant changes in DA or DA turnover in female rat STR were detected at any age following chronic Pb^{2+} exposure.

Increases in DA and DA turnover were not associated with altered TH expression or activation

TH is the rate-limiting enzyme in DA synthesis (**Fig. 2.6**). TH activity is tightly regulated by a number of mechanisms including phosphorylation, gene expression, and feedback inhibition (Dunkley, Bobrovskaya et al. 2004, Daubner, Le et al. 2011, Tekin, Roskoski et al. 2014). TH activity can be altered in response to changes in the environment and TH activity has been described in different regions of the rat brain as early as 2 weeks following chronic Pb^{2+} exposure (Chin, Ryu et al. 1992, Ramesh and Jadhav 1998). Phosphorylation acts as a short-term regulator of TH activity and there is evidence to suggest that TH phosphorylation is affected by Pb^{2+} exposure (Leret, Garcia-Uceda et al. 2002). Phosphorylation at the serine 40 site plays a pivotal role in the modulation of feedback inhibition of TH by catecholamines by altering TH conformation which, in turn, facilitates the release of the bound catecholamine and ultimately increases TH activity 20-fold (Dunkley, Bobrovskaya et al. 2004, Daubner, Le et al. 2011, Tekin, Roskoski et al. 2014). In this study, pser40TH levels were compared between exposed and control rats as a surrogate marker for enzymatic activity of TH. To determine if the increases in DA and DA metabolites observed in PN28 and PN50 male rats could be due to Pb^{2+}-induced alteration of TH expression and phosphorylation, we measured TH and pser40TH levels as well as pser40TH/TH ratio in STR using Western blots.

We found no significant differences in TH ($p=0.58$, $F_{2,15}=0.57$), pser40TH ($p=0.57$, $F_{2,14}= 0.58$), or pser40TH/TH ratio ($p=0.31$, $F_{2,14}= 1.28$) in the male STR at PN14 between control and exposed groups (**Fig. 2.7A-C**) or in TH ($p=0.73$, $F_{2,15}=0.32$), pser40TH ($p=0.67$, $F_{2,15}=0.42$), or pser40TH/TH ($p=0.72$, $F_{2,15}=0.34$) in male STR at PN28 between control and exposed groups (**Fig. 2.7D-F**). Furthermore, we found no significant differences in TH ($p=0.10$, $F_{2,12}= 2.76$), pser40TH. ($p=0.26$, $F_{2,15}= 1.48$), or pser40TH/TH ($p=0.41$, $F_{2,15}=0.95$) in male STR at PN50 between control and exposed groups (**Fig. 2.7G-I**). Overall, chronic Pb^{2+} exposure had no

significant impact on TH or pser40TH levels or pser40TH/TH ratio in male rats at all ages studied (**Fig. 2.11A-I**).

Similarly, we detected no significant differences in TH ($p=0.30$, $F_{2,13}= 1.30$), pser40TH ($p=0.80$, $F_{2,13}= 0.23$), or pser40TH/TH ratio ($p=0.63$, $F_{2,14}=0.47$) in the female STR at PN14 between control and exposed groups (**Fig. 2.8A-C**). We also did not observe any significant changes in TH ($p=0.24$, $F_{2,13}= 1.59$), pser40TH ($p=0.054$, $F_{2,14}= 3.61$), or pser40TH/TH ($p=0.12$, $F_{2,13}= 2.48$) in female STR at PN28 between control and exposed groups (**Fig. 2.8D-F**). In contrast, we did find a significant increase in pser40TH levels ($p=0.02$, $F_{2,14}= 5.35$) in female rats exposed to 1500ppm PbAC diets versus those on 0ppm and 180ppm PbAC diet at PN50 (**Fig. 2.8G-I**).

Altered VMAT2 levels in STR of PN14 and PN28 female rats on 180ppm PbAC diet

DA is transported into vesicles via VMAT2 to prevent degradation (Fleckenstein, Volz et al. 2009). To determine if the increases in DA and DA metabolites observed in PN28 and PN50 were associated by any changes to VMAT2 levels, we measured VMAT2 levels in the OT, NAC, and STR using quantitative autoradiography (See **Fig.2.12-14** for representative autoradiograms of regions analyzed). After adjusting for multiple comparisons, we found no significant changes in VMAT2 levels in male rats at PN14, PN28, and PN50 in the OT ($p=0.03$, $F_{2,14} =4.71$ at PN14; $p=0.15$, $F_{2,12} =2.23$ at PN28; $p=0.22$, $F_{2,15} =1.70$ at PN50) (**Fig. 2.9A-C**), NAC ($p=0.24$, $F_{2,15}= 1.58$ at PN14; $p=0.87$, $F_{2,15}=0.14$ at PN28; $p=0.95$, $F_{2,17}= 0.05$ at PN50) (**Fig.2.10-C**), or STR ($p=0.02$, $F_{2,15}=4.83$ at PN14; $p=0.55$, $F_{2,16}=0.55$ at PN28; $p=0.39$, $F_{2,17} =0.99$ at PN50) (**Fig.2.11A-C**).

After adjusting for multiple comparisons, we also did not observe any significant changes in VMAT2 levels in female rats at PN14, PN28, and PN50 female rat OT ($p=0.15$, $F_{2,14}= 2.16$ at PN14; $p=0.02$, $F_{2,16}= 4.78$ at PN28; $p=0.40$, $F_{2,17}=0.97$at PN50) (**Fig.2.9D-F**) or NAC ($p=0.10$, $F_{2,15}= 2.64$ at PN14; $p=0.38$, $F_{2,17}= 1.0$ at PN28; $p=0.27$, $F_{2,16}= 1.40$ at PN50) (**Fig.2.10D-F**). A significant decrease in VMAT2 levels was observed in the STR of PN14 female rats exposed to 180ppm diet versus females on control or 1500ppm diet ($p=0.0014$, $F_{2,14}= 10.96$) (**Fig.2.11D**). However, a moderate increase in VMAT2 levels was detected in this region at PN28 in females exposed to 180ppm PbAC diet versus females on control or 1500ppm diet ($p=0.01$, $F_{2,14} = 5.42$) (**Fig.2.11E**). No significant difference in VMAT2 levels were detected in the STR of female rats at PN50 ($p=0.44$, $F_{2,15}=0.86$) (**Fig.2.11F**).

Table 2.1

A)

Control	Males	Females	Average
PN14	0.4 ± 0.1 N=9	0.4 ± 0.1 N=5	0.4 ± 0.0 N=14
PN28	0.4 ± 0.1 N=14	0.7 ± 0.2 N=17	0.6 ± 0.1 N=31
PN50	0.6 ± 0.1 N=47	0.6 ± 0.2 N=23	0.6 ± 0.1 N=70

B)

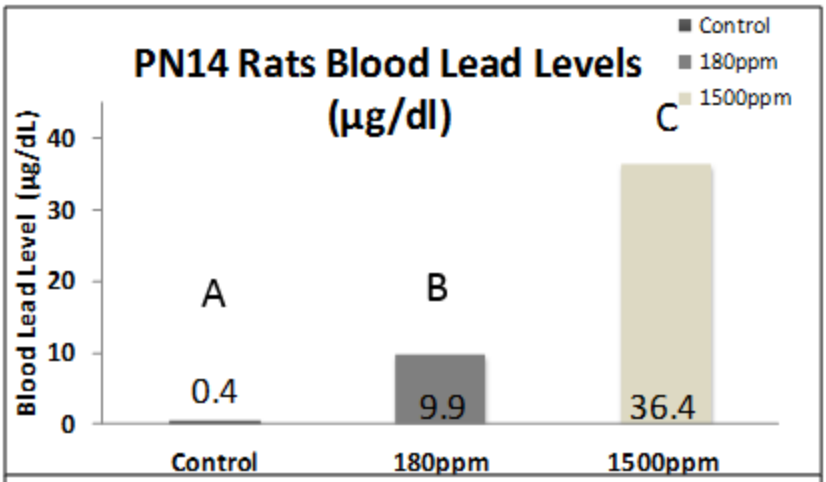
180ppm	Males	Females	Average
PN14	9.7± 0.9 N=7	10.3 ± 1.3 N=3	9.9 ± 0.7 N=10
PN28	6.8 ± 0.3 N=24	7.1 ± 0.5 N=12	7.0 ± 0.3 N=12
PN50	4.4 ± 0.2 N=28	4.5 ± 0.3 N=12	4.4 ± 0.2 N=40

C)

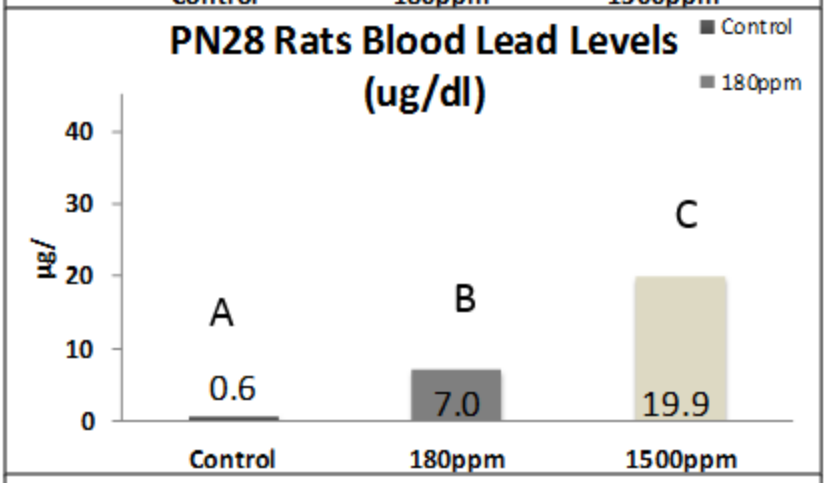
1500ppm	Males	Females	Average
PN14	36.1 ± 2.9 N=8	36.9 ± 3.9 N=4	36.0 ± 3.9 N=12
PN28	17.7 ± 0.5 N=13	22.5 ± 1.9 N=7	19.9 ± 1.7 N=6
PN50	22.2 ± 0.9 N=47	21.5 ± 1.2 N=23	22.0 ± 0.7 N=70

Table 2.1. BLL measured across ages and sex from control and Pb^{2+} -exposed rats. (A) displays averaged BLL measured for rats fed control chow. (B) and (C) display averaged BLL measured for rats chronically exposed to 180ppm and 1500ppm PbAC, respectively. Each value is presented as BLL mean± SEM of at least 3 animals. To minimize litter effects, BLL from only one male and/or one female were collected for each age and exposure.

A)


PN14 Rats Blood Lead Levels (µg/dl)
Control
180ppm
1500ppm
Blood Lead Level (µg/dL)
40
30
20
10
0
A
B
C
0.4
9.9
36.4
Control
180ppm
1500ppm

B)


PN28 Rats Blood Lead Levels (ug/dl)
Control
180ppm
µg/
40
30
20
10
0
A
B
C
0.6
7.0
19.9
Control
180ppm
1500ppm

C)

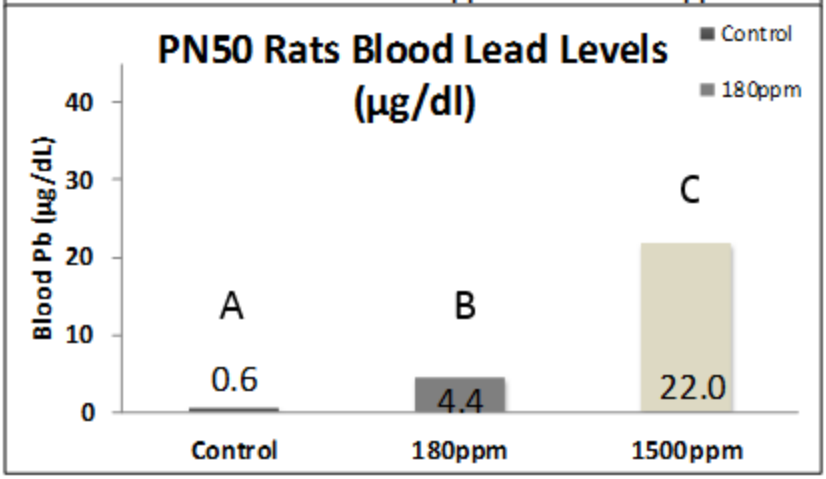
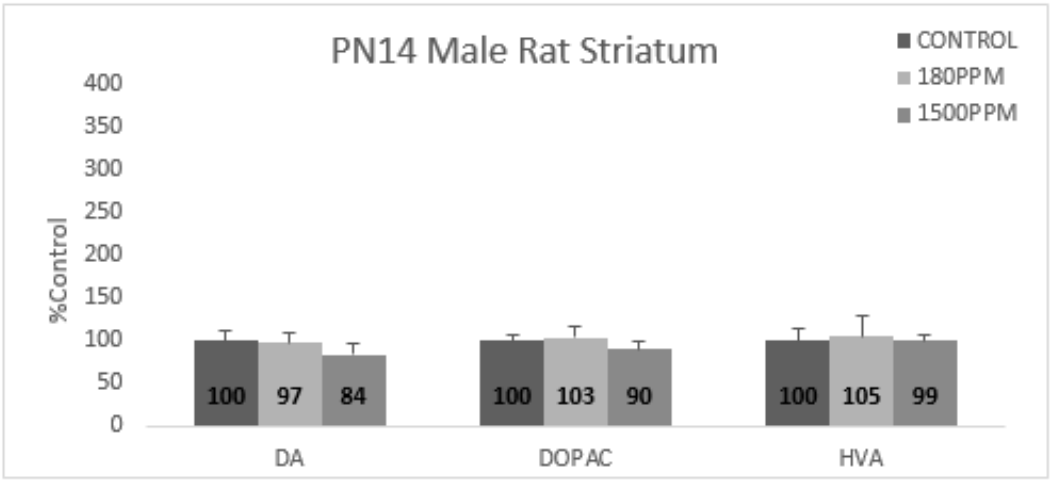
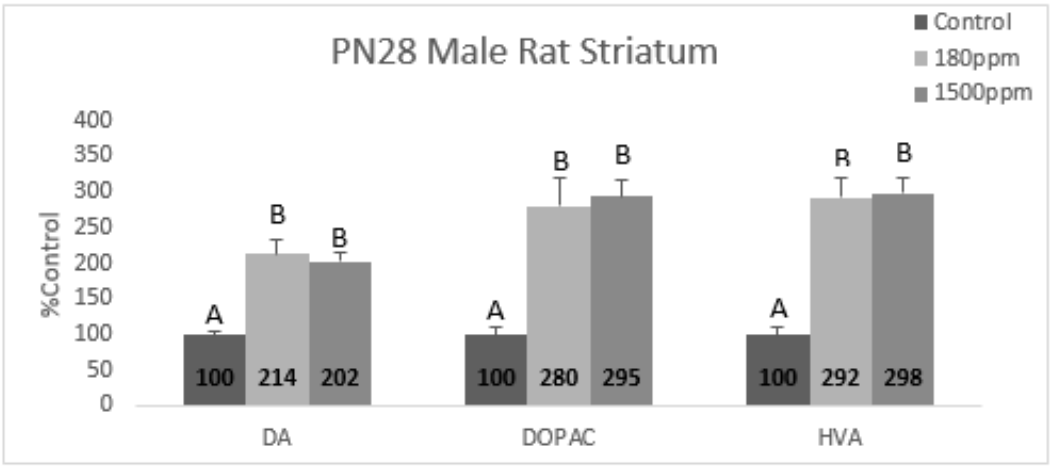

PN50 Rats Blood Lead Levels (µg/dl)
Control
180ppm
Blood Pb (µg/dL)
40
30
20
10
0
A
B
C
0.6
4.4
22.0
Control
180ppm
1500ppm

Figure 2.1. BLL for control and exposed rats at PN14, PN28, and PN50 **A)** At PN14, the resulting blood Pb^{2+} levels in these animals was 0.4±0.0 µg/dl (n=14) for control, 9.9±0.7µg/dl (n=10) for rats on 180ppm PbAC diet, and 36.0 ± 3.9µg/dl for rats on 1500ppm PbAC diet (n=12). BLL were significantly different across all groups ($p<0.0001$, $F_{2,33}= 207.6$). **B)** At PN28, the resulting blood Pb^{2+} levels in these animals was 0.6±0.1 µg/dl (n=31) for control, 7.0±0.3µg/dl (n=12) for rats on 180ppm PbAC diet, and 19.9 ± 1.7µg/dl for rats on 1500ppm PbAC (n=6). BLL were significantly different across all groups ($p<0.0001$, $F_{2,56}= 173.3$). **C)** At PN50, the resulting blood PbAC levels in these animals was 0.6±0.1 µg/dl (n=70) for control, 4.4±0.2µg/dl (n=40) for rats on 180ppm PbAC diet, and 22.0 ± 0.7µg/dl (n=70) for rats on 1500ppm PbAC diet (n=70). BLL were significantly different across all groups ($p<0.0001$, $F_{2,191} = 426.7$). Statistically significant differences among exposure groups are indicated by different letters labelling bars. There is no statistically significant difference between bars labelled with the same letters

A)


PN14 Male Rat Striatum
CONTROL
180PPM
1500PPM
%Control
400
350
300
250
200
150
100
50
0
100
97
84
100
103
90
100
105
99
DA
DOPAC
HVA

B)


PN28 Male Rat Striatum
Control
180ppm
1500ppm
%Control
400
350
300
250
200
150
100
50
0
A
B
B
100
214
202
A
B
B
100
280
295
A
B
B
100
292
298
DA
DOPAC
HVA

C)

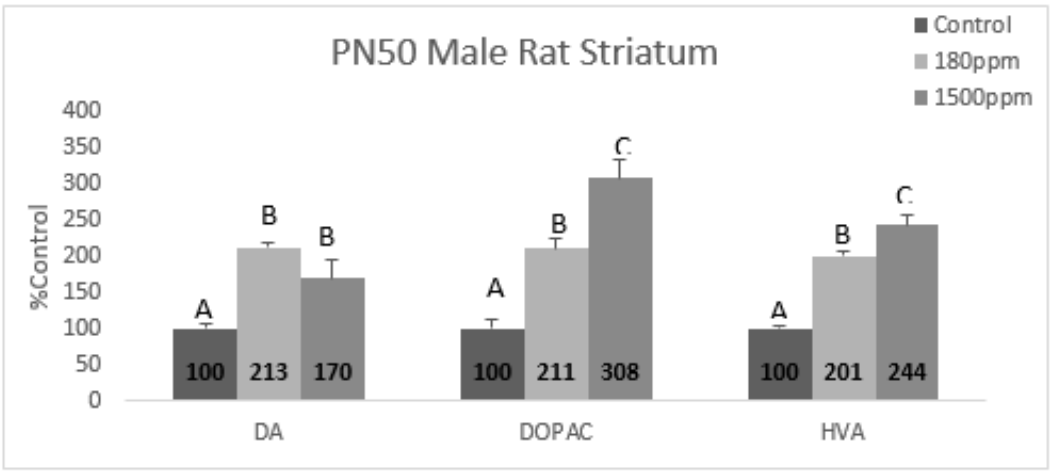

PN50 Male Rat Striatum
Control
180ppm
1500ppm
%Control
400
350
300
250
200
150
100
50
0
A
B
B
100
213
170
A
B
C
100
211
308
A
B
C
100
201
244
DA
DOPAC
HVA

Figure 2.2. DA, DOPAC, and HVA levels in control and Pb^{2+}-exposed male rat STR.

(A) No significant changes in DA (P=0.53, $F_{2,14}$=0.66, control n=5, 180ppm n=6, 1500ppm n=6), DOPAC (p=0.68, $F_{2,13}$=0.40, control n=5, 180ppm n=5, 1500ppm n=6), or HVA (p=0.98, $F_{2,14}$=0.02, control n=5, 180ppm n=6, 1500ppm n=6) levels were detected in the STR in Pb^{2+} -exposed versus control PN14 male rats. (B) Significant changes in DA (P=0.0005, $F_{2,11}$=16.49, control n=4, 180ppm n=5, 1500ppm n=5), DOPAC (p=0.0002, $F_{2,13}$=18.51, control n=6, 180ppm n=5, 1500ppm n=5), and HVA (p=<0.0001, $F_{2,14}$=29.10, control n=6, 180ppm n=5, 1500ppm n=6) levels were detected in the STR in Pb^{2+}-exposed versus control PN28 male rats. (C) Significant changes in DA (P=0.0004, $F_{2,13}$=15.14, control n=6, 180ppm n=5, 1500ppm n=5) levels were detected in the STR in Pb^{2+}-exposed versus control PN50 male rats. A dose-dependent effect was detected for DOPAC (p<0.0001, $F_{2,15}$=30.82, control n=6, 180ppm n=6, 1500ppm n=6),) and HVA (p=<0.0001, $F_{2,10}$=55.59, control n=4, 180ppm n=5, 1500ppm n=4) for Pb^{2+}-exposed PN50 rats.

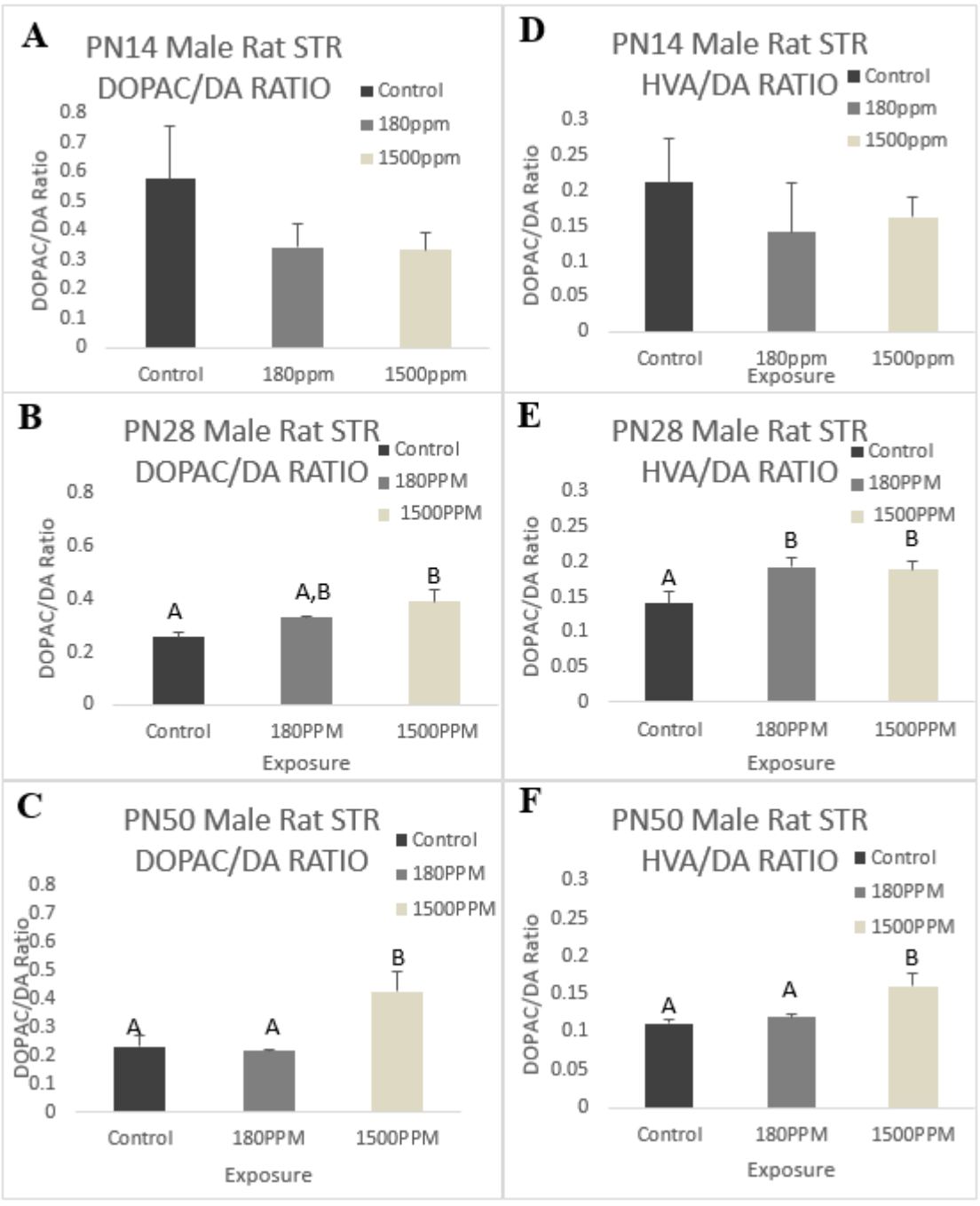
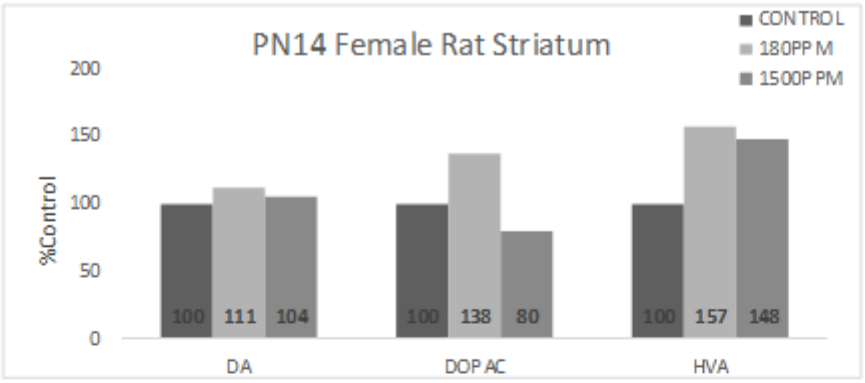
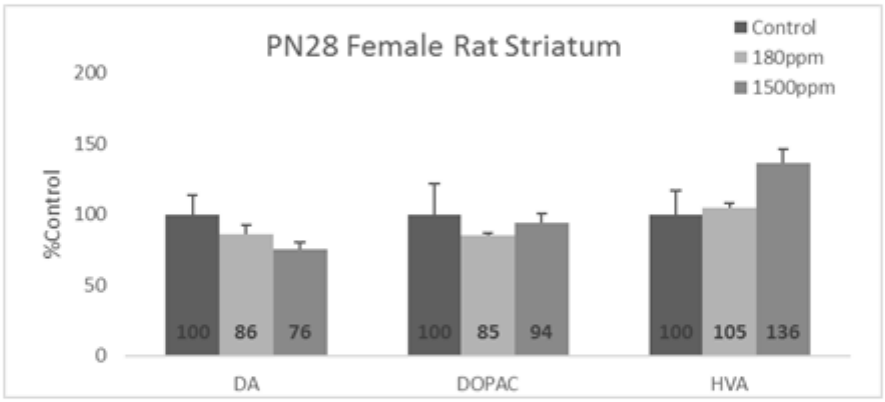

A
PN14 Male Rat STR
DOPAC/DA RATIO
Control
180ppm
1500ppm
DOPAC/DA Ratio
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
180ppm
1500ppm
D
PN14 Male Rat STR
HVA/DA RATIO
Control
180ppm
1500ppm
DOPAC/DA Ratio
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
180ppm
1500ppm
Exposure
B
PN28 Male Rat STR
DOPAC/DA RATIO
Control
180PPM
1500PPM
DOPAC/DA Ratio
0.8
0.6
0.4
0.2
0
A
A,B
B
Control
180PPM
1500PPM
Exposure
E
PN28 Male Rat STR
HVA/DA RATIO
Control
180PPM
1500PPM
DOPAC/DA Ratio
0.3
0.25
0.2
0.15
0.1
0.05
0
A
B
B
Control
180PPM
1500PPM
Exposure
C
PN50 Male Rat STR
DOPAC/DA RATIO
Control
180PPM
1500PPM
DOPAC/DA Ratio
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
A
A
B
Control
180PPM
1500PPM
Exposure
F
PN50 Male Rat STR
HVA/DA RATIO
Control
180PPM
1500PPM
DOPAC/DA Ratio
0.3
0.25
0.2
0.15
0.1
0.05
0
A
A
B
Control
180PPM
1500PPM
Exposure

Figure 2.3. DOPAC/DA and HVA/DA ratios in control and Pb^{2+}-exposed male rat STR. (A) No significant changes in DOPAC/DA ratio were detected in PN14 male rat STR ($p=0.42$, $F_{2, 14} = 0.92$, control n=6, 180ppm n=6, 1500ppm n=5). (B) A significant increase in DOPAC/DA ratio was detected in the STR of PN28 male rats exposed to 1500ppm diet versus control animals ($p=0.04$, $F_{2, 12} = 4.13$, control n=6, 180ppm n=3, 1500ppm n=6). (C) The DOPAC/DA ratio was significantly increased in the STR of PN50 male rats exposed to 1500ppm diet versus control animals and animals exposed to 180ppm diet ($p=0.01$, $F_{2, 13} = 6.41$, control n=6, 180ppm n=5, 1500ppm n=5). (D) No significant changes in HVA/DA ratio were detected in PN14 male rat ($p=0.71$, $F_{2, 10} = 0.35$, control n=5, 180ppm n=5, 1500ppm n=3). (E) A significant increase in HVA/DA ratio was detected in the STR of PN28 male rats exposed to 180ppm and 1500ppm diet versus control animals ($p=0.001$, $F_{2, 11} = 13.78$, control n=5, 180ppm n=4, 1500ppm n=5). (F) The HVA/DA ratio was significantly increased in the STR of PN50 male rats exposed to 1500ppm diet versus control animals and animals exposed to 180ppm diet ($p=0.01$, $F_{2, 12} = 6.41$, control n=5, 180ppm n=5, 1500ppm n=5).

A)


PN14 Female Rat Striatum
CONTROL
180PPM
1500PPM
%Control
200
150
100
50
0
100
111
104
100
138
80
100
157
148
DA
DOPAC
HVA

B)


PN28 Female Rat Striatum
Control
180ppm
1500ppm
%Control
200
150
100
50
0
100
86
76
100
85
94
100
105
136
DA
DOPAC
HVA

C)

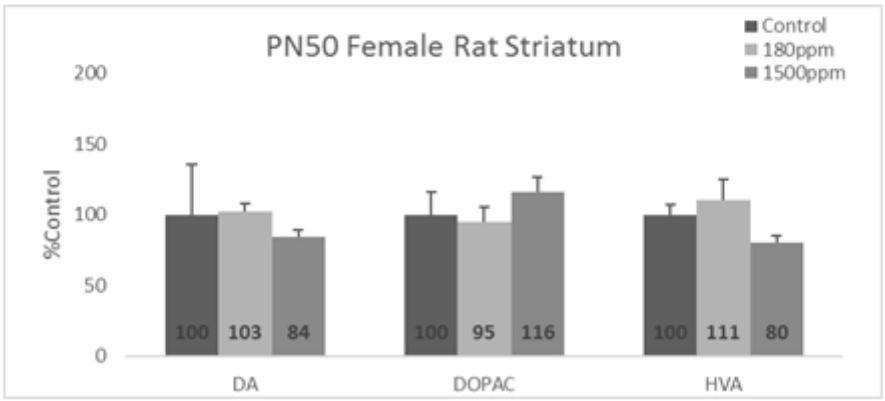

PN50 Female Rat Striatum
Control
180ppm
1500ppm
%Control
200
150
100
50
0
100
103
84
100
95
116
100
111
80
DA
DOPAC
HVA

Figure 2.4. DA, DOPAC, and HVA levels in control and Pb^{2+}-exposed female rat STR. (A) No significant changes in DA (p=0.90, $F_{2,13}$ =0.11, control n=6, 180ppm n=5, 1500ppm n=5), DOPAC (p=0.054, $F_{2,14}$ =3.62, control n=6, 180ppm n=6, 1500ppm n=5), or HVA (p=0.059, $F_{2,12}$=3.63, control n=5, 180ppm n=5, 1500ppm n=5) levels were detected in the STR in Pb^{2+}-exposed versus control PN14 female. (B) No significant changes in DA (p=0.30, $F_{2,12}$ =1.32, control n=5, 180ppm n=6, 1500ppm n=5), DOPAC (p=0.77, $F_{2,13}$ =.26, control n=5, 180ppm n=5, 1500ppm n=5), or HVA (p=0.20, $F_{2,11}$= 1.88, control n=4, 180ppm n=5, 1500ppm n=5) levels were detected in the STR in Pb^{2+}-exposed versus control PN28 females. (C) No significant changes in DA (p=0.78, $F_{2,13}$= .25, control n=5, 180ppm n=6, 1500ppm n=5), DOPAC (p=0.48, $F_{2,12}$ =0.77, control n=5, 180ppm n=5, 1500ppm n=5), or HVA (p=0.13, $F_{2,11}$ =2.44, control n=4, 180ppm n=5, 1500ppm n=5) levels were detected in the STR in Pb^{2+}-exposed versus control PN50 females.

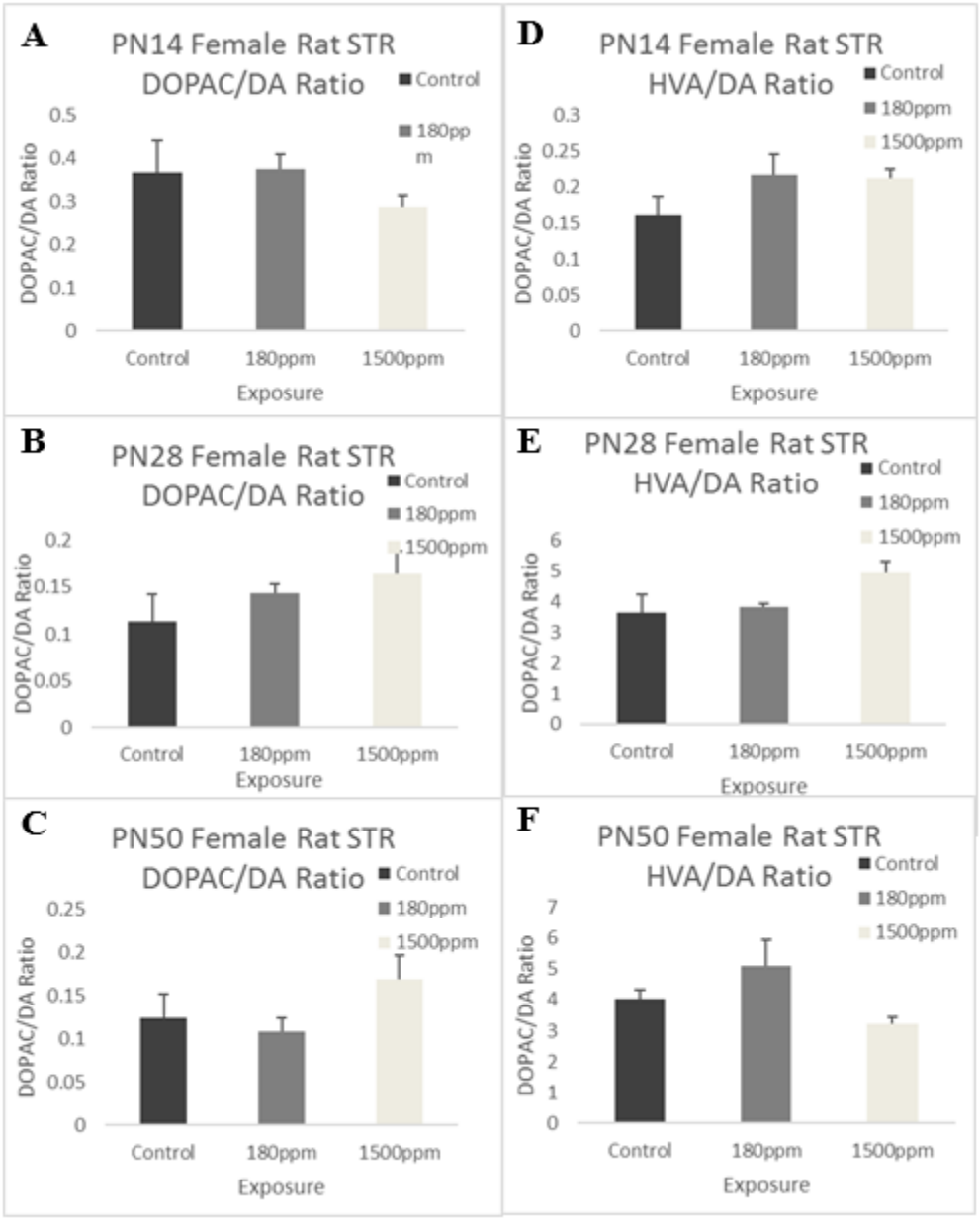

A
PN14 Female Rat STR
DOPAC/DA Ratio
Control
180ppm
DOPAC/DA Ratio
0.5
0.4
0.3
0.2
0.1
0
Control
180ppm
1500ppm
Exposure
D
PN14 Female Rat STR
HVA/DA Ratio
Control
180ppm
1500ppm
DOPAC/DA Ratio
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
180ppm
1500ppm
Exposure
B
PN28 Female Rat STR
DOPAC/DA Ratio
Control
180ppm
1500ppm
DOPAC/DA Ratio
0.2
0.15
0.1
0.05
0
Control
180ppm
1500ppm
Exposure
E
PN28 Female Rat STR
HVA/DA Ratio
Control
180ppm
1500ppm
DOPAC/DA Ratio
6
5
4
3
2
1
0
Control
180ppm
1500ppm
Exposure
C
PN50 Female Rat STR
DOPAC/DA Ratio
Control
180ppm
1500ppm
DOPAC/DA Ratio
0.25
0.2
0.15
0.1
0.05
0
Control
180ppm
1500ppm
Exposure
F
PN50 Female Rat STR
HVA/DA Ratio
Control
180ppm
1500ppm
DOPAC/DA Ratio
7
6
5
4
3
2
1
0
Control
180ppm
1500ppm
Exposure

Figure 2.5. DOPAC/DA and HVA/DA ratios in control and Pb^{2+}-exposed female rat STR. (A) No significant change in DOPAC/DA ratio was detected in the STR of exposed PN14 female rats versus control (p=0.40, $F_{2,12}$ =0.99, control n=5, 180ppm n=5, 1500ppm n=5). (B) No significant change in DOPAC/DA ratio was detected in the STR of exposed PN28 female rats versus control (p=0.57, $F_{2,14}$ =0.58, control n=6, 180ppm n=6, 1500ppm n=5). (C) No significant change in DOPAC/DA ratio was detected in the STR of exposed PN50 female rats versus control (p=0.23, $F_{2,13}$ =1.63, control n=5, 180ppm n=5, 1500ppm n=6). (D) No significant change in HVA/DA ratio was detected in the STR of exposed PN14 female rats versus control (p=0.24, F2,13 =1.60, control n=5, 180ppm n=6, 1500ppm n=5). (E) No significant change in HVA/DA ratio was detected in the STR of exposed PN28female rats versus control (p=0.20, F2,11 =1.88, control n=6, 180ppm n=4, 1500ppm n=4). (F) No significant change in HVA/DA ratio was detected in the STR of exposed PN50 female rats versus control (p=0.11, F2,12 =2.73, control n=4, 180ppm n=6, 1500ppm n=5).

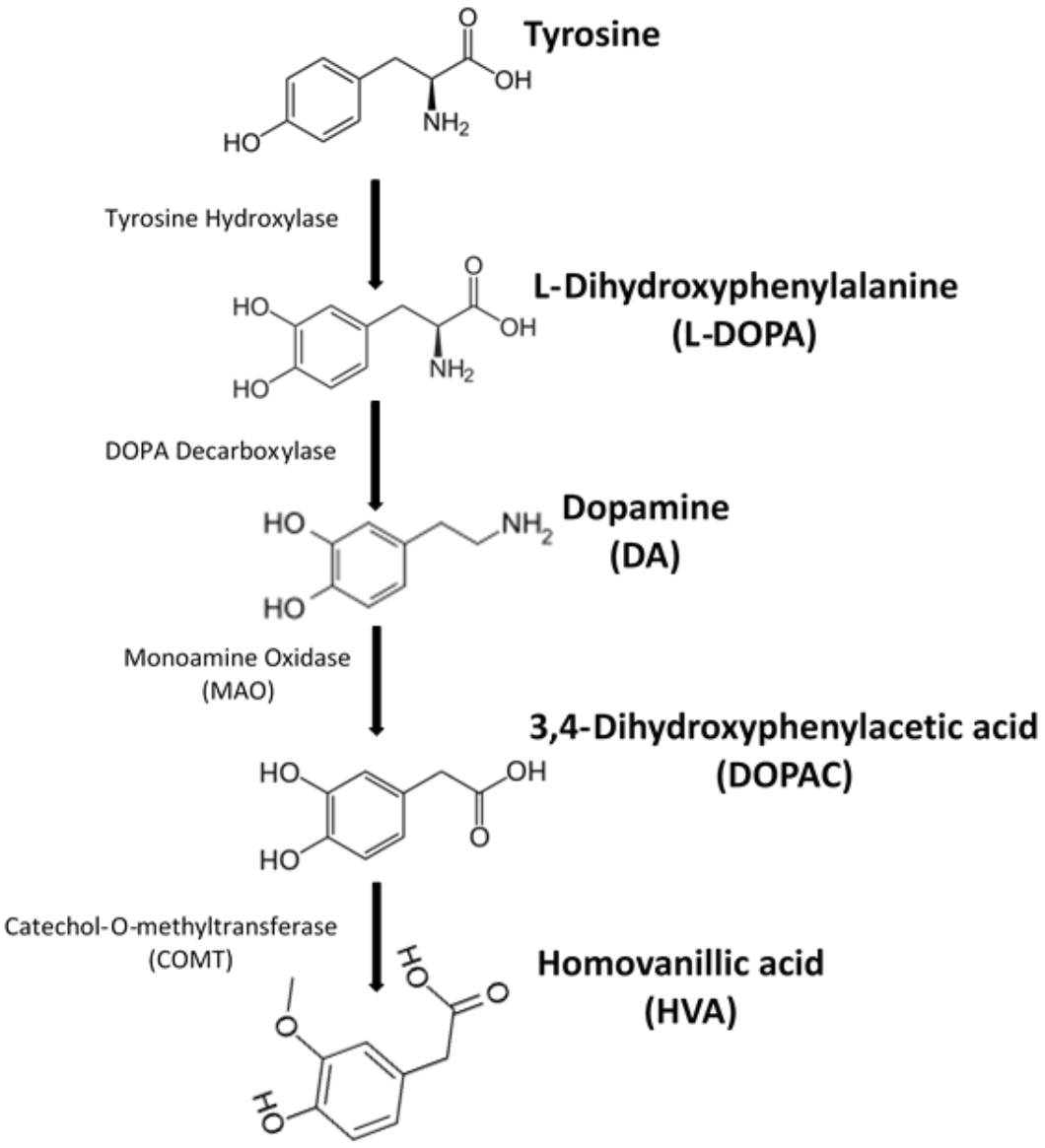


Figure 2.6: DA Synthesis and Metabolism

DA synthesis begins with the addition of a hydroxyl group to tyrosine to form DA precursor L-DOPA by TH, the rate-limiting enzyme in DA synthesis. DOPA decarboxylase then removes a carboxyl group from L-DOPA, resulting in DA synthesis. DA is metabolized into DOPAC by MAO through oxidative deamination. Methylation of DOPAC by COMT results in synthesis of DA metabolite HVA. DOPAC and HVA levels can be utilized as markers for dopamine turnover.

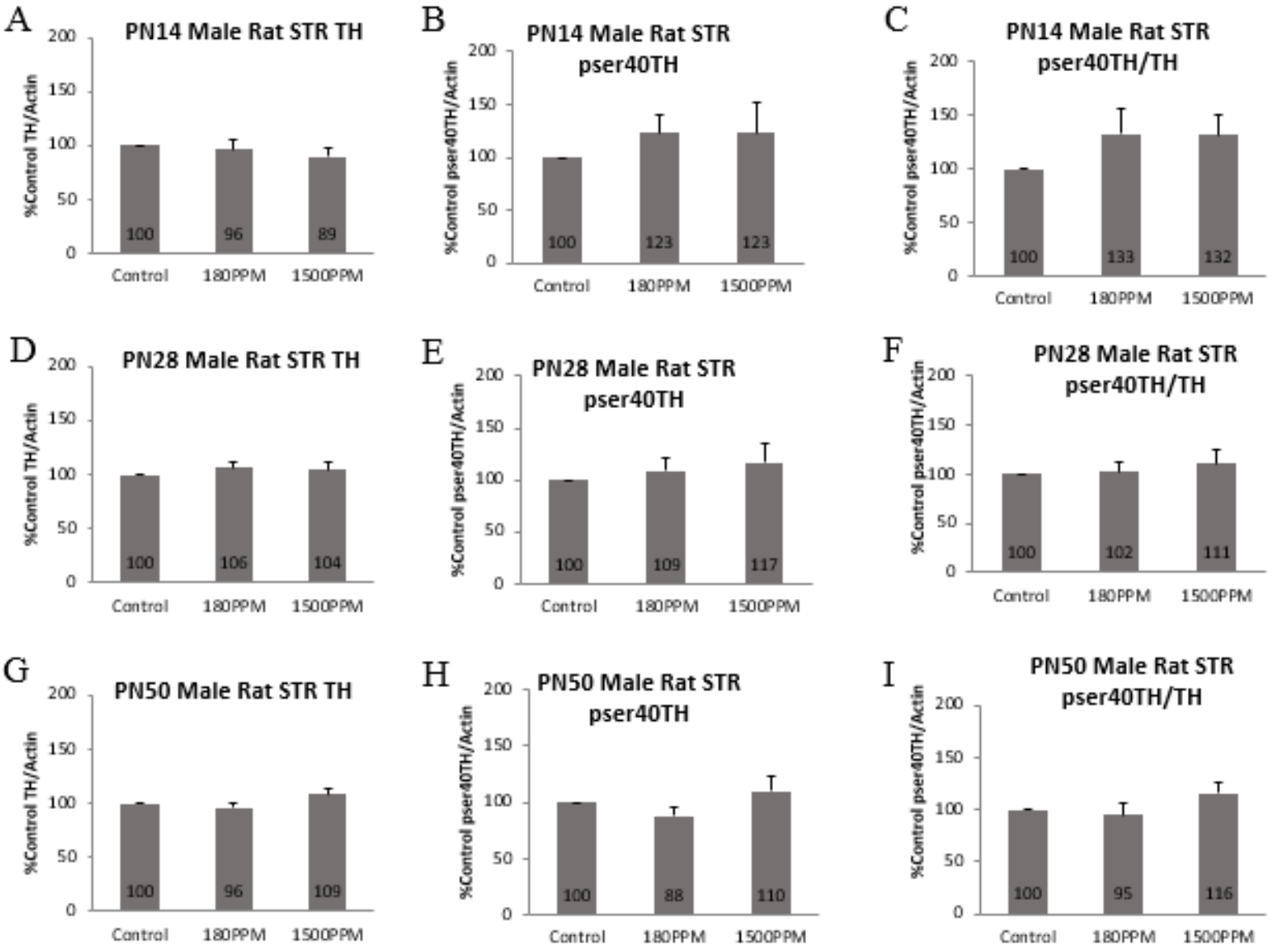

A
PN14 Male Rat STR TH
%Control TH/Actin
200
150
100
50
0
100
96
89
Control
180PPM
1500PPM
B
PN14 Male Rat STR pser40TH
%Control pser40TH/Actin
100
123
123
C
PN14 Male Rat STR pser40TH/TH
%Control pser40TH/Actin
100
133
132
D
PN28 Male Rat STR TH
%Control TH/Actin
100
106
104
E
PN28 Male Rat STR pser40TH
%Control pser40TH/Actin
100
109
117
F
PN28 Male Rat STR pser40TH/TH
%Control pser40TH/Actin
100
102
111
G
PN50 Male Rat STR TH
%Control TH/Actin
100
96
109
H
PN50 Male Rat STR pser40TH
%Control pser40TH/Actin
100
88
110
I
PN50 Male Rat STR pser40TH/TH
%Control pser40TH/Actin
100
95
116

Figure 2.7. Phosphorylation of TH at serine 40 site in male rat STR. A) No significant changes in TH were detected in The STR of PN14 males ($p=0.58$, $F_{2,15}=0.57$) (control n=6, 180ppm n=6, 1500ppm n=6). B) No significant changes in pser40TH were detected in the STR of PN14 males ($p=0.57$, $F_{2,14}= 0.58$) (control n=6, 180ppm n=6, 1500ppm n=5). C) No significant changes in pser40TH/TH ratio were detected in The STR of PN14 males ($p=0.31$, $F_{2,14}= 1.28$) (control n=6, 180ppm n=6, 1500ppm n=5). D) No significant changes in TH were detected in PN28 male STR ($p=0.73$, $F_{2,15}=0.32$) (control n=6, 180ppm n=6, 1500ppm n=6). E) No significant changes in pser40TH were detected in PN28 male STR ($p=0.67$, $F_{2,15}= 0.42$) (control n=6, 180ppm n=6, 1500ppm n=6). F) No significant changes in pser40TH/TH ratio were detected in PN28 male STR ($p=0.72$, $F_{2,15}=0.34$) (control n=6, 180ppm n=6, 1500ppm n=6). H) No significant changes in TH were detected in PN50 male STR ($p=0.10$, $F_{2,12}= 2.76$) (control n=6, 180ppm n=4, 1500ppm n=5). I) No significant changes in pser40TH were detected in PN50 male STR ($p=0.26$, $F_{2,15}= 1.48$) (control n=6, 180ppm n=6, 1500ppm n=6). J) No significant changes in pser40TH/TH ratio were detected in PN50 male STR ($p=0.41$, $F_{2,15}=0.95$) (control n=6, 180ppm n=5, 1500ppm n=5).

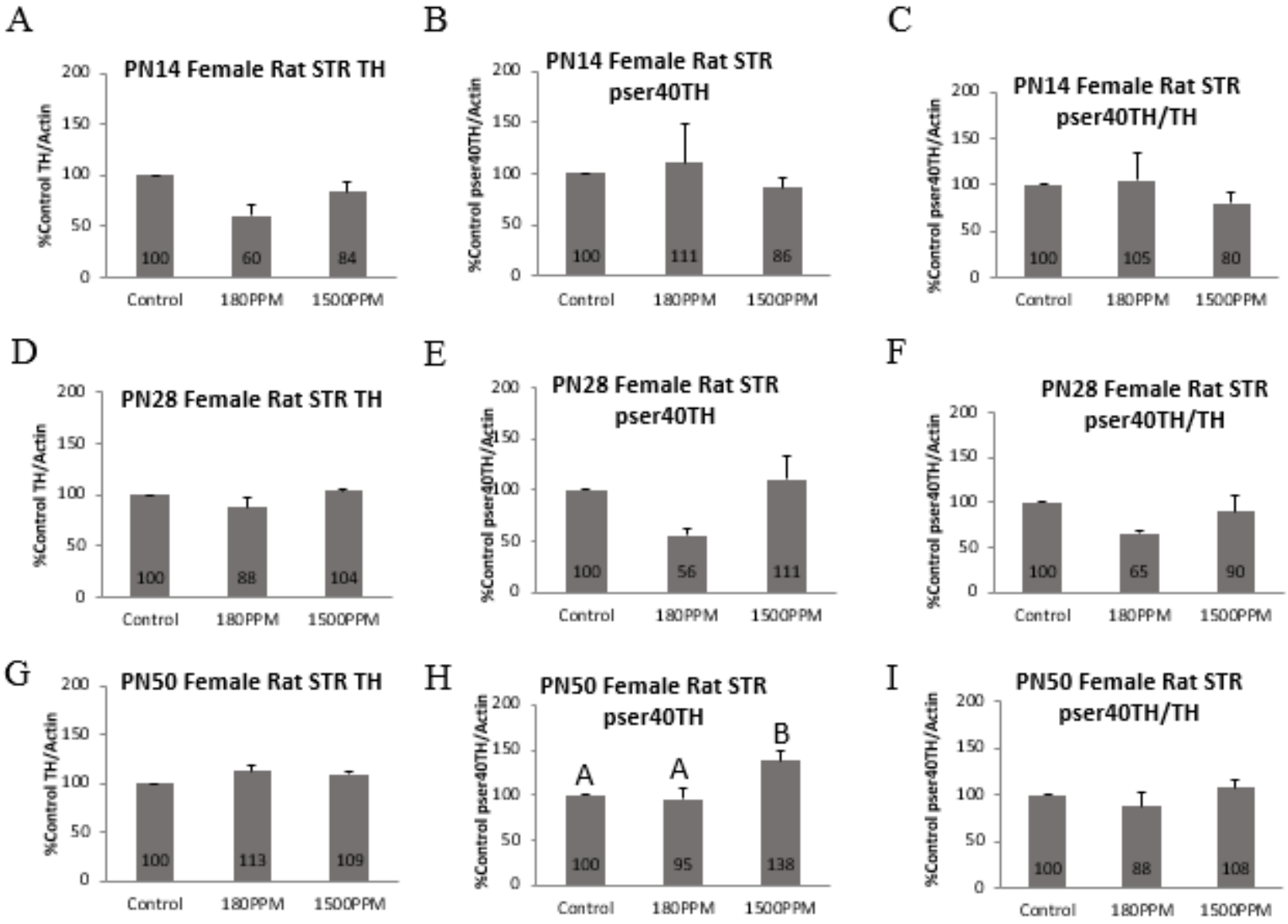

A
PN14 Female Rat STR TH
%Control TH/Actin
200
150
100
50
0
100
60
84
Control
180PPM
1500PPM
B
PN14 Female Rat STR pser40TH
%Control pser40TH/Actin
100
111
86
C
PN14 Female Rat STR pser40TH/TH
100
105
80
D
PN28 Female Rat STR TH
100
88
104
E
PN28 Female Rat STR pser40TH
100
56
111
F
PN28 Female Rat STR pser40TH/TH
100
65
90
G
PN50 Female Rat STR TH
100
113
109
H
PN50 Female Rat STR pser40TH
A
A
B
100
95
138
I
PN50 Female Rat STR pser40TH/TH
100
88
108

Figure 2.8. Phosphorylation of TH at serine 40 site in female rat STR. A) No significant changes in TH were detected in STR of PN14 females ($p=0.30$, $F_{2,13}= 1.30$) (control n=6, 180ppm n=5, 1500ppm n=5). B) No significant changes in pser40TH were detected in STR of PN14 females ($p=0.80$, $F_{2,13}= 0.23$) (control n=6, 180ppm n=6, 1500ppm n=4). C) No significant changes in pser40TH/TH ratio were detected in PN14 female STR ($p=0.63$, $F_{2,14}=0.47$) (control n=6, 180ppm n=6, 1500ppm n=5). D) No significant changes in TH were detected in STR of PN28 females ($p=0.24$, $F_{2,13}= 1.59$) (control n=6, 180ppm n=6, 1500ppm n=4). E) No significant changes in pser40TH were detected in STR of PN28 females ($p=0.054$, $F_{2,14}= 3.61$) (control n=6, 180ppm n=5, 1500ppm n=6). F) No significant changes in pser40TH/TH ratio were detected in STR of PN28 females ($p=0.12$, $F_{2,13}= 2.48$) (control n=6, 180ppm n=5, 1500ppm n=5). G) No significant changes in TH were detected in the STR of PN50 females ($p=0.09$, $F_{2,15}= 2.77$) (control n=6, 180ppm n=6, 1500ppm n=6). H) A significant increase in pser40TH was detected in the STR of PN50 female rats on 1500ppm diet (38% increase versus control, 43% increase versus 180ppm females) ($p=0.02$, $F_{2,14}= 5.35$) (control n=6, 180ppm n=6, 1500ppm n=5). I) No significant changes in pser40TH/TH ratio were detected in STR of PN50 females ($p=0.40$, $F_{2,14}= 0.90$) (control n=6, 180ppm n=6, 1500ppm n=5).

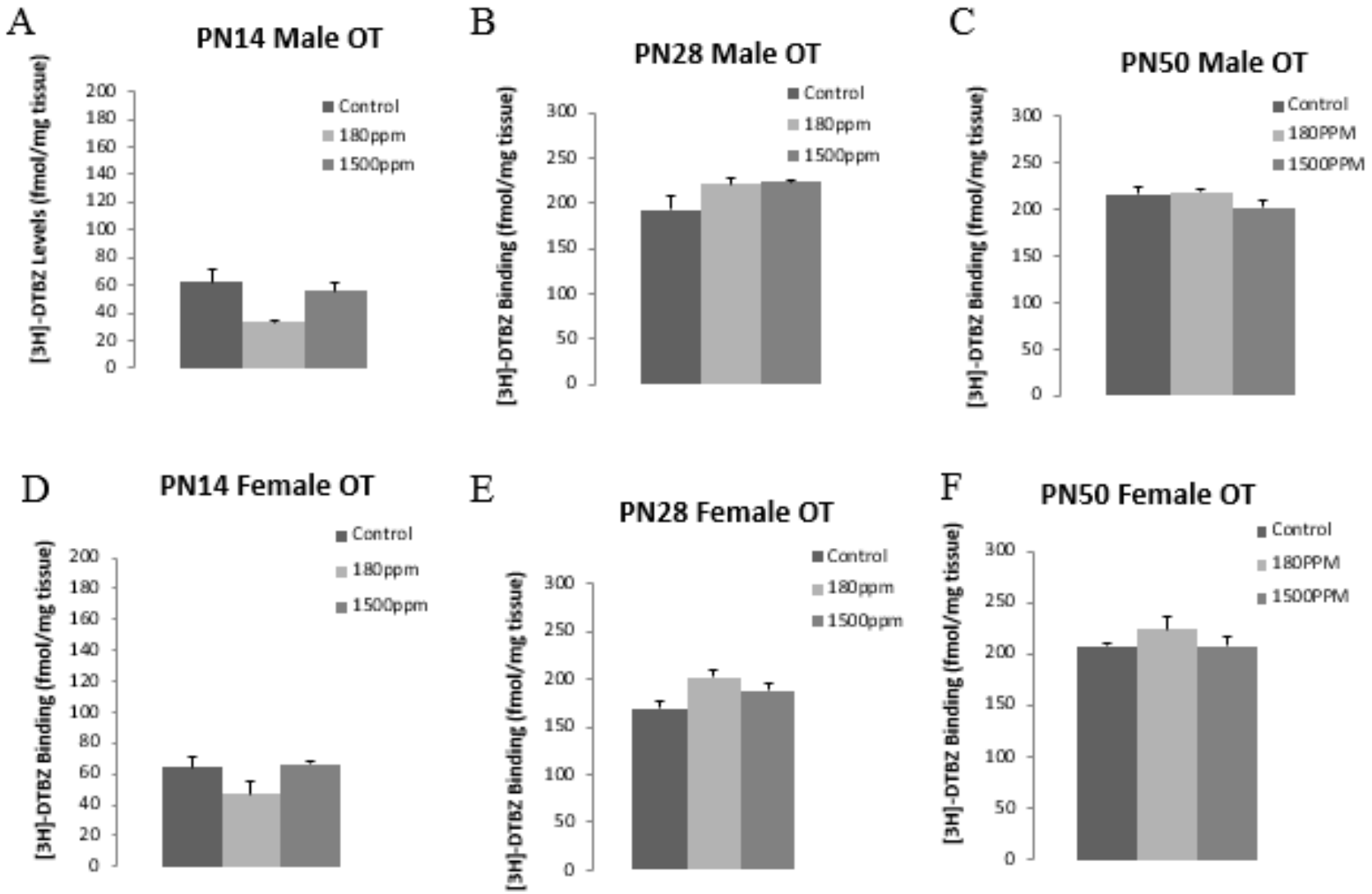

A
PN14 Male OT
[3H]-DTBZ Levels (fmol/mg tissue)
Control
180ppm
1500ppm
B
PN28 Male OT
[3H]-DTBZ Binding (fmol/mg tissue)
Control
180ppm
1500ppm
C
PN50 Male OT
[3H]-DTBZ Binding (fmol/mg tissue)
Control
180PPM
1500PPM
D
PN14 Female OT
[3H]-DTBZ Binding (fmol/mg tissue)
Control
180ppm
1500ppm
E
PN28 Female OT
[3H]-DTBZ Binding (fmol/mg tissue)
Control
180ppm
1500ppm
F
PN50 Female OT
[3H]-DTBZ Binding (fmol/mg tissue)
Control
180PPM
1500PPM

Figure 2.9. VMAT2 levels in the OT. A) At PN14, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the male rat OT ($p=0.03$, $F_{2,14} =4.71$) (control n= 5, 180ppm n= 5, 1500ppm n=6). B) At PN28, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the male rat OT ($p=0.15$, $F_{2,12} =2.23$) (control n= 6, 180ppm n= 6, 1500ppm n=3). C) At PN50, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the male rat OT ($p=0.22$, $F_{2,15} =1.70$) (control n=6, 180ppm n= 5, 1500ppm n=7). D) At PN14, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the female rat OT ($p=0.15$, $F_{2,14}= 2.16$) (control n= 6, 180ppm n= 7, 1500ppm n=4). E) At PN28, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the female rat OT ($p=0.02$, $F_{2,16}= 4.78$) (control n= 8, 180ppm n= 5, 1500ppm n=6). F) At PN50, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the female rat OT ($p=0.40$, $F_{2,17}= 0.97$) (control n=6, 180ppm n= 7, 1500ppm n=7).

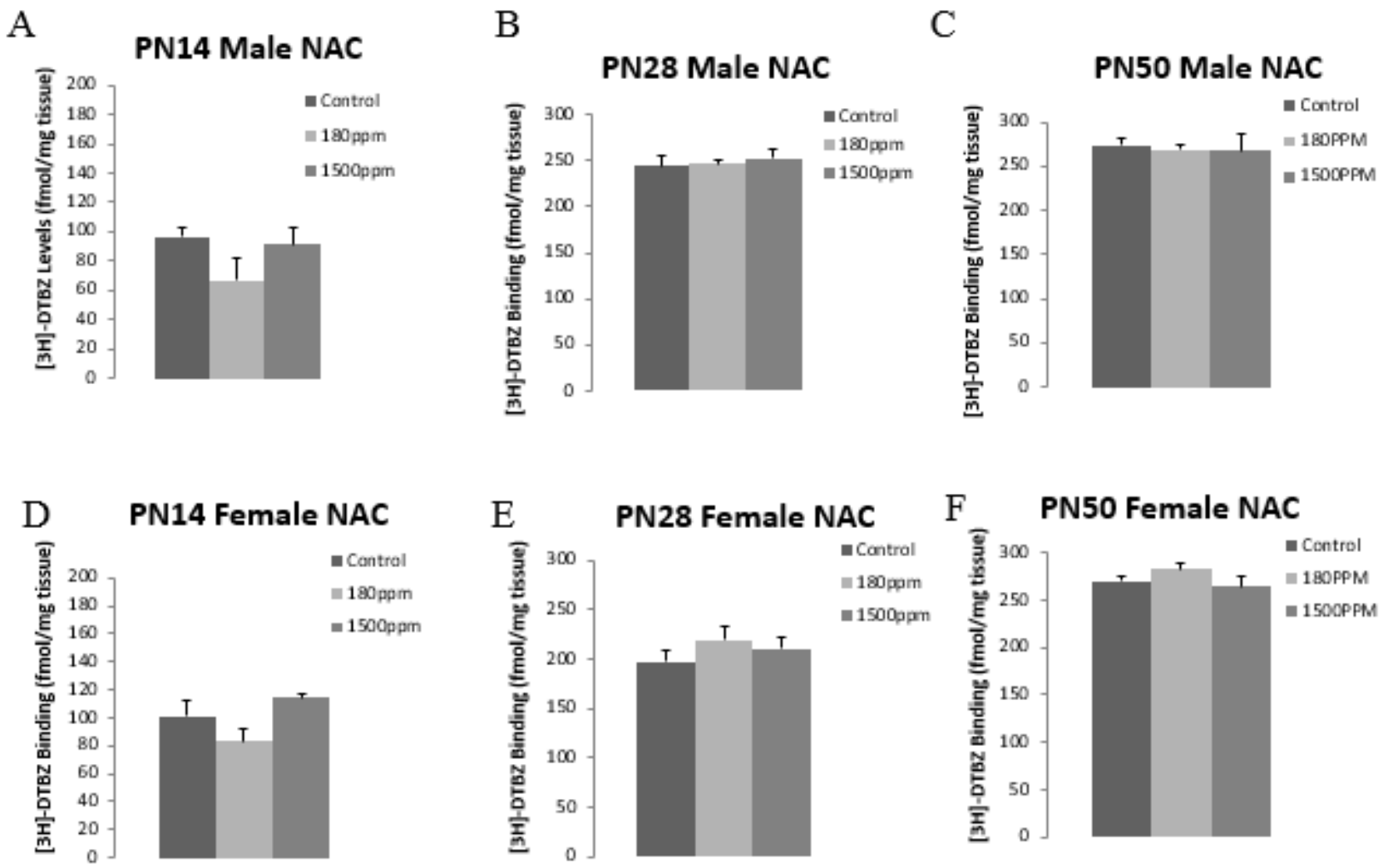

A
PN14 Male NAC
[3H]-DTBZ Levels (fmol/mg tissue)
Control
180ppm
1500ppm
B
PN28 Male NAC
[3H]-DTBZ Binding (fmol/mg tissue)
Control
180ppm
1500ppm
C
PN50 Male NAC
[3H]-DTBZ Binding (fmol/mg tissue)
Control
180PPM
1500PPM
D
PN14 Female NAC
[3H]-DTBZ Binding (fmol/mg tissue)
Control
180ppm
1500ppm
E
PN28 Female NAC
[3H]-DTBZ Binding (fmol/mg tissue)
Control
180ppm
1500ppm
F
PN50 Female NAC
[3H]-DTBZ Binding (fmol/mg tissue)
Control
180PPM
1500PPM

Figure 2.10. VMAT2 levels in the male rat NAC. A) At PN14, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the male rat NAC ($p=0.24$, $F_{2,15}= 1.58$) (control n= 5, 180ppm n= 7, 1500ppm n=6). B) At PN28, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the male rat NAC ($p=0.87$, $F_{2,15}=0.14$) (control n= 8, 180ppm n= 5, 1500ppm n=5). C) At PN50, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the male rat NAC ($p=0.95$, $F_{2,17}= 0.05$) (control n=7, 180ppm n= 5, 1500ppm n=8). D) At PN14, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the female rat NAC ($p=0.10$, $F_{2,15}=2.64$) (control n= 7, 180ppm n= 6, 1500ppm n=5). E) At PN28, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the female rat NAC ($p=0.38$, $F_{2,17}= 1.0$) (control n= 8, 180ppm n= 6, 1500ppm n=6). F) At PN50, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the female rat NAC ($p=0.27$, $F_{2,16}= 1.40$) (control n=6, 180ppm n= 6, 1500ppm n=7).

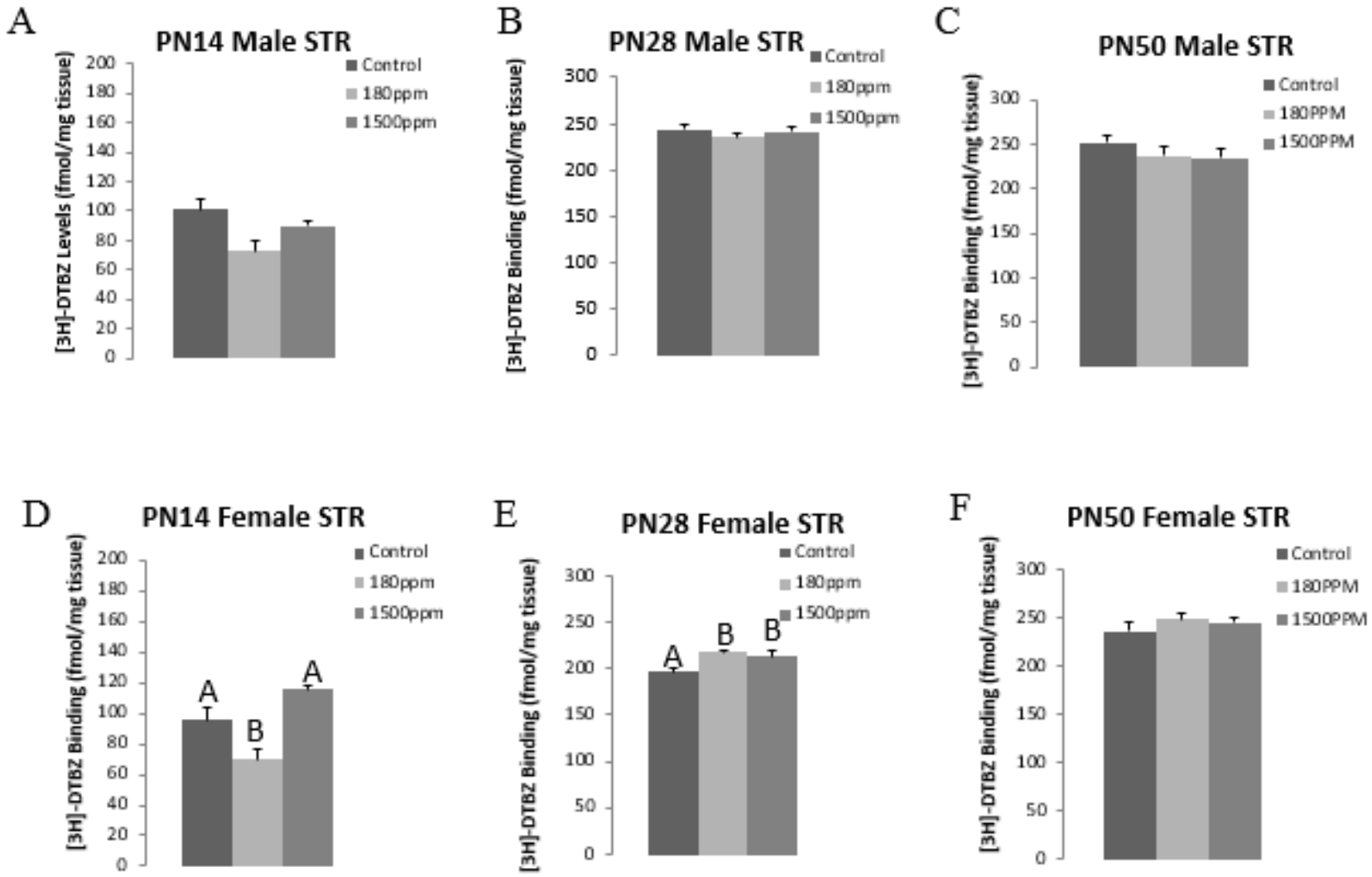

A
PN14 Male STR
[3H]-DTBZ Levels (fmol/mg tissue)
Control
180ppm
1500ppm
B
PN28 Male STR
[3H]-DTBZ Binding (fmol/mg tissue)
C
PN50 Male STR
180PPM
1500PPM
D
PN14 Female STR
E
PN28 Female STR
F
PN50 Female STR

Figure 2.11. VMAT2 levels in the male rat STR. A) At PN14, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the male rat STR ($p=0.02$, $F_{2,15}= 4.83$) (control n= 6, 180ppm n= 6, 1500ppm n=6). B) At PN28, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the male rat STR ($p=0.55$, $F_{2,16}= 0.55$) (control n= 9, 180ppm n= 7, 1500ppm n=3). C) At PN50, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the male rat STR ($p=0.39$, $F_{2,17} =0.99$) (control n=6, 180ppm n= 6, 1500ppm n=8). D) At PN14, when adjusting for multiple comparisons, a significant decrease in VMAT2 levels was detected in the STR of female rats on 180ppm diet versus female rats on control or 1500ppm diet. VMAT2 levels were 17% lower in the STR of females on 180ppm diet versus control ($p=0.0014$, $F_{2,14}= 10.96$) (control n= 6, 180ppm n= 6, 1500ppm n=5). B) At PN28, when adjusting for multiple comparisons, a significant increase in VMAT2 levels were detected in the STR of female rats on 180ppm and 1500ppm diets versus control ($p=0.01$). STR VMAT2 levels increased 10% in females on 180ppm diet and 8% in female rats on 1500ppm diet versus STR of female controls ($p=0.01$, $F_{2,14} = 5.42$) (control n= 7, 180ppm n= 4, 1500ppm n=6). C) At PN50, when adjusting for multiple comparisons, no significant changes in VMAT2 levels were detected in the female rat STR ($p=0.44$, $F_{2,15}= 0.86$) (control n=6, 180ppm n= 6, 1500ppm n=6).

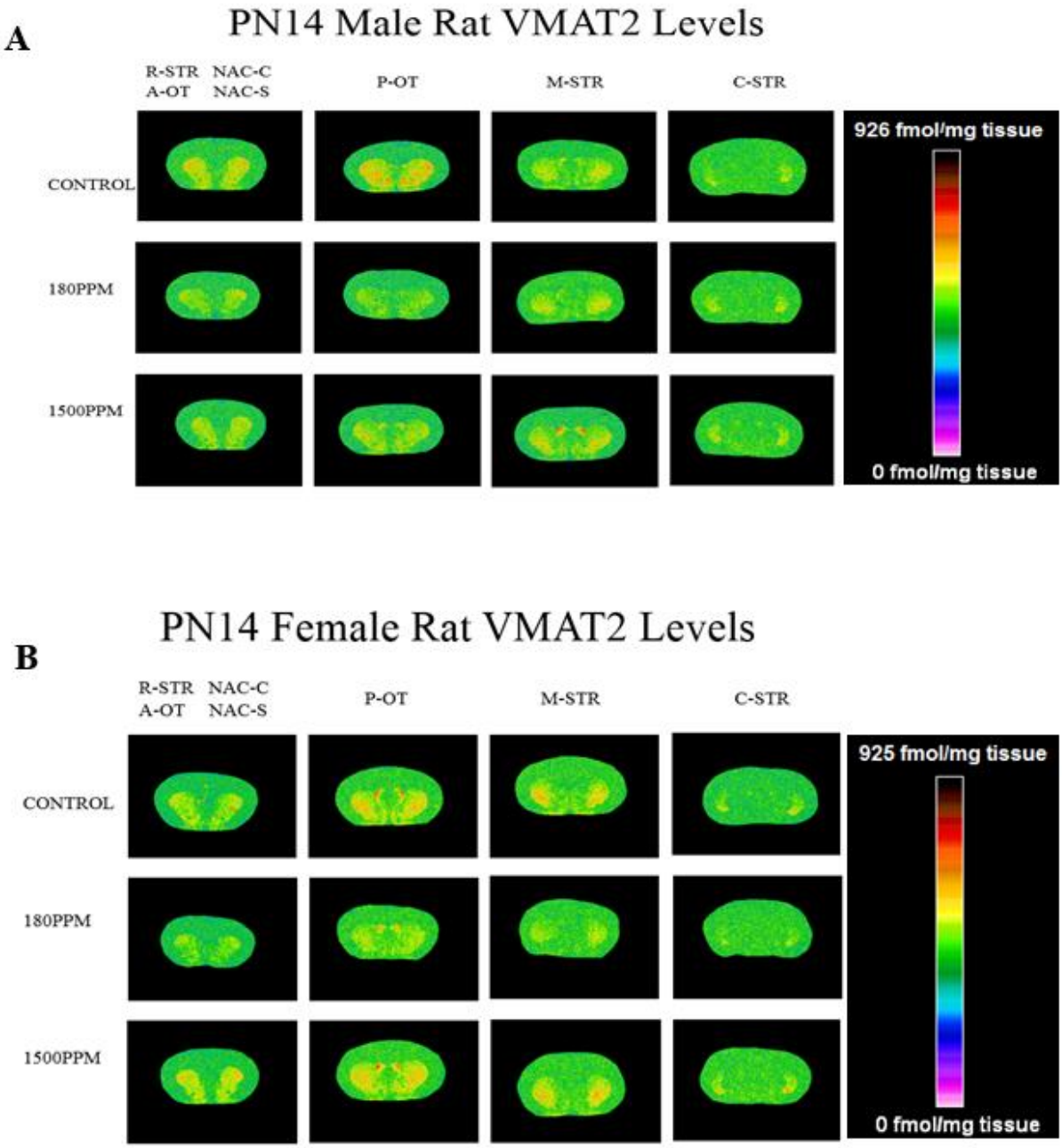


Figure 2.12. Representative autoradiograms illustrating [3H]-DTBZ binding in OT, NAC, and STR of control and Pb^{2+}-exposed male (A) and female (B) rats at PN14.

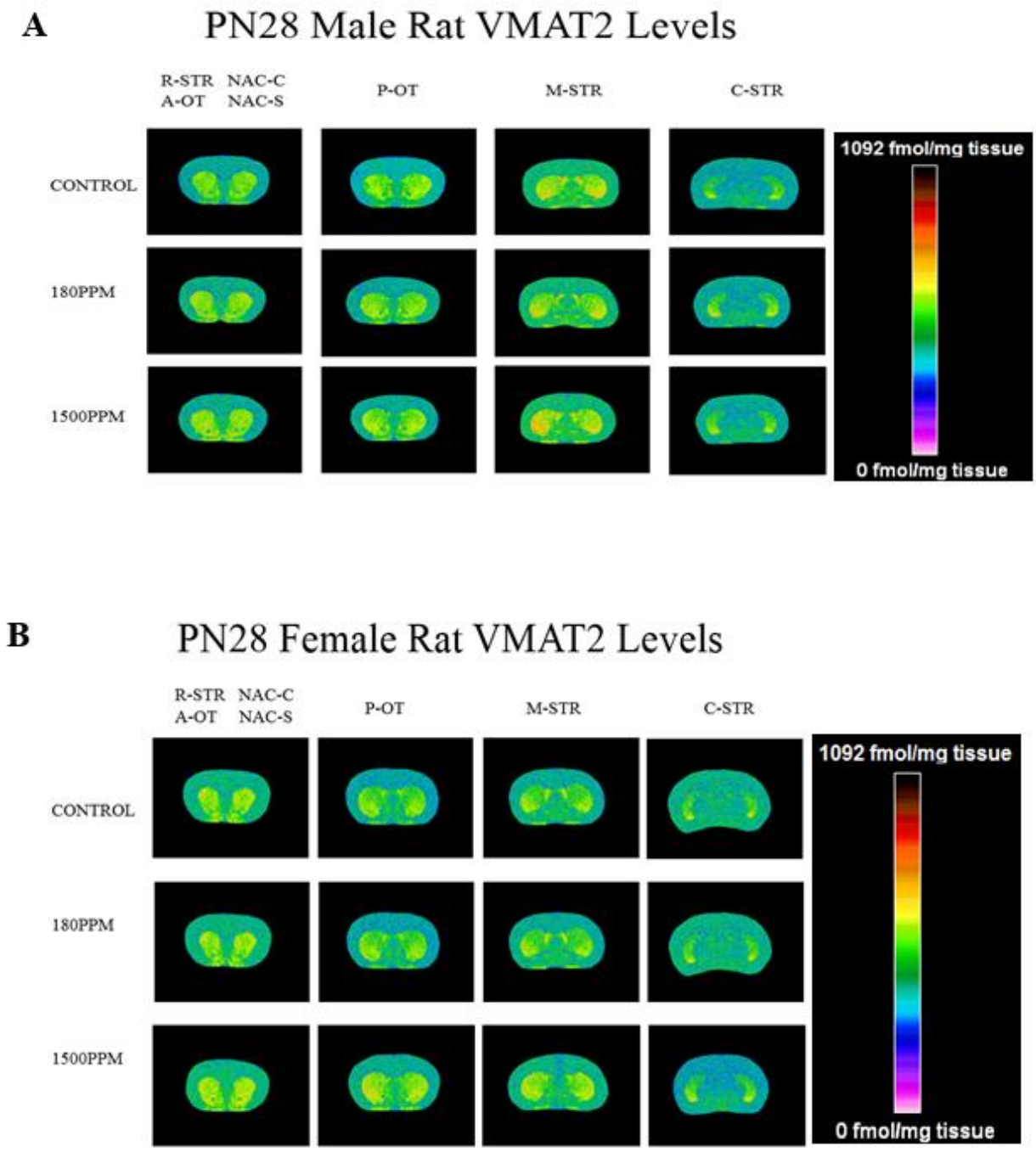


Figure 2.13. Representative autoradiograms illustrating [3H]-DTBZ binding in OT, NAC, and STR of control and Pb^{2+}-exposed male (A) and female (B) rats at PN28.

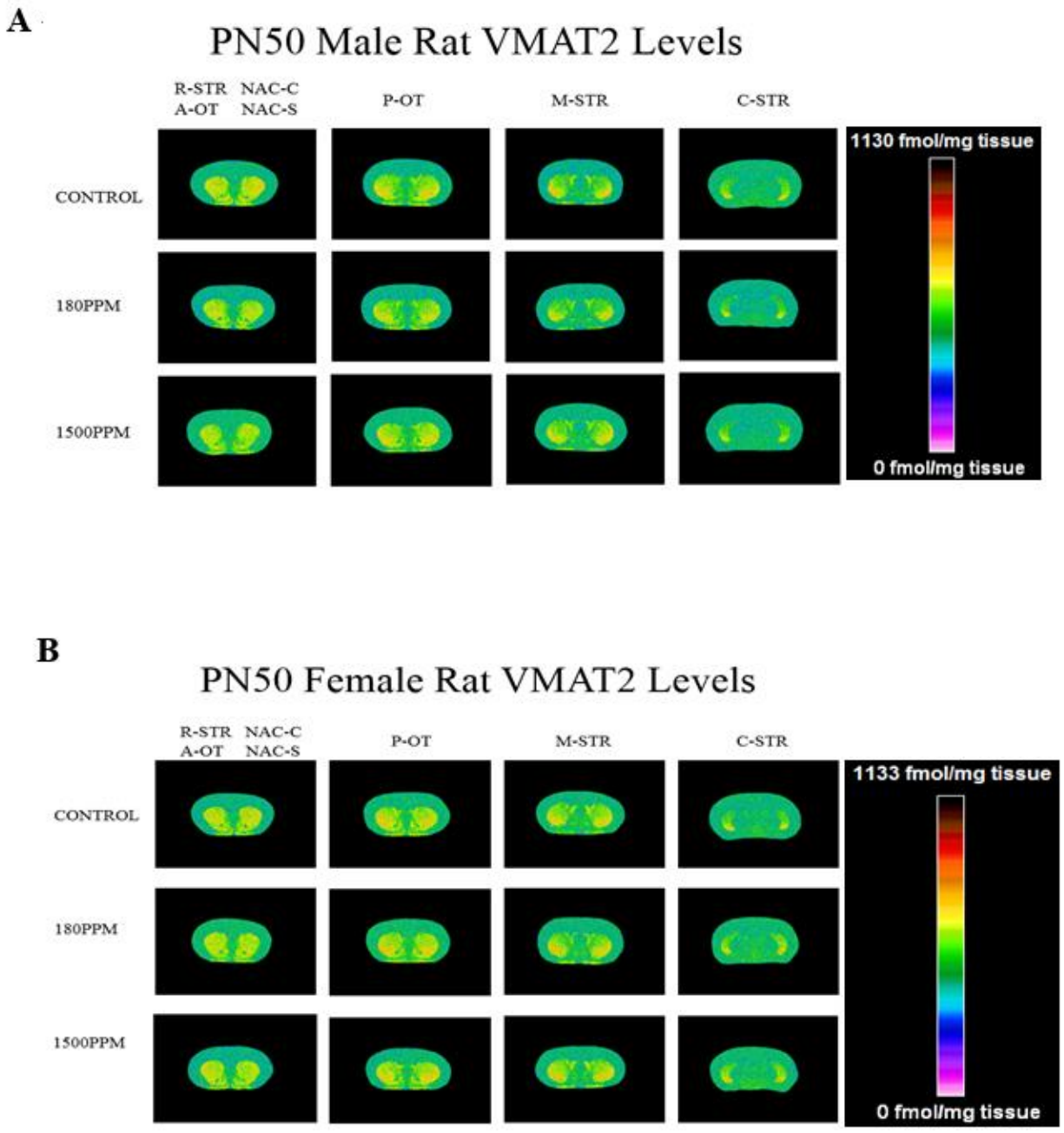


Figure 2.14. Representative autoradiograms illustrating [3H]-DTBZ binding in OT, NAC, and STR of control and Pb^{2+}-exposed male (A) and female (B) rats at PN50.

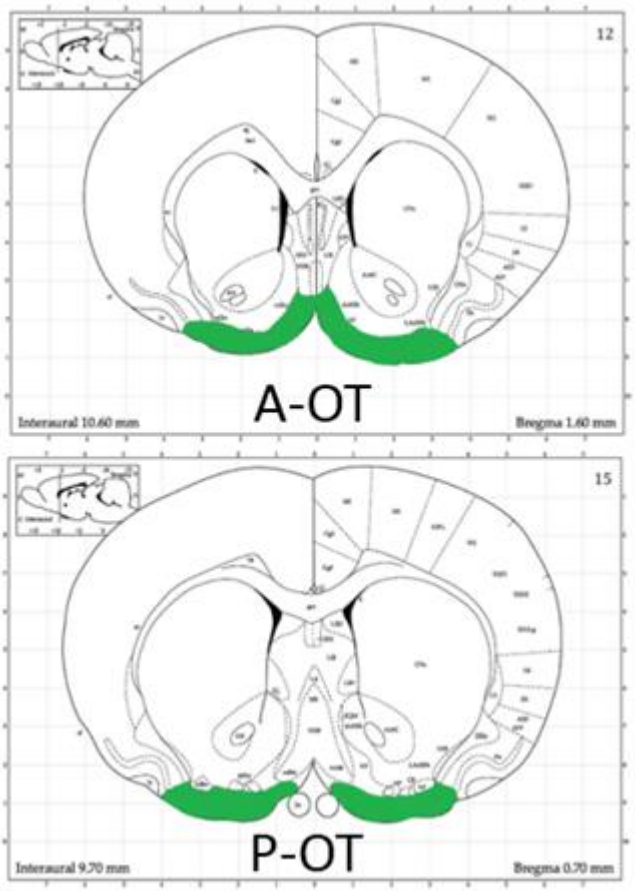


Figure 2.15. Anatomical representation of OT regions (highlighted in green) from which [3H]-DTBZ binding were measured. Regions highlighted are A-OT at Bregma 1.60mm and P-OT at 0.70mm Bregma. Images adapted from (Paxinos and Watson 1998).

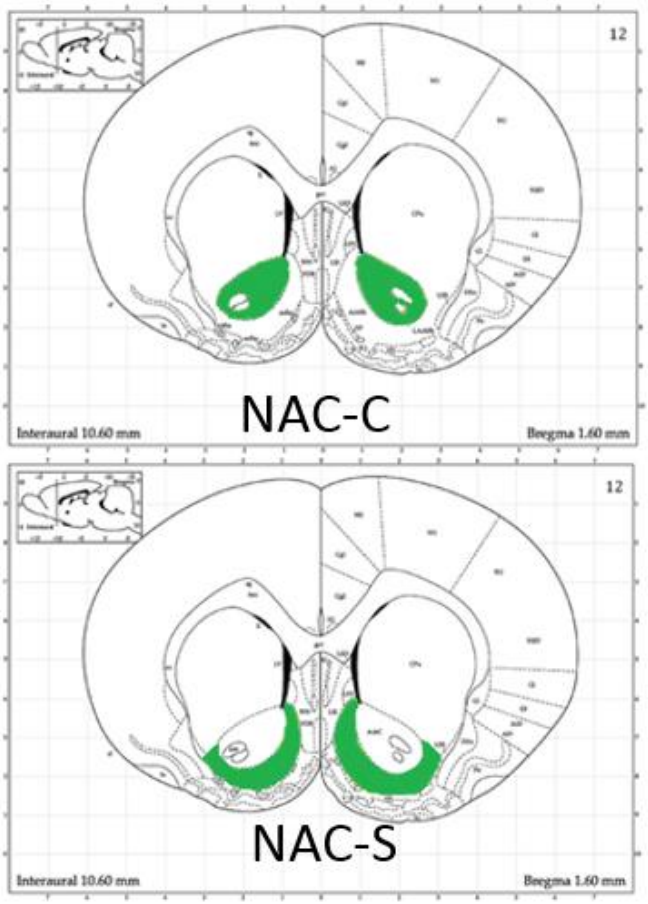


Figure 2.16. Anatomical representation of NAC regions (highlighted in green) from which [3H]-DTBZ binding were measured. Regions highlighted are NAC-C and NAC-S at Bregma 1.60mm. Images adapted from (Paxinos and Watson 1998).

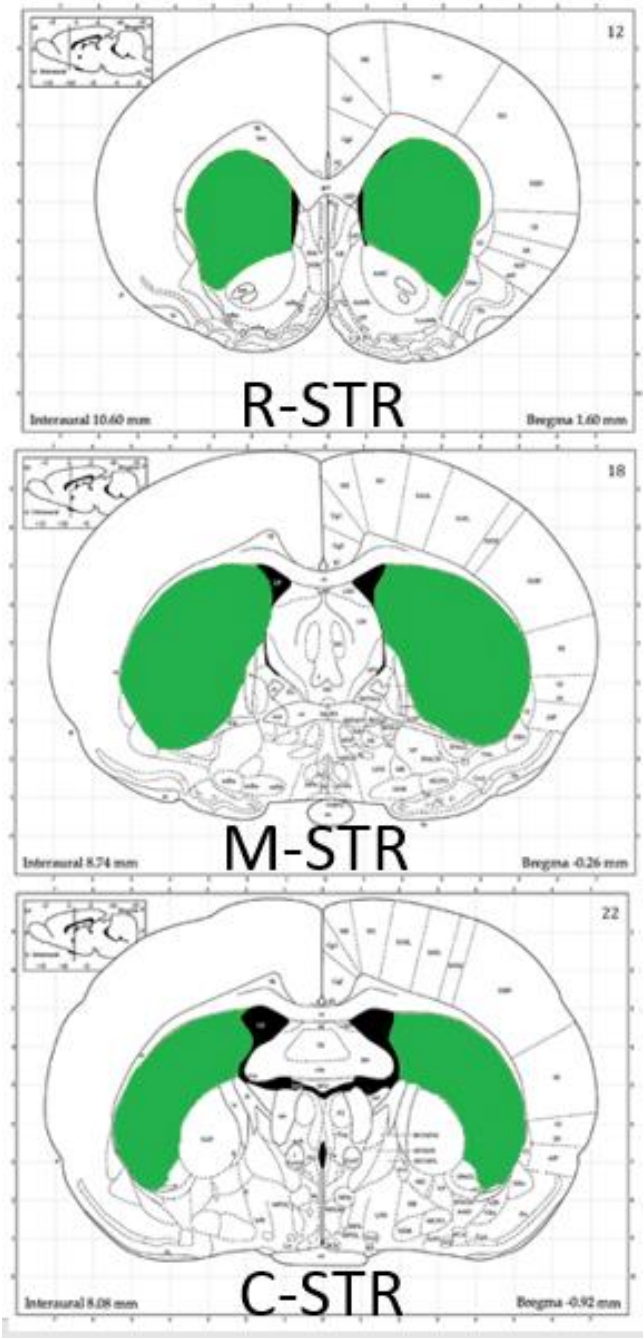


Figure 2.17. Anatomical representation of STR regions (highlighted in green) from which [3H]-DTBZ binding were measured. Regions highlighted are R-STR at Bregma 1.60mm, M-STR at Bregma -0.26mm, and C-STR at Bregma -0.92mm. Images adapted from (Paxinos and Watson 1998).

Discussion

In the present study, we observed increased DA, DA metabolites, and DA turnover in the STR of adolescent and young adult male rats with BLL as low as 4.4µg/dl following chronic Pb^{2+} exposure. Dysregulation of DA levels by biological or pharmacological means has been demonstrated to impact motor activity and other processes regulated by the DAergic system (Jones and Miller 2008). Furthermore, dysregulation of synaptic DA levels in the synaptic cleft may disrupt DAergic circuits underlying reward, motivation, conditioning of habits, and executive function (Volkow, Wang et al. 2011, Volkow, Wang et al. 2011a). This, in turn, may enhance the motivational value of a drug and impair inhibition of actions associated with the desire to take it. The loss of control over drug intake may, in turn, increase the risk for drug addiction (Volkow and Li 2004, Baler and Volkow 2006, Volkow, Wang et al. 2011a). Previous studies suggest that altered mesostriatal and mesolimbic system signaling resulting from excess DA in regions like the STR may underlie the changes in behavior and locomotor activity in Pb^{2+}-intoxicated animals (Zuch, O'Mara et al. 1998, Guilarte, Opler et al. 2012, Stansfield, Ruby et al. 2015).

It is important to note that the increased sensitization to cocaine's psychostimulant effects in female rats with BLL as low as 4.2µg/dl has not been found to be associated with changes in DA, DA metabolites, or DA turnover in females at any age or exposure group (Stansfield, Ruby et al. 2015). It is possible that estrous cycle-dependent variations in progesterone and estrogen levels can underlie some sex-dependent differences in basal DA levels and DA-mediated functions and behaviors (Becker and Rudick 1999). Fluctuations in estrogen and progesterone levels throughout the estrous cycle have been demonstrated to affect release and reuptake of DA

in mesolimbic and mesostriatal DAergic systems (Becker and Beer 1986, Thompson, Thomas et al. 1997).

DA receptor levels and autoreceptor function can also be modulated by hormone fluctuations (Thompson, Thomas et al. 1997, Bobzean, DeNobrega et al. 2014). In the present study, estrous cycle was not taken into consideration when animals were sacrificed and brains collected for analysis. It is possible that females were collected at different stages of the estrous cycle. As a result, potential Pb^{2+}-induced effects in females could have been masked by progesterone- or estrogen-induced effects (Westwood 2008).

TH expression and activation are subject to various short-term and long-term regulatory mechanisms such as feedback inhibition by DA and other catecholamines, phosphorylation of serine residues, D2R autoreceptor activation, and transcriptional regulation (Salvatore, Garcia-Espana et al. 2000, Dunkley, Bobrovskaya et al. 2004, Fujisawa and Okuno 2005). Phosphorylation of TH alters its conformation, resulting in a 300-fold decrease in DA affinity, increasing TH activity. Since phosphorylation of TH at its serine 40 residue increases its activity 20-fold, pser40TH levels were used as a surrogate marker for enzymatic activity of TH (Daubner, Le et al. 2011). The increases in DA observed in adolescent and young adult male rat STR observed in this study were not associated with altered TH expression or activation, as no changes in TH, pser40TH, or pser40TH/TH levels were detected in these animals. Interestingly, increased pser40TH levels were detected in the STR of females on 1500ppm PbAC diet, raising the possibility that TH activity was modulated in our model in a sex-specific manner. As pser40TH levels are an indirect measure of TH activation, further studies of Pb^{2+} impact on direct TH activity are needed.

DA levels in the synapse are tightly regulated by a number of factors, including autoreceptor-mediated regulation of DA release, DA metabolism, reuptake by dopamine transporter (DAT), and repackaging of free intracellular DA into synaptic vesicles via VMAT2 (Walters, Ruskin et al. 2000, Duchemin, Zhang et al. 2009). VMAT2 and DAT both serve as markers for DAergic neurons and have been used to assess presynaptic terminal integrity and function (Stephenson, Childs et al. 2007, Sun, Kouranova et al. 2013). Furthermore, cocaine acts by blocking DAT, preventing DA reuptake and the resulting increase in extracellular DA underlies the psychostimulant effects of cocaine (Volkow, Wang et al. 1997, Miller, Gainetdinov et al. 1999, Volkow, Wang et al. 2000). In our previous study, we observed no changes to either VMAT2 or DAT levels in Pb^{2+}-exposed male rats at PN50 exhibiting increased sensitivity to cocaine's psychostimulant effects, indicating that this increased sensitivity was not mediated by an altered presynaptic environment (Guilarte, Nihei et al. 2003, Stansfield, Ruby et al. 2015). This hypothesis is supported by the observation in our more recent work demonstrating that administration of D1 antagonist SCH23390 prior to injection of cocaine results in a complete block of cocaine-induced locomotor activity in Pb^{2+}-treated animals, suggesting that increased sensitization of Pb^{2+}-exposed animals may be mediated, at least in part, via postsynaptic D1R receptors (Stansfield, Ruby et al. 2015).

Attempts to determine dopamine transporter (DAT) levels in the STR through autoradiography and Western blots in the present study were unsuccessful. We intend to address the impact of chronic Pb^{2+} exposure on DAT across sex, age, and exposure groups analyzed in this study in a future study. As in our previous work, VMAT2 levels were unchanged in exposed males at PN50, PN28, and PN14 and females at PN50 following exposure to either 180ppm or 15000ppm PbAC diet. VMAT2 levels were significantly decreased in the STR of PN14 females

and significantly increased in PN28 females following chronic Pb^{2+} exposure (10% increase for rats on 180ppm PbAC diet versus control, 8% increase for rats on 1500ppm PbAC diet versus control).

Further investigation will be required to elucidate the functional significance of and mechanism(s) underlying the decrease in STR VMAT2 levels at PN14 in females on 180ppm PbAC diet versus control and the increase in STR VMAT2 levels at PN28 in Pb^{2+}-exposed female rats versus control in the absence of changes in DA, DA turnover, and TH expression. The DAergic system undergoes extensive changes and is most vulnerable to Pb^{2+} neurotoxicity from gestational day 19 to PN21 as terminal fields of the DA system are established during this period (Leret, Garcia-Uceda et al. 2002). One potential explanation for changes in VMAT2 levels observed, based on the multiple-hit hypothesis proposed by Cory-Slechta (2005), is that low-level Pb^{2+} exposure may act with another age-dependent factor to alter compensatory mechanisms within the mesolimbic system, leading to altered VMAT2 expression across ages (Cory-Slechta 2005). However, this theory does not adequately explain the regional differences in VMAT2 expression observed in PN14 and PN28 females.

We did not detect any changes in VMAT2 levels in the OT and NAC. However, previous studies have demonstrated that these regions are vulnerable to Pb^{2+} toxicity (Lasley, Greenland et al. 1984, Nation, Frye et al. 1989, Pokora, Richfield et al. 1996, Ramesh and Jadhav 1998, Zuch, O'Mara et al. 1998, White, Cory-Slechta et al. 2007). As such, future studies should be undertaken to determine if DA levels and turnover, as well as TH and pser40TH, are altered in the OT and NAC of male and female rats at the ages and BLLs analyzed in the current study. The results of this study provide further evidence that the drug sensitization observed by Stansfield (2015) was mediated by a hyperactive DAergic state. Furthermore, they suggest that

increases in DA, DA metabolites, and DA turnover in animals may underlie the increased sensitization to cocaine our laboratory previously observed in animals with BLLs as low as 4.4µg/dl. Though we lacked the statistical power to compare effects between age and sex, our results also suggest that Pb^{2+} alters the DAergic system in a time-, sex-dependent manner.

References

Antonio, M. T. and M. L. Leret (2000). "Study of the neurochemical alterations produced in discrete brain areas by perinatal low-level lead exposure." Life Sci **67**(6): 635-642.

Bacopoulos, N. G., S. E. Hattox and R. H. Roth (1979). "3,4-Dihydroxyphenylacetic acid and homovanillic acid in rat plasma: possible indicators of central dopaminergic activity." Eur J Pharmacol **56**(3): 225-236.

Baler, R. D. and N. D. Volkow (2006). "Drug addiction: the neurobiology of disrupted self-control." Trends Mol Med **12**(12): 559-566.

Becker, J. B. and M. E. Beer (1986). "The influence of estrogen on nigrostriatal dopamine activity: behavioral and neurochemical evidence for both pre- and postsynaptic components." Behav Brain Res **19**(1): 27-33.

Becker, J. B. and C. N. Rudick (1999). "Rapid effects of estrogen or progesterone on the amphetamine-induced increase in striatal dopamine are enhanced by estrogen priming: a microdialysis study." Pharmacol Biochem Behav **64**(1): 53-57.

Beery, A. K. and I. Zucker (2011). "Sex bias in neuroscience and biomedical research." Neurosci Biobehav Rev **35**(3): 565-572.

Bobzean, S. A., A. K. DeNobrega and L. I. Perrotti (2014). "Sex differences in the neurobiology of drug addiction." Exp Neurol **259**: 64-74.

Broening, H. W. and S. William Jr (1998). Chapter 12 - Ontogeny of Neurotransmitters: Monoamines A2 - Chang, William SlikkerLouis W. Handbook of Developmental Neurotoxicology. San Diego, Academic Press**:** 245-256.

CDC, Centers for Disease Control and Prevention (2012, 7 Jun 2012). "CDC Response to Advisory Committee on Childhood Lead Poisoning Prevention Recommendations in "Low Level Lead Exposure Harms Children: A Renewed Call for Primary Prevention."." Retrieved 12/10/15, from http://www.cdc.gov/nceh/lead/ACCLPP/Final_Document_030712.pdf

CDC, Centers for Disease Control and Prevention (2015, December 8, 2015). "Lead." Retrieved December 15, 2015, from http://www.cdc.gov/nceh/lead/.

CDC, Centers for Disease Control and Prevention (2016). "Number of Children Tested and Confirmed BLL's ≥10 µg/dL by State, Year, and BLL Group, Children < 72 Months Old."

Chin, K., J. H. Ryu, J. H. Cheong, K. H. Ko and Y. Kuroiwa (1992). "Selective effect of chronic lead ingestion on tyrosine hydroxylase activity in brain regions of rats." J Toxicol Sci **17**(4): 197-210.

Cory-Slechta, D. A. (1995). "Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter system functions." Annu Rev Pharmacol Toxicol **35**: 391-415.

Cory-Slechta, D. A. (2005). "Studying toxicants as single chemicals: does this strategy adequately identify neurotoxic risk?" Neurotoxicology **26**(4): 491-510.

Cory-Slechta, D. A., M. B. Virgolini, M. Thiruchelvam, D. D. Weston and M. R. Bauter (2004). "Maternal stress modulates the effects of developmental lead exposure." Environ Health Perspect **112**(6): 717-730.

Daubner, S. C., T. Le and S. Wang (2011). "Tyrosine hydroxylase and regulation of dopamine synthesis." Arch Biochem Biophys **508**(1): 1-12.

Devi, C. B., G. H. Reddy, R. P. Prasanthi, C. S. Chetty and G. R. Reddy (2005). "Developmental lead exposure alters mitochondrial monoamine oxidase and synaptosomal catecholamine levels in rat brain." Int J Dev Neurosci **23**(4): 375-381.

Duchemin, A. M., H. Zhang, N. H. Neff and M. Hadjiconstantinou (2009). "Increased expression of VMAT2 in dopaminergic neurons during nicotine withdrawal." Neurosci Lett **467**(2): 182-186.

Dunkley, P. R., L. Bobrovskaya, M. E. Graham, E. I. von Nagy-Felsobuki and P. W. Dickson (2004). "Tyrosine hydroxylase phosphorylation: regulation and consequences." J Neurochem **91**(5): 1025-1043.

Fleckenstein, A. E., T. J. Volz and G. R. Hanson (2009). "Psychostimulant-induced alterations in vesicular monoamine transporter-2 function: neurotoxic and therapeutic implications." Neuropharmacology **56 Suppl 1**: 133-138.

Fujisawa, H. and S. Okuno (2005). "Regulatory mechanism of tyrosine hydroxylase activity." Biochem Biophys Res Commun **338**(1): 271-276.

Gedeon, Y., G. T. Ramesh, P. J. Wellman and A. L. Jadhav (2001). "Changes in mesocorticolimbic dopamine and D1/D2 receptor levels after low level lead exposure: a time course study." Toxicol Lett **123**(2-3): 217-226.

Guilarte, T. R., M. K. Nihei, J. L. McGlothan and A. S. Howard (2003). "Methamphetamine-induced deficits of brain monoaminergic neuronal markers: distal axotomy or neuronal plasticity." Neuroscience **122**(2): 499-513.

Guilarte, T. R., M. Opler and M. Pletnikov (2012). "Is lead exposure in early life an environmental risk factor for Schizophrenia? Neurobiological connections and testable hypotheses." Neurotoxicology **33**(3): 560-574.

Guillot, T. S. and G. W. Miller (2009). "Protective actions of the vesicular monoamine transporter 2 (VMAT2) in monoaminergic neurons." Mol Neurobiol **39**(2): 149-170.

Jones, D. C. and G. W. Miller (2008). "The effects of environmental neurotoxicants on the dopaminergic system: A possible role in drug addiction." Biochem Pharmacol **76**(5): 569-581.

Kala, S. V. and A. L. Jadhav (1995). "Low level lead exposure decreases in vivo release of dopamine in the rat nucleus accumbens: a microdialysis study." J Neurochem **65**(4): 1631-1635.

Lasley, S. M., R. D. Greenland, D. J. Minnema and I. A. Michaelson (1984). "Influence of chronic inorganic lead exposure on regional dopamine and 5-hydroxytryptamine turnover in rat brain." Neurochem Res **9**(12): 1675-1688.

Leret, M. L., F. Garcia-Uceda and M. T. Antonio (2002). "Effects of maternal lead administration on monoaminergic, GABAergic and glutamatergic systems." Brain Res Bull **58**(5): 469-473.

Lindgren, N., A. Usiello, M. Goiny, J. Haycock, E. Erbs, P. Greengard, T. Hokfelt, E. Borrelli and G. Fisone (2003). "Distinct roles of dopamine D2L and D2S receptor isoforms in the regulation of protein phosphorylation at presynaptic and postsynaptic sites." Proc Natl Acad Sci U S A **100**(7): 4305-4309.

Ma, T., H. H. Chen and I. K. Ho (1999). "Effects of chronic lead (Pb) exposure on neurobehavioral function and dopaminergic neurotransmitter receptors in rats." Toxicol Lett **105**(2): 111-121.

Mignot, E. and D. Laude (1985). "Study of dopamine turnover by monitoring the decline of dopamine metabolites in rat CSF after alpha-methyl-p-tyrosine." J Neurochem **45**(5): 1527-1533.

Miller, G. W., R. R. Gainetdinov, A. I. Levey and M. G. Caron (1999). "Dopamine transporters and neuronal injury." Trends Pharmacol Sci **20**(10): 424-429.

Nation, J. R., D. M. Baker, B. Taylor and D. E. Clark (1986). "Dietary lead increases ethanol consumption in the rat." Behav Neurosci **100**(4): 525-530.

Nation, J. R., A. L. Cardon, H. M. Heard, R. Valles and G. R. Bratton (2003). "Perinatal lead exposure and relapse to drug-seeking behavior in the rat: a cocaine reinstatement study." Psychopharmacology (Berl) **168**(1-2): 236-243.

Nation, J. R., G. D. Frye, J. Von Stultz and G. R. Bratton (1989). "Effects of combined lead and cadmium exposure: changes in schedule-controlled responding and in dopamine, serotonin, and their metabolites." Behav Neurosci **103**(5): 1108-1114.

Nation, J. R., K. R. Smith and G. R. Bratton (2004). "Early developmental lead exposure increases sensitivity to cocaine in a self-administration paradigm." Pharmacol Biochem Behav **77**(1): 127-135.

Nowak, P., G. Szczerbak, D. Nitka, R. M. Kostrzewa, J. Josko and R. Brus (2008). "Cortical dopaminergic neurotransmission in rats intoxicated with lead during pregnancy. Nitric oxide and hydroxyl radicals formation involvement." Neurotoxicol Teratol **30**(5): 428-432.

Paxinos, G. and C. Watson (1998). The Rat Brain in Stereotaxic Coordinates, Academic Press.

Pokora, M. J., E. K. Richfield and D. A. Cory-Slechta (1996). "Preferential vulnerability of nucleus accumbens dopamine binding sites to low-level lead exposure: time course of effects and interactions with chronic dopamine agonist treatments." J Neurochem **67**(4): 1540-1550.

Ramesh, G. T. and A. L. Jadhav (1998). "Region-specific alterations in tyrosine hydroxylase activity in rats exposed to lead." Mol Cell Biochem **189**(1-2): 19-24.

Salvatore, M. F., A. Garcia-Espana, M. Goldstein, A. Y. Deutch and J. W. Haycock (2000). "Stoichiometry of tyrosine hydroxylase phosphorylation in the nigrostriatal and mesolimbic systems in vivo: effects of acute haloperidol and related compounds." J Neurochem **75**(1): 225-232.

Sheleg, M., C. L. Yochum, G. C. Wagner, R. Zhou and J. R. Richardson (2013). "Ephrin-A5 deficiency alters sensorimotor and monoaminergic development." Behav Brain Res **236**(1): 139-147.

Stansfield KH, M. J., Guilarte TR. (2015). Early Life Lead Exposure and Sensitization to Cocaine: A Novel Pathway to Addiction. . Society of Toxicology. San Diego, CA.

Stansfield, K. H., K. N. Ruby, B. D. Soares, J. L. McGlothan, X. Liu and T. R. Guilarte (2015). "Early-life lead exposure recapitulates the selective loss of parvalbumin-positive GABAergic interneurons and subcortical dopamine system hyperactivity present in schizophrenia." Transl Psychiatry **5**: e522.

Stephenson, D. T., M. A. Childs, Q. Li, S. Carvajal-Gonzalez, A. Opsahl, M. Tengowski, M. D. Meglasson, K. Merchant and M. E. Emborg (2007). "Differential loss of presynaptic dopaminergic markers in Parkinsonian monkeys." Cell Transplant **16**(3): 229-244.

Sun, J., E. Kouranova, X. Cui, R. H. Mach and J. Xu (2013). "Regulation of dopamine presynaptic markers and receptors in the striatum of DJ-1 and Pink1 knockout rats." Neurosci Lett **557 Pt B**: 123-128.

Szczerbak, G., P. Nowak, R. M. Kostrzewa and R. Brus (2007). "Maternal lead exposure produces long-term enhancement of dopaminergic reactivity in rat offspring." Neurochem Res **32**(10): 1791-1798.

Tekin, I., R. Roskoski, Jr., N. Carkaci-Salli and K. E. Vrana (2014). "Complex molecular regulation of tyrosine hydroxylase." J Neural Transm **121**(12): 1451-1481.

Thompson, J., N. Thomas, A. Singleton, M. Piggott, S. Lloyd, E. K. Perry, C. M. Morris, R. H. Perry, I. N. Ferrier and J. A. Court (1997). "D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele." Pharmacogenetics **7**(6): 479-484.

Volkow, N. and T. K. Li (2005). "The neuroscience of addiction." Nat Neurosci **8**(11): 1429-1430.

Volkow, N. D., G. F. Koob and A. T. McLellan (2016). "Neurobiologic Advances from the Brain Disease Model of Addiction." N Engl J Med **374**(4): 363-371.

Volkow, N. D. and T. K. Li (2004). "Drug addiction: the neurobiology of behaviour gone awry." Nat Rev Neurosci **5**(12): 963-970.

Volkow, N. D., G. J. Wang and R. D. Baler (2011a). "Reward, dopamine and the control of food intake: implications for obesity." Trends Cogn Sci **15**(1): 37-46.

Volkow, N. D., G. J. Wang, M. W. Fischman, R. Foltin, J. S. Fowler, D. Franceschi, M. Franceschi, J. Logan, S. J. Gatley, C. Wong, Y. S. Ding, R. Hitzemann and N. Pappas (2000). "Effects of route of administration on cocaine induced dopamine transporter blockade in the human brain." Life Sci **67**(12): 1507-1515.

Volkow, N. D., G. J. Wang, M. W. Fischman, R. W. Foltin, J. S. Fowler, N. N. Abumrad, S. Vitkun, J. Logan, S. J. Gatley, N. Pappas, R. Hitzemann and C. E. Shea (1997). "Relationship between subjective effects of cocaine and dopamine transporter occupancy." Nature **386**(6627): 827-830.

Volkow, N. D., G. J. Wang, J. S. Fowler, D. Tomasi and F. Telang (2011). "Addiction: beyond dopamine reward circuitry." Proc Natl Acad Sci U S A **108**(37): 15037-15042.

Walters, J. R., D. N. Ruskin, K. A. Allers and D. A. Bergstrom (2000). "Pre- and postsynaptic aspects of dopamine-mediated transmission." Trends Neurosci **23**(10 Suppl): S41-47.

Westwood, F. R. (2008). "The female rat reproductive cycle: a practical histological guide to staging." Toxicol Pathol **36**(3): 375-384.

White, L. D., D. A. Cory-Slechta, M. E. Gilbert, E. Tiffany-Castiglioni, N. H. Zawia, M. Virgolini, A. Rossi-George, S. M. Lasley, Y. C. Qian and M. R. Basha (2007). "New and evolving concepts in the neurotoxicology of lead." Toxicol Appl Pharmacol **225**(1): 1-27.

Wilson, J. M. and S. J. Kish (1996). "The vesicular monoamine transporter, in contrast to the dopamine transporter, is not altered by chronic cocaine self-administration in the rat." J Neurosci **16**(10): 3507-3510.

Zuch, C. L., D. J. O'Mara and D. A. Cory-Slechta (1998). "Low-level lead exposure selectively enhances dopamine overflow in nucleus accumbens: an in vivo electrochemistry time course assessment." Toxicol Appl Pharmacol **150**(1): 174-185.

Chapter 3: Chronic Lead Exposure Increases D1 Dopamine Receptor Levels in Rat Olfactory Tubercle, Nucleus Accumbens, and Dorsal Striatum

Abstract

There is growing evidence that lead (Pb^{2+}) exposure induces dopaminergic (DAergic) system hyperactivity by increasing dopamine (DA) levels and DA turnover. This may, in turn, increase susceptibility to drug addiction. Studies described in Chapter 2 demonstrated that chronic exposure to Pb^{2+} increases DA, 3,4-Dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and DA turnover in the dorsal striatum (STR) of adolescent and young adult male rats exposed to Pb^{2+}. As DAergic neurons of both the mesostriatal and mesolimbic systems project to the STR, DAergic signaling in both of these systems may be affected. The downstream effects of DA on these systems are dependent on whether DA binds to a D1- or D2-like DA receptor subtype (D1R, D2R) at the synapse. Whereas D1R activation results in excitatory downstream signaling processes, activation of D2R by DA results in inhibitory downstream signaling processes. In the present study, we used quantitative receptor autoradiography to measure D1R and D2R levels in the STR, nucleus accumbens (NAC), and olfactory tubercle (OT) of juvenile (PN14), adolescent (PN28), and young adult (PN50) male and female rats following chronic exposure to 180ppm Pb^{2+} acetate (PbAC) diet and 1500ppm PbAC in diet to elucidate the impact of Pb^{2+} on DAergic signaling processes.

We observed that chronic exposure to Pb^{2+} led to significant increases in OT D1R levels in male and female rats at PN28. Significant increases in D1R levels were also detected in the NAC and STR of male rats at PN28 following chronic Pb^{2+} exposure. A significant increase in D2R levels was also detected in the STR of PN14 male rats exposed to 180ppm PbAC diet. The results of this study expand upon previous findings by demonstrating increased expression of D1R in adolescent female OT as well as in male rat STR, NAC, and OT. To our knowledge, this is the first study to describe increased D1R levels in the OT elicited by chronic Pb^{2+} exposure.

Furthermore, our results suggest that Pb^{2+} exposure-induced D1R upregulation in the OT is a likely mechanism underlying the increased sensitivity to cocaine's psychostimulant effects observed in Pb^{2+}-exposed rats in previous work by our laboratory (Stansfield, Ruby et al. 2015).

Introduction

The prevalence of low-level Pb^{2+} exposure has historically been disproportionately higher in low-income and minority populations in urban areas (Miller, Nation et al. 2000, Nation, Smith et al. 2004, Sanders, Liu et al. 2009). These populations are also at a higher risk for drug addiction (Ensminger, Anthony et al. 1997, Ensminger, Juon et al. 2002). Not all individuals who use drugs become addicted and there is considerable evidence that genetic, as well as environmental, factors increase vulnerability to drug addiction (Volkow and Li 2004, Volkow and Wise 2005, Volkow, Wang et al. 2011). Environmental Pb^{2+} exposure has been associated with increased risk for drug addiction (Fishbein, Todd et al. 2008, Jones and Miller 2008). Furthermore, a number of studies have demonstrated that Pb^{2+} exposure sensitizes animals to addictive drugs (Miller, Nation et al. 2000, Nation, Miller et al. 2000, Miller, Nation et al. 2001, Nation, Cardon et al. 2003, Nation, Smith et al. 2004).

The dopaminergic (DAergic) system underlies many processes affected by Pb^{2+} exposure, among them attention, reward, and addiction (Cory-Slechta 1995, Koepp, Gunn et al. 1998, Jones and Miller 2008). Disruption of DAergic signaling underlying reward, motivation, executive function and conditioning may increase the risk for drug addiction, by enhancing a drug's motivational value and by impairing inhibition of actions associated with the desire to take a drug (Volkow, Wang et al. 2011, Volkow, Wang et al. 2011). DA release, synthesis, turnover, and metabolism are altered by Pb^{2+} as evidenced by the results in the previous chapter,

as well as other work by our laboratory and others (Nation, Frye et al. 1989, Cory-Slechta 1995, Zuch, O'Mara et al. 1998, Leret, Garcia-Uceda et al. 2002, Devi, Reddy et al. 2005, Szczerbak, Nowak et al. 2007, Stansfield, Ruby et al. 2015). Altered expression and function of DA receptors and DA transporters has also been described, as well as impaired D2 autoreceptor-mediated regulation of DA synthesis in presynaptic neurons (Lasley and Lane 1988, Cory-Slechta 1995, Pokora, Richfield et al. 1996, Zuch, O'Mara et al. 1998)

Previously, our laboratory demonstrated that Pb^{2+} exposure increases DA turnover and D2R levels in the striatum of Pb^{2+}-exposed young adult male rats with blood Pb^{2+} levels (BLL) of 22.2µg/dl (Stansfield, Ruby et al. 2015) We also demonstrated increased locomotor sensitization to cocaine in the same animal model. Further work by our laboratory has demonstrated that male rats with BLLs as low as 4.5µg/dl exhibited increased locomotor activity following a 5mg/kg (low dose) injection of cocaine compared to control animals receiving the same cocaine dose. A 15mg/kg (high dose) cocaine injection, however, resulted in similar levels of cocaine-induced locomotor activity between animals with BLLs of 4.5µg/dl and control animals. Administration of the D1-like DA receptor (D1R) antagonist SCH-23390 completely blocked this effect and administration of D2-like DA receptor (D2R) antagonist Raclopride dampened the effect, indicating that the increased stimulatory effects of cocaine in Pb^{2+}-treated rats may be primarily mediated by D1R (Stansfield KH 2015). However, the manner in which Pb^{2+} exposure alters DA receptor ontogeny is still unclear.

DA regulates the flow of information through the STR and other regions of the DAergic system such as the nucleus accumbens (NAC) and olfactory tubercle (OT). Downstream effects of DA are dependent on its activation of D1- or D2-like DA receptor (D1R and D2R, respectively) subtypes. Activation of D1R stimulates cyclic adenosine monophosphate (cAMP)

production, which increases protein kinase A (PKA) activity. Increased PKA activity, in turn, results in increased phosphorylation of dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32) at its threonine-34 (Thr34) residue (Beaulieu and Gainetdinov 2011). Following phosphorylation of Thr34, DARPP-32 acts as a potent protein phosphatase 1 (PP-1) inhibitor (Nairn, Svenningsson et al. 2004). Inhibition of PP-1, in turn, enhances phosphorylation of extracellular-signal regulated kinases (ERK) and results in increased activation of transcription factors such as cAMP response element binding protein (CREB), which can ultimately influence gene expression and long-term synaptic plasticity in regions associated with reward and inhibition control (Beaulieu and Gainetdinov 2011, Volkow, Koob et al. 2016). Activation of D2R, on the other hand, results in decreased cAMP production which, in turn, results in decreased PKA activity. Decreased PKA activity can lead to decreased phosphorylation of substrates such as CREB (Koob and Volkow 2010, Beaulieu and Gainetdinov 2011).

These two opposing systems work together to regulate a large number of central nervous system processes and are vulnerable to the neurotoxic effects of Pb^{2+} (Cory-Slechta and Widzowski 1991, Nishi, Snyder et al. 1997, Miller, Nation et al. 2001, Nation, Smith et al. 2004, Surmeier, Ding et al. 2007, Szczerbak, Nowak et al. 2007). However, there is considerable disagreement in the literature concerning the manner in which these signaling systems are affected (Cory-Slechta, Crofton et al. 2001, Nation, Smith et al. 2004, Jones and Miller 2008)

The present study seeks to elucidate the altered neurobiology that may underlie the increased sensitivity of Pb^{2+}-exposed rats to the psychostimulant effects of cocaine. As we previously demonstrated that sensitivity to cocaine's psychostimulant effects was blocked by administration of a D1R antagonist and dampened by the administration of a D2-like DA receptor (D2R) antagonist (Stansfield KH 2015), we used quantitative receptor autoradiography

to measure D1R and D2R levels in the STR, NAC, and OT, as these brain regions are known to be affected by psychostimulants and involved in the onset of drug addiction (Ikemoto 2002, Ikemoto 2003, Ikemoto 2007, Ikemoto 2010). The present study also sought to address limitations in our current understanding of Pb^{2+}'s effects on DA receptor ontogeny by analyzing the effects of Pb^{2+} in juvenile (postnatal day 14, PN14), adolescent (PN28), and young adult (PN50) male and female rats following chronic exposure to 180ppm or 1500ppm PbAC.

Materials and Methods

Animals

All animal studies were approved by the Columbia University Medical Center Animal Care and Use Committee and have been carried out in accordance with the Guide for Care and Use of Laboratory Animals as stated by the U.S. National Institutes of Health. Long-Evans rats were purchased from Charles River, Inc. and fed 0-, 180-, or 1500ppm PbAC diet. PbAC diet was prepared by and purchased from Dyets (Dyets, Bethlehem, PA, USA). Diet comprised of Purina RMH 1000 diet with PbAC incorporated into chow mix. Food and water were provided *ad libitum* to animals. Dams were initiated on Pb^{2+} diet 10 days prior to mating with Long-Evans male rats, which were maintained on control diet at all times. Litters were culled to 10 pups per litter at postnatal day PN 1-2 and weaned on PN21. Upon weaning, rats were maintained on same diet as their respective mother. Rats were maintained on a 12-hour light-dark cycle until sacrificed at PN14, PN28, and PN50. For PN14 age group, all pups from a litter were euthanized on the same day.

Blood Pb^{2+} levels

Rats were anesthetized with a 25mg/kg dose of pentobarbital. Blood was collected transcardially from rats for each age group. BLL were measured using a Magellan Pb^{2+}Care analyzer using manufacturer's instructions (ESA Laboratories, Chelmsford, MA, USA). BLL were averaged between litters in each exposure group at each age and for each sex.

Tissue Collection

For D1R and D2R quantitative autoradiography, rat brains were harvested immediately after decapitation, snap frozen, and then stored at -80ºC until used. One male and one female brain was used per litter for statistical purposes. Therefore, the litter was the statistical unit.

Quantitative Autoradiography

Fresh-frozen brains were sectioned at 20-micron thickness in the coronal plane on a freezing cryostat (Leica Biosystems) and thaw-mounted on poly-L-lysine-coated slides. Slides were stored at − 20 °C until used.

D1R Autoradiography

For D1R autoradiography, slides were pre-incubated in 50mM Tris buffer (pH 7.4) at room temperature for 20 minutes. For total binding, slides were incubated in Tris buffer with [^{3}H]-SCH23390 (1.36nM, 1.1nM and 1.39nM for PN14, PN28 and PN50 rats, respectively) in Tris buffer (pH 7.4) for 30 minutes at room temperature. Nonspecific binding was determined by adding 5µM butaclamol to the buffer. Slides were rinsed twice in buffer at 4^{O}C and then dipped once in dH_2O at 4^{O}C. The slides were then dried at room temperature overnight.

D2R Autoradiography

For D2R autoradiography, slides were incubated in [^{3}H]-Raclopride (3.0nM for PN14 and PN50, 2.9nM for PN28) in 170mM Tris-HCl buffer (pH 7.4) for 30 min at room temperature for total binding. Nonspecific binding was determined in the presence of 10 μM haloperidol (D2R antagonist). The slides were then washed for 1min in buffer at 4 °C four times, and then quickly dipped in dH20 at 4 °C. Slides were then dried at room temperature overnight.

For both D1R and D2R autoradiography, after drying overnight, slides were apposed to Kodak Biomax MR film, MR-1, for 6 weeks. [^{3}H]-Microscales (Amersham, Arlington Heights, IL, USA) were included with each film to allow for quantitative analysis of images. Images were captured and analyzed using MCID Imaging software (MCID, InterFocus Imaging, Cambridgeshire, UK). A rat brain atlas (Paxinos and Watson 1998) was used to define regions in the OT, NAC, and STR to be analyzed. D1R and D2R levels in OT were determined by averaging binding intensity measurements for anterior OT (A-OT) (at Bregma 1.60mm) and posterior OT (P-OT) (at Bregma 0.70mm) (**Fig. 2.15**). D1R and D2R levels in NAC were determined by averaging binding intensity measurements for the NAC core (NAC-C) and NAC shell (NAC-S) (both at Bregma 1.60) (**Fig. 2.16**). D1R and D2R levels in STR were determined by averaging binding intensity measurements for rostral STR (R-STR) (at Bregma 1.60mm), middle STR (M-STR) (at Bregma -0.26mm), and caudal STR (C-STR) (at Bregma -0.92mm) (**Fig. 2.17).** For representative D1R autoradiograms of regions measured, see **Fig. 3.7A, Fig. 3.8A,** and **Fig. 3.9A** for male and **Fig. 3.7B, Fig. 3.8B,** and **Fig. 3.9B** for female rat images. For representative D2R autoradiograms of regions measured, see **Fig. 3.10A, Fig. 3.11A,** and **Fig. 3.12A** for male and **Fig. 3.10B, Fig. 3.11B,** and **Fig. 3.12B** for female rat images.

Data and Statistical Analysis

Statistical analysis was performed using one-way ANOVA with post hoc Tukey's test (Graphpad Software, Inc.). A Bonferroni correction was used for statistical analysis of D1R and D2R autoradiography data to account for multiple comparison across three brain. For autoradiography analysis of OT, NAC, and STR, values of $p \leq .017$ were considered statistically significant. D1R and D2R levels for OT, NAC, and STR are presented as femtomoles per milligram tissue (fmol/mg tissue). To calculate outliers, data for each group was divided into quartiles. Outliers were defined as any data point more than 1.5 interquartile ranges below the first quartile or above the third quartile.

Results

BLLs in our rat model of Pb^{2+} exposure

The exposure paradigm utilized in this study resulted in BLLs that were within the range of those detected in children aged 1-5 in recent NHANES surveys irrespective of age, sex, and exposure of animal, as demonstrated in the previous chapter (see **Table 2.1A-C** and **Fig. 2.1A-C** for BLL data) (CDC 2012, CDC 2016).

Increased D1R levels in OT, NAC, and STR of rats following chronic exposure to Pb^{2+}

Increased D1R levels in young adolescent rat OT following chronic exposure to Pb^{2+}

At PN14, after adjusting for multiple comparisons, no significant changes in D1R levels were detected in exposed male ($p=0.03$, $F_{2,13} =6.39$) or female ($p=0.35$, $F_{2,14}= 1.13$) rat OT versus control (**Fig. 3.1A**, **Fig. 3.1D**). At PN28, significant increases in D1R levels were detected

in exposed male (17% and 48% increase for rats on 180ppm and 1500ppm PbAC diet, respectively) ($p=0.0001$, $F_{2,14}=19.80$) and female (41% increase for rats on 1500ppm PbAC diet versus control) ($p=0.0005$, $F_{2,16}=12.45$) rat OT versus control (**Fig. 3.1B**, **Fig. 3.1E**). Furthermore, a dose-dependent increase in D1R levels was detected in the OT of exposed male rats at PN28 versus control. At PN50, no significant changes in D1R levels were detected in exposed male ($p=0.09$, $F_{2,14}=2.82$) or female ($p=0.64$, $F_{2,14}= 0.47$) rat OT versus control (**Fig. 3.1C**, **Fig. 3.1F**).

Increased D1R levels in young adolescent male rat NAC following chronic exposure to Pb^{2+}

At PN14, no significant changes in D1R levels were detected in exposed male ($p=0.09$, $F_{2,16}= 2.72$) or female ($p=0.61$, $F_{2,18}= 0.51$) rat NAC versus control (**Fig. 3.2A**, **Fig. 3.2D**). A 21% increase in D1R levels was detected at PN28 in male rat NAC for males on 1500ppm PbAC diet versus control ($p=0.0026$, $F_{2,15}=9.03$) (**Fig. 3.2B**). However, no changes in D1R levels were detected in exposed female rat NAC versus control ($p=0.39$, $F_{2,15}= 1.0$) at PN28 (**Fig. 3.2E**). No differences in D1R levels were detected in either exposed male ($p=0.2$, $F_{2,16}= 1.78$) or exposed female ($p=0.29$, $F_{2,12}= 1.37$) rat NAC at PN50 versus control (**Fig. 3.2C**, **Fig. 3.2F**).

Increased D1R levels in young adolescent male rat STR following chronic exposure to Pb^{2+}

At PN14, no significant changes in D1R levels were detected in exposed male ($p=0.07$, $F_{2,16}= 3.13$) or female ($p=0.04$, $F_{2,17}= 3.83$) rat STR versus control (**Fig. 3.3A**, **Fig. 3.3D**). A 22% increase in D1R levels was detected in the STR of PN28 males exposed to 1500ppm PbAC diet ($p=0.006$, $F_{2,17}=7.02$) versus control (**Fig. 3.3B)**. No significant changes in D1R levels were detected in exposed female rat STR at PN28 versus control (**Fig. 3.3E**). At PN50, no significant

changes in D1R levels were detected in exposed male (p=0.49, $F_{2,16}$ =.75) or female (p=0.16, $F_{2,15}$=2.07) rat STR versus control (**Fig. 3.3C**, **Fig. 3.3F**).

Increased D2R levels in juvenile male rat STR following chronic exposure to Pb^{2+}

No detectable changes in OT D2R levels following chronic exposure to Pb^{2+}

At PN14, no significant changes in D2R levels were detected in exposed male (p=0.18, $F_{2,15}$=1.94) or female (p=0.15, $F_{2,15}$= 2.12) rat OT versus control (**Fig. 3.4A**, **Fig. 3.4D**). No significant changes in D2R levels were detected in exposed male (p=0.77, $F_{2,18}$=0.26) or female (p=0.67, $F_{2,13}$=0.41) rat OT at PN28 versus control (**Fig. 3.4B**, **Fig. 3.4E**). At PN50, no significant changes in D2R levels were detected in exposed male (p=0.29, $F_{2,16}$ =1.32) or female (p=0.43, $F_{2,14}$=0.89) rat OT versus control (**Fig. 3.4C**, **Fig. 3.4F**).

No detectable changes in NAC D2R levels following chronic exposure to Pb^{2}

No significant changes in D2R levels were detected in exposed male (p=0.13, $F_{2,17}$= 2.33) or female (p=0.52, $F_{2,14}$= 0.68) rat NAC at PN14 versus control (**Fig. 3.5A**, **Fig. 3.5D**). At PN28, no significant changes in D2R levels were detected in exposed male (p=0.86, $F_{2,15}$= 2.33) or female (p=0.11, $F_{2,14}$= 2.62) rat NAC versus control (**Fig. 3.5B**, **Fig. 3.5E**). At PN50, no significant changes in D2R levels were detected in exposed male (p=0.90, $F_{2,18}$= 1.78) or female (p=0.02, $F_{2,16}$= 4.98) rat NAC versus control (**Fig. 3.5C**, **Fig. 3.5F**).

Increased D2R levels in juvenile male rat STR following chronic exposure to Pb^{2+}

A 30% increase in D2R levels was detected in the STR of PN14 male rats exposed to 180ppm PbAC diet (p=0.01, $F_{2,15}$=5.05) versus control (**Fig. 3.6A**). After adjusting for multiple

comparisons, no significant changes in D2R levels were detected in exposed female rat STR ($p=0.02$, $F_{2,13}= 5.24$) at PN14 versus control (**Fig. 3.6D**). At PN28, no significant changes in D2R levels were detected in exposed male ($p=0.03$, $F_{2,15} =4.63$) or female ($p=0.80$, $F_{2,16}=0.22$) rat STR versus control after adjusting for multiple comparisons (**Fig. 3.6B**, **Fig. 3.6E**). No significant change in D2R levels was detected in exposed male ($p=0.46$, $F_{2,14}=0.82$) or female ($p=0.35$, $F_{2,17}=1.12$) rat STR at PN50 versus control (**Fig. 3.6C**, **Fig. 3.6F**).

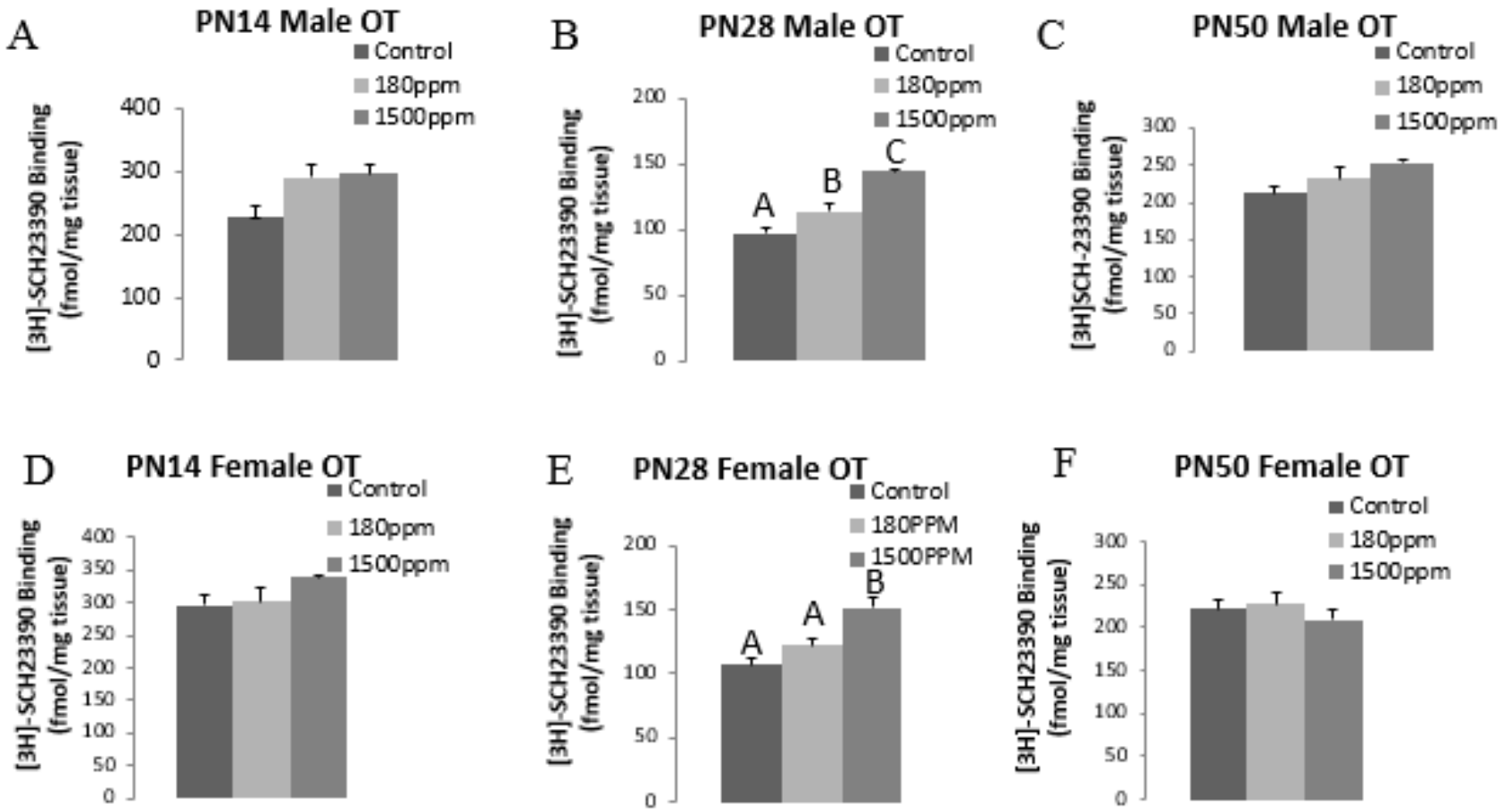

A
PN14 Male OT
Control
180ppm
1500ppm
[3H]-SCH23390 Binding (fmol/mg tissue)
0
100
200
300
400
B
PN28 Male OT
Control
180ppm
1500ppm
[3H]-SCH23390 Binding (fmol/mg tissue)
0
50
100
150
200
A
B
C
C
PN50 Male OT
Control
180ppm
1500ppm
[3H]SCH-23390 Binding (fmol/mg tissue)
0
50
100
150
200
250
300
D
PN14 Female OT
Control
180ppm
1500ppm
[3H]-SCH23390 Binding (fmol/mg tissue)
0
50
100
150
200
250
300
350
400
E
PN28 Female OT
Control
180PPM
1500PPM
[3H]-SCH23390 Binding (fmol/mg tissue)
0
50
100
150
200
A
A
B
F
PN50 Female OT
Control
180ppm
1500ppm
[3H]-SCH23390 Binding (fmol/mg tissue)
0
50
100
150
200
250
300

Figure 3.1 D1R levels in the male and female rat OT. A) At PN14, after adjusting for multiple comparisons, no significant changes in D1R levels were detected in the OT ($p=0.03$, $F_{2,13}=6.39$) (control n= 5, 180ppm n= 5, 1500ppm n=6). B) At PN28, a highly significant dose-dependent increase in D1R levels was detected in the OT of rats on PbAC (17% and 48% for rats on 180ppm and 1500ppm PbAC diet, respectively) versus control diet ($p=0.0001$, $F_{2,14}=19.80$) (control n= 7, 180ppm n= 6, 1500ppm n=4). C) At PN50, no significant changes in D1R levels were detected in the OT ($p=0.09$, $F_{2,14}=2.82$) (control n=6, 180ppm n= 6, 1500ppm n=5). D) At PN14, no significant changes in D1R levels were detected in the OT ($p=0.35$, $F_{2,14}= 1.13$) (control n= 5, 180ppm n= 7, 1500ppm n=5). E) At PN28, a 41% increase in D1R levels was detected in the STR of female rats exposed to 1500ppm PbAC versus control diet ($p=0.0005$, $F_{2,16}=12.45$) (control n=7, 180ppm n= 6, 1500ppm n=6). F) At PN50, no significant changes in D1R levels were detected in the OT ($p=0.64$, $F_{2,14}= .47$) (control n=7, 180ppm n= 6, 1500ppm n=4).

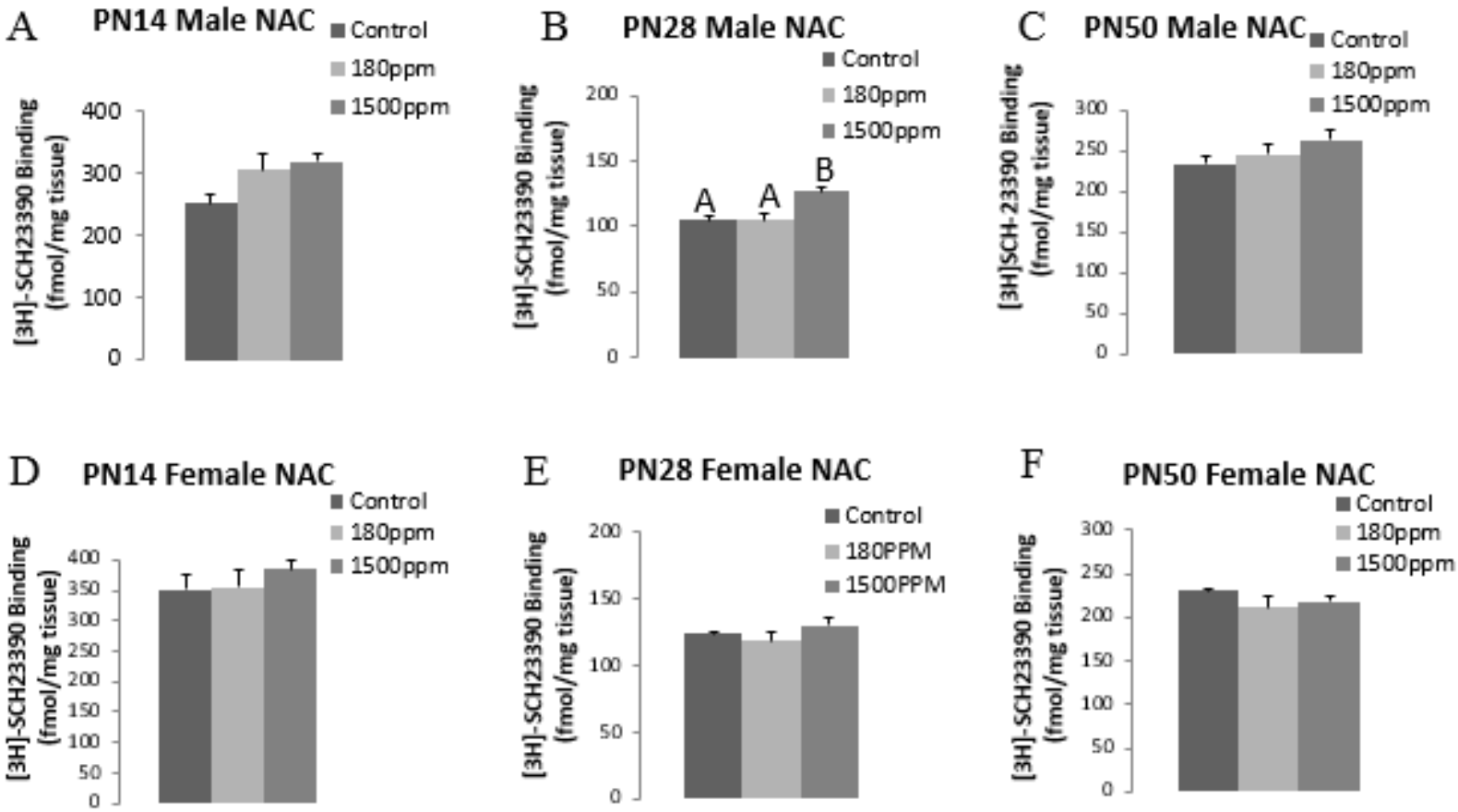

A
PN14 Male NAC
Control
180ppm
1500ppm
[3H]-SCH23390 Binding (fmol/mg tissue)
400
300
200
100
0
B
PN28 Male NAC
Control
180ppm
1500ppm
[3H]-SCH23390 Binding (fmol/mg tissue)
200
150
100
50
0
A
A
B
C
PN50 Male NAC
Control
180ppm
1500ppm
[3H]SCH-23390 Binding (fmol/mg tissue)
300
250
200
150
100
50
0
D
PN14 Female NAC
Control
180ppm
1500ppm
[3H]-SCH23390 Binding (fmol/mg tissue)
400
350
300
250
200
150
100
50
0
E
PN28 Female NAC
Control
180PPM
1500PPM
[3H]-SCH23390 Binding (fmol/mg tissue)
200
150
100
50
0
F
PN50 Female NAC
Control
180ppm
1500ppm
[3H]-SCH23390 Binding (fmol/mg tissue)
300
250
200
150
100
50
0

Figure 3.2. D1R levels in the male and female rat NAC. A) At PN14, no significant changes in D1R levels were detected in the NAC ($p=0.09$, $F_{2,16}= 2.72$) (control n= 6, 180ppm n= 7, 1500ppm n=6). B) At PN28, a 21% increase in D1R levels was detected in the NAC of male rats exposed to 1500ppm PbAc versus control diet ($p=0.0026$, $F_{2,15}=9.03$) (control n= 7, 180ppm n= 6, 1500ppm n=5). C) At PN50, no significant changes in D1R levels were detected in the NAC ($p=0.2$, $F_{2,16}= 1.78$) (control n=7, 180ppm n= 6, 1500ppm n=6). D) At PN14, no significant changes in D1R levels were detected in the NAC ($p=0.61$, $F_{2,18}= 0.51$) (control n= 7, 180ppm n= 7, 1500ppm n=7). E) At PN28, no significant changes in D1R levels were detected in the NAC ($p=0.39$, $F_{2,15}= 1.0$) (control n= 6, 180ppm n= 6, 1500ppm n=6). F) At PN50, no significant changes in D1R levels were detected in the NAC ($p=0.29$, $F_{2,12}= 1.37$) (control n=6, 180ppm n=5, 1500ppm n=4).

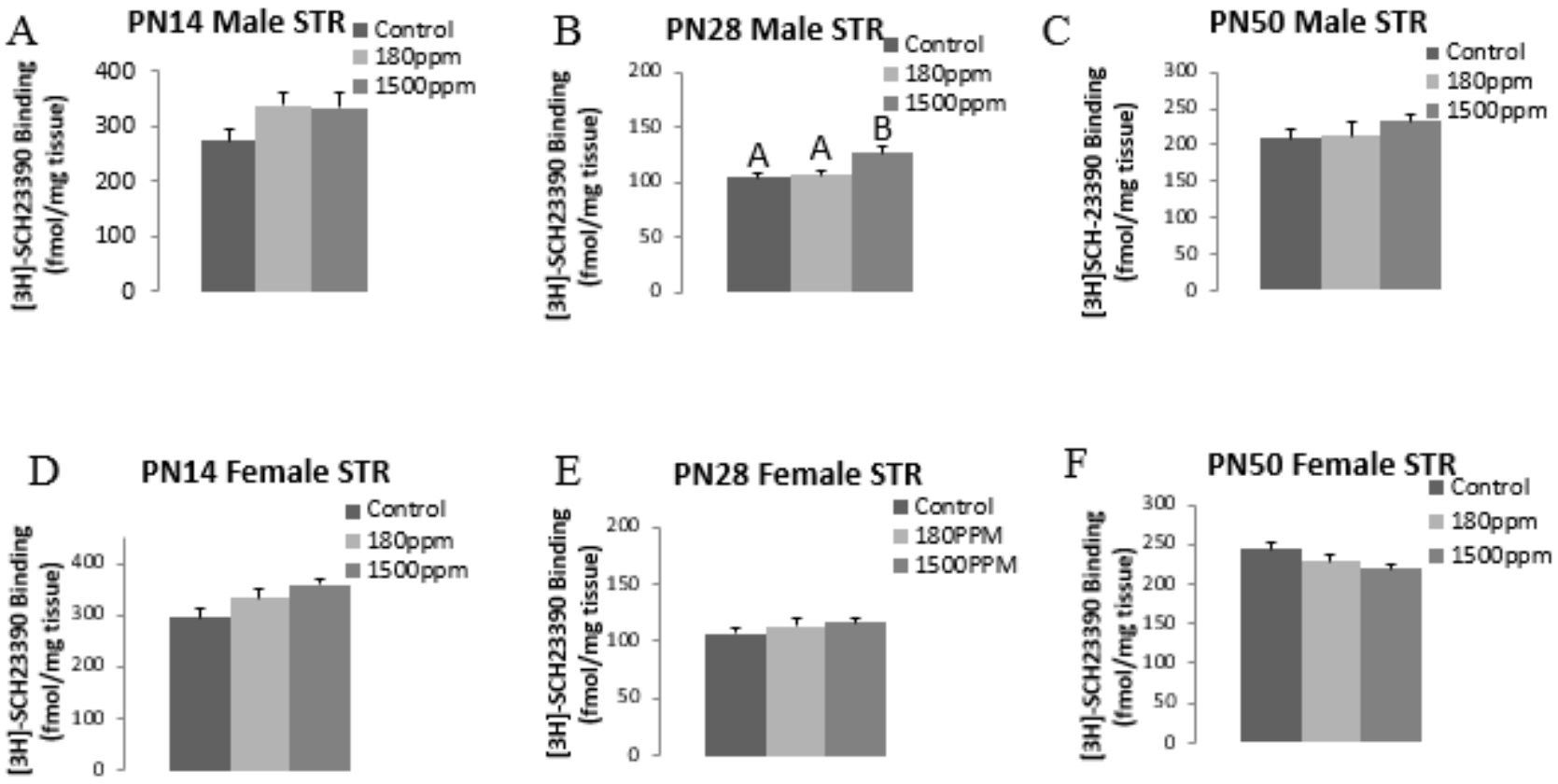

A
PN14 Male STR
[3H]-SCH23390 Binding (fmol/mg tissue)
Control
180ppm
1500ppm
B
PN28 Male STR
A
A
B
C
PN50 Male STR
D
PN14 Female STR
E
PN28 Female STR
180PPM
1500PPM
F
PN50 Female STR

Figure 3.3. D1R levels in the male and female rat STR. A) At PN14, no significant changes in D1R levels were detected in the STR ($p=0.07$, $F_{2,16}= 3.13$) (control n= 7, 180ppm n= 6, 1500ppm n=6). B) At PN28, a 22% increase in D1R levels was detected in the STR of male rats exposed to 1500ppm PbAC versus control diet ($p=0.006$, $F_{2,17}=7.02$) (control n=8, 180ppm n= 6, 1500ppm n=6). C) At PN50, no significant changes in D1R levels were detected in the STR ($p=0.49$, $F_{2,16} =0.75$) (control n=5, 180ppm n= 7, 1500ppm n=7). D) At PN14, no significant changes in D1R levels were detected in the STR ($p=0.04$, $F_{2,17}= 3.83$) (control n=6, 180ppm n= 7, 1500ppm n=7). E) At PN28, no significant changes in D1R levels were detected in the STR ($p=0.35$, $F_{2,16}= 1.11$) (control n=8, 180ppm n= 5, 1500ppm n=6). F) At PN50, no significant changes in D1R levels were detected in the STR ($p=0.16$, $F_{2,15}=2.07$) (control n=7, 180ppm n= 6, 1500ppm n=5).

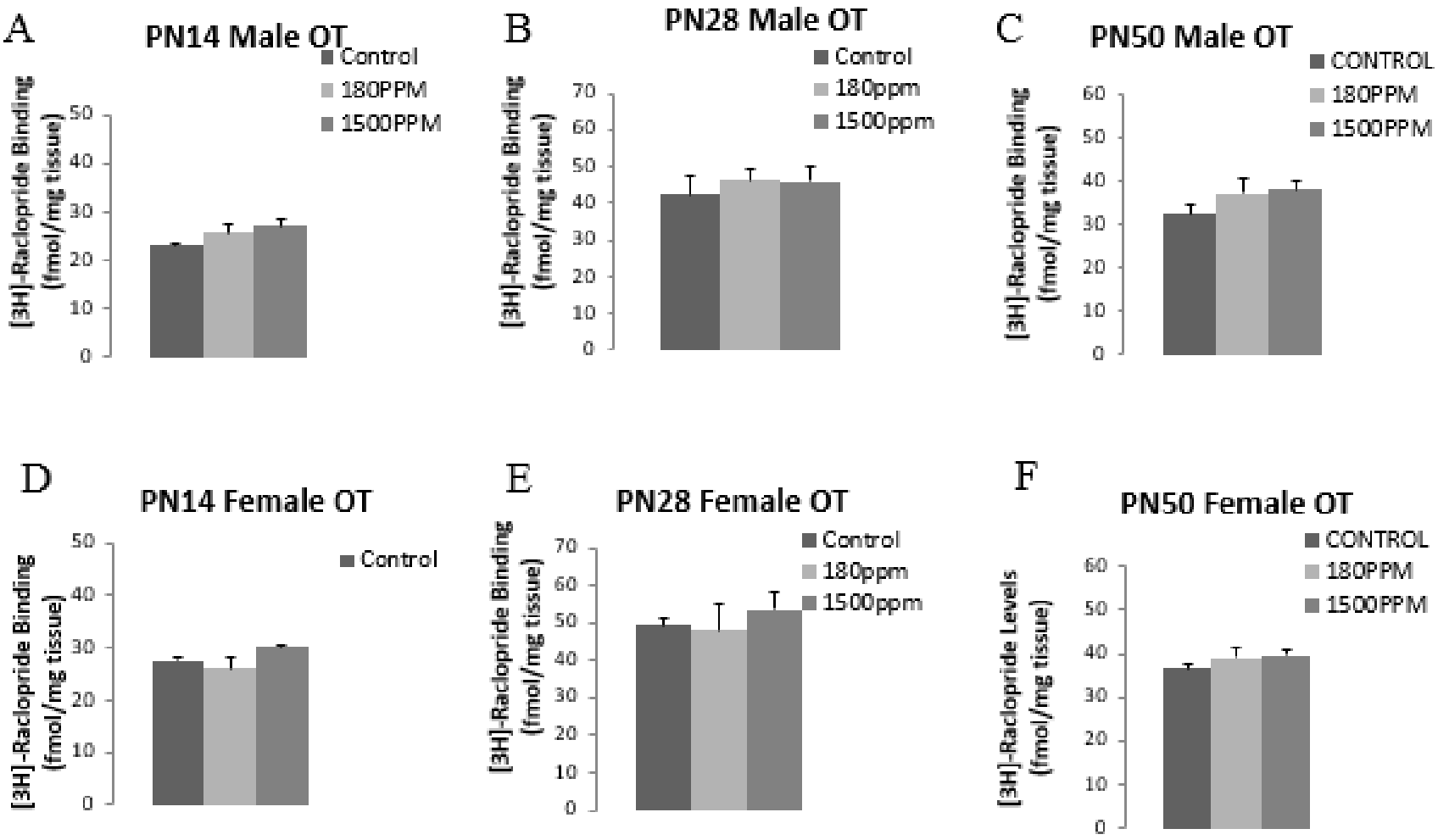

A
PN14 Male OT
Control
180PPM
1500PPM
[3H]-Raclopride Binding (fmol/mg tissue)
0
10
20
30
40
50
B
PN28 Male OT
Control
180ppm
1500ppm
[3H]-Raclopride Binding (fmol/mg tissue)
0
10
20
30
40
50
60
70
C
PN50 Male OT
CONTROL
180PPM
1500PPM
[3H]-Raclopride Binding (fmol/mg tissue)
0
10
20
30
40
50
60
D
PN14 Female OT
Control
[3H]-Raclopride Binding (fmol/mg tissue)
0
10
20
30
40
50
E
PN28 Female OT
Control
180ppm
1500ppm
[3H]-Raclopride Binding (fmol/mg tissue)
0
10
20
30
40
50
60
70
F
PN50 Female OT
CONTROL
180PPM
1500PPM
[3H]-Raclopride Levels (fmol/mg tissue)
0
10
20
30
40
50
60

Figure 3.4. D2R levels in the male and female rat OT. A) At PN14, no significant changes in D2R levels were detected in the OT ($p=0.18$, $F_{2,15}=1.94$) (control n= 5, 180ppm n= 7, 1500ppm n=6). B) At PN28, no significant changes in D2R levels were detected in the OT ($p=0.77$, $F_{2,18}=0.26$) (control n= 8, 180ppm n= 6, 1500ppm n=7. C) At PN50, no significant changes in D2R levels were detected in the OT ($p=0.29$, $F_{2,16}=1.32$) (control n=6, 180ppm n= 5, 1500ppm n=8). D) At PN14, no significant changes in D2R levels were detected in the OT ($p=0.15$, $F_{2,15}=2.12$) (control n= 5, 180ppm n= 7, 1500ppm n=6). E) At PN28, no significant changes in D2R levels were detected in the OT ($p=0.67$, $F_{2,13}= 0.41$) (control n= 7, 180ppm n= 4, 1500ppm n=5). F) At PN50, no significant changes in D2R levels were detected in the OT ($p=0.43$, $F_{2,14}=0.89$) (control n=6, 180ppm n= 6, 1500ppm n=5).

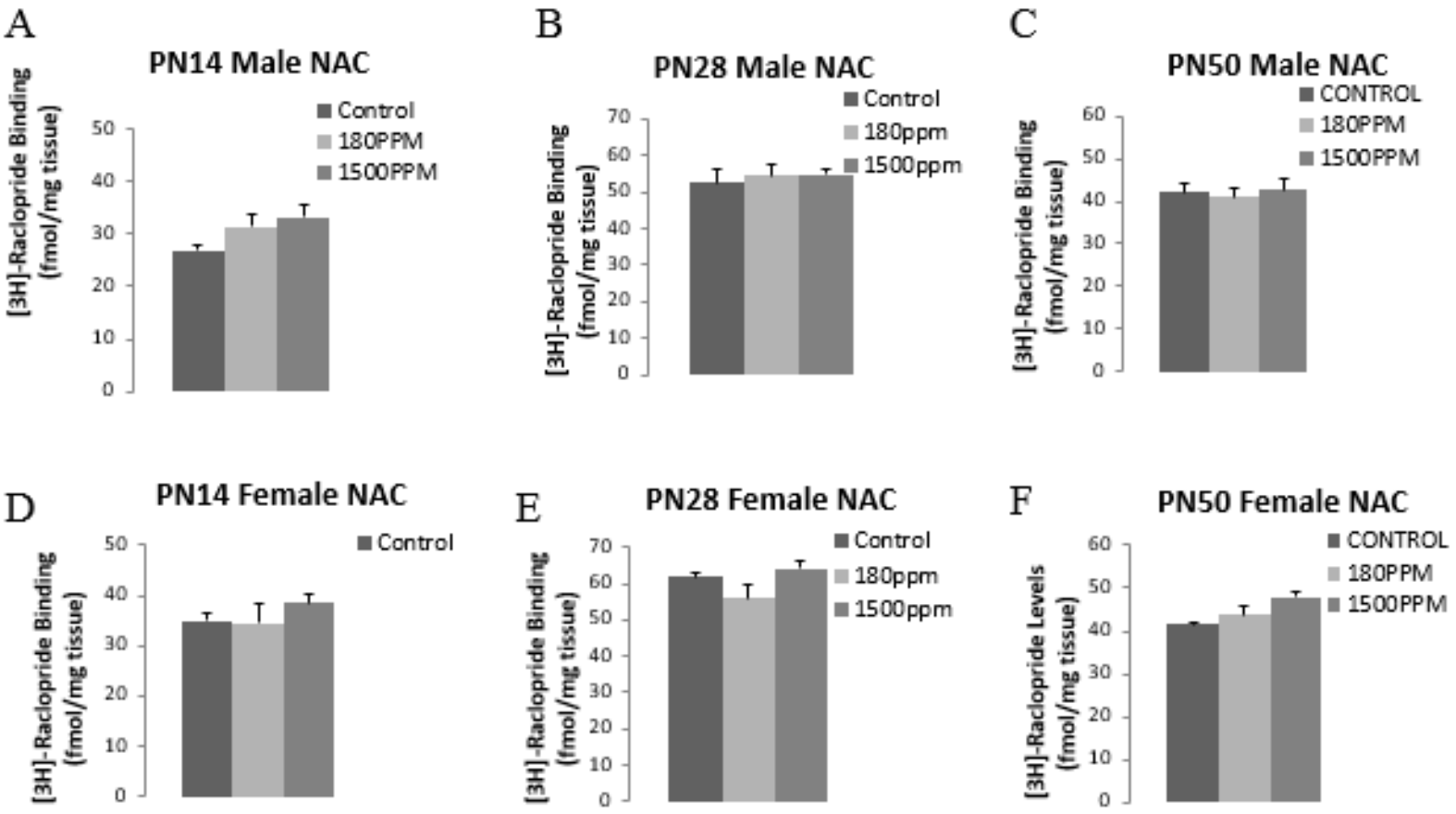

A
PN14 Male NAC
[3H]-Raclopride Binding (fmol/mg tissue)
Control
180PPM
1500PPM
B
PN28 Male NAC
[3H]-Raclopride Binding (fmol/mg tissue)
Control
180ppm
1500ppm
C
PN50 Male NAC
[3H]-Raclopride Binding (fmol/mg tissue)
CONTROL
180PPM
1500PPM
D
PN14 Female NAC
[3H]-Raclopride Binding (fmol/mg tissue)
Control
E
PN28 Female NAC
[3H]-Raclopride Binding (fmol/mg tissue)
Control
180ppm
1500ppm
F
PN50 Female NAC
[3H]-Raclopride Levels (fmol/mg tissue)
CONTROL
180PPM
1500PPM

Figure 3.5. D2R levels in the male and female rat NAC. A) At PN14, no significant changes in D2R levels were detected in the NAC ($p=0.13$, $F_{2,17}= 2.33$) (control n= 6, 180ppm n= 7, 1500ppm n=7). B) At PN28, no significant changes in D2R levels were detected in the NAC ($p=0.86$, $F_{2,15}= 2.33$) (control n= 7, 180ppm n= 6, 1500ppm n=5). C) At PN50, no significant changes in D2R levels were detected in the NAC ($p=0.90$, $F_{2,18}= 1.78$) (control n=7, 180ppm n= 6, 1500ppm n=8). D) At PN14, no significant changes in D2R levels were detected in the NAC ($p=0.52$, $F_{2,14}=0.68$) (control n= 5, 180ppm n= 6, 1500ppm n=6). E) At PN28, no significant changes in D2R levels were detected in the NAC ($p=0.11$, $F_{2,14}= 2.62$) (control n= 6, 180ppm n= 6, 1500ppm n=5). F) At PN50, after adjusting for multiple comparisons, no significant changes in D2R levels were detected in the NAC ($p=0.02$, $F_{2,16}= 4.98$) (control n=6, 180ppm n=6, 1500ppm n=7).

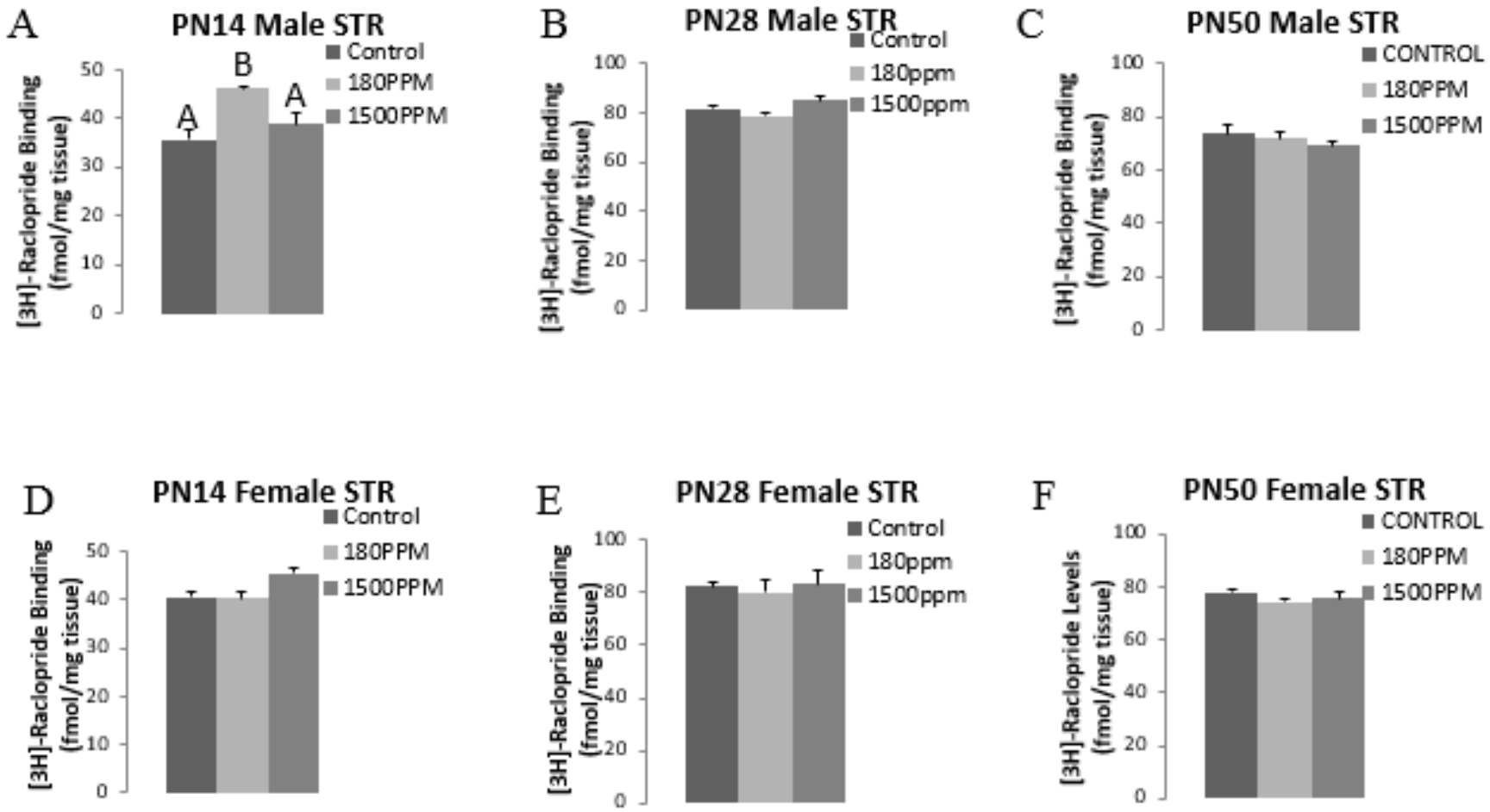

A
PN14 Male STR
[3H]-Raclopride Binding (fmol/mg tissue)
Control
180PPM
1500PPM
A
B
A
B
PN28 Male STR
[3H]-Raclopride Binding (fmol/mg tissue)
Control
180ppm
1500ppm
C
PN50 Male STR
[3H]-Raclopride Binding (fmol/mg tissue)
CONTROL
180PPM
1500PPM
D
PN14 Female STR
[3H]-Raclopride Binding (fmol/mg tissue)
Control
180PPM
1500PPM
E
PN28 Female STR
[3H]-Raclopride Binding (fmol/mg tissue)
Control
180ppm
1500ppm
F
PN50 Female STR
[3H]-Raclopride Levels (fmol/mg tissue)
CONTROL
180PPM
1500PPM

Figure 3.6. D2R levels in the male rat STR. A) At PN14, a significant increase in D2R levels was detected in the STR of male rats exposed to 180ppm ($p=0.01$, $F_{2,15}=5.05$) (control n=6, 180ppm n= 5, 1500ppm n=7). B) At PN28, after adjusting for multiple comparisons, no significant changes in D2R levels were detected in the STR ($p=0.03$, $F_{2,15}=4.63$) (control n=6, 180ppm n= 6, 1500ppm n=6). C) At PN50, no significant changes in D2R levels were detected in the STR ($p=0.46$, $F_{2,14}=0.82$) (control n=7, 180ppm n= 5, 1500ppm n=5). D) At PN14, after adjusting for multiple comparisons, no significant changes in D2R levels were detected in the STR ($p=0.02$, $F_{2,13}= 5.24$) (control n=5, 180ppm n= 5, 1500ppm n=6). E) At PN28, no significant changes in D2R levels were detected in the STR ($p=0.80$, $F_{2,16}= 0.22$) (control n=7, 180ppm n= 6, 1500ppm n=6). F) At PN50, no significant changes in D2R levels were detected in the STR ($p=0.35$, $F_{2,17}=1.12$) (control n=7, 180ppm n= 6, 1500ppm n=7).

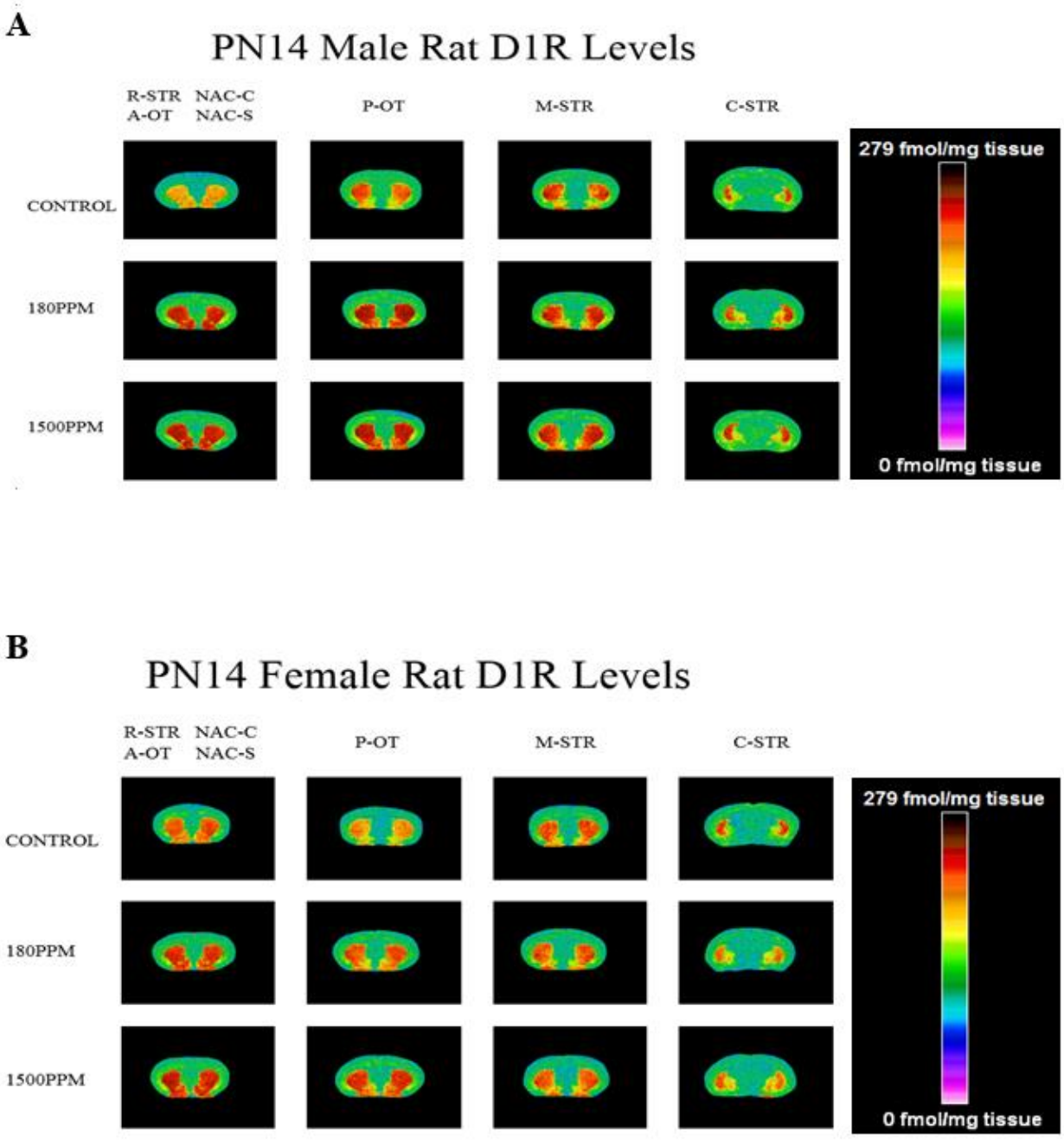


Figure 3.7. Representative autoradiograms illustrating [^{3}H]-SCH23390 binding in OT, NAC, and STR of control and Pb^{2+}-exposed male (A) and female (B) rats at PN14.

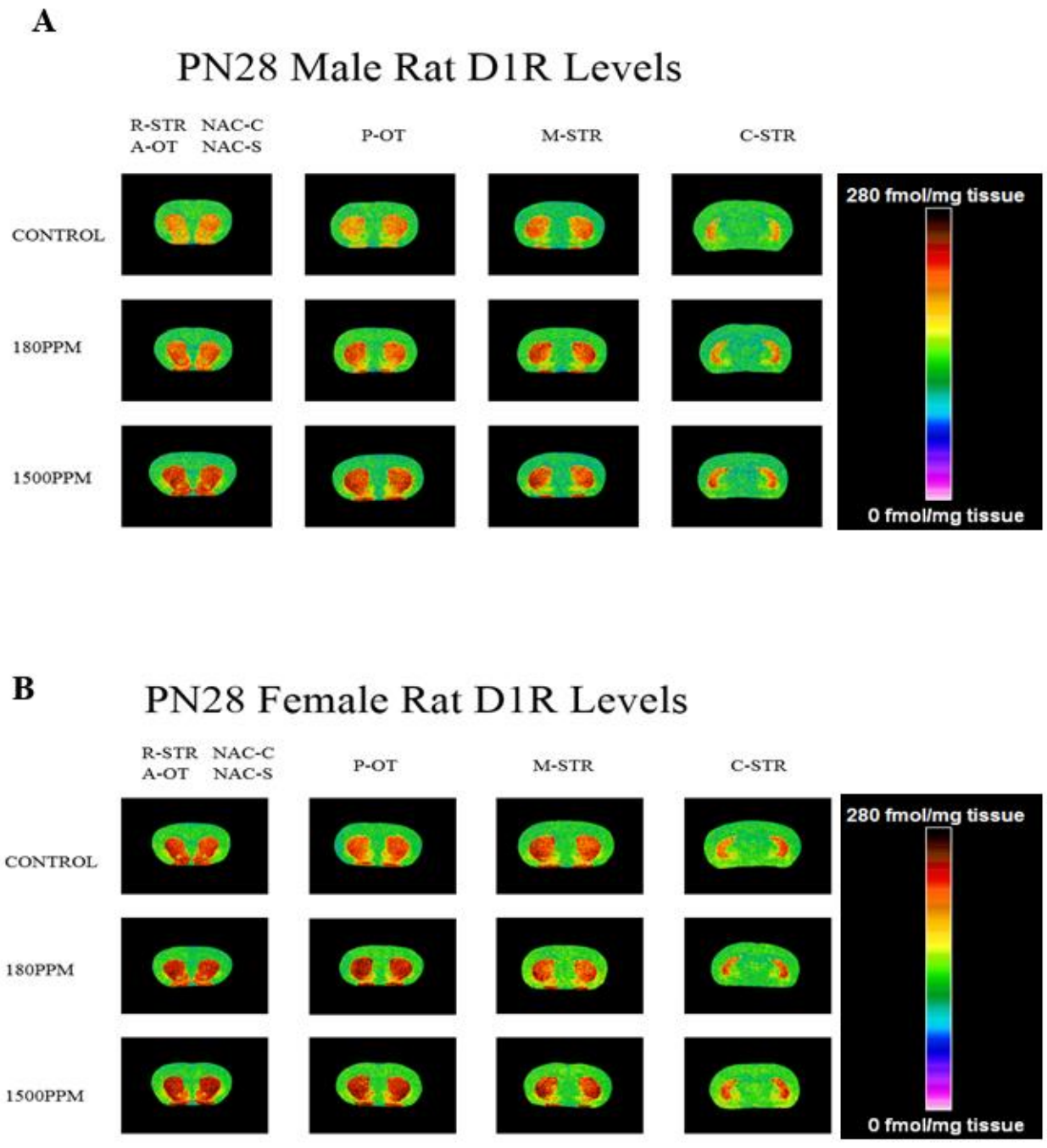


Figure 3.8. Representative autoradiograms illustrating [^{3}H]-SCH23390 binding in OT, NAC, and STR of control and Pb^{2+}-exposed male (A) and female (B) rats at PN28.

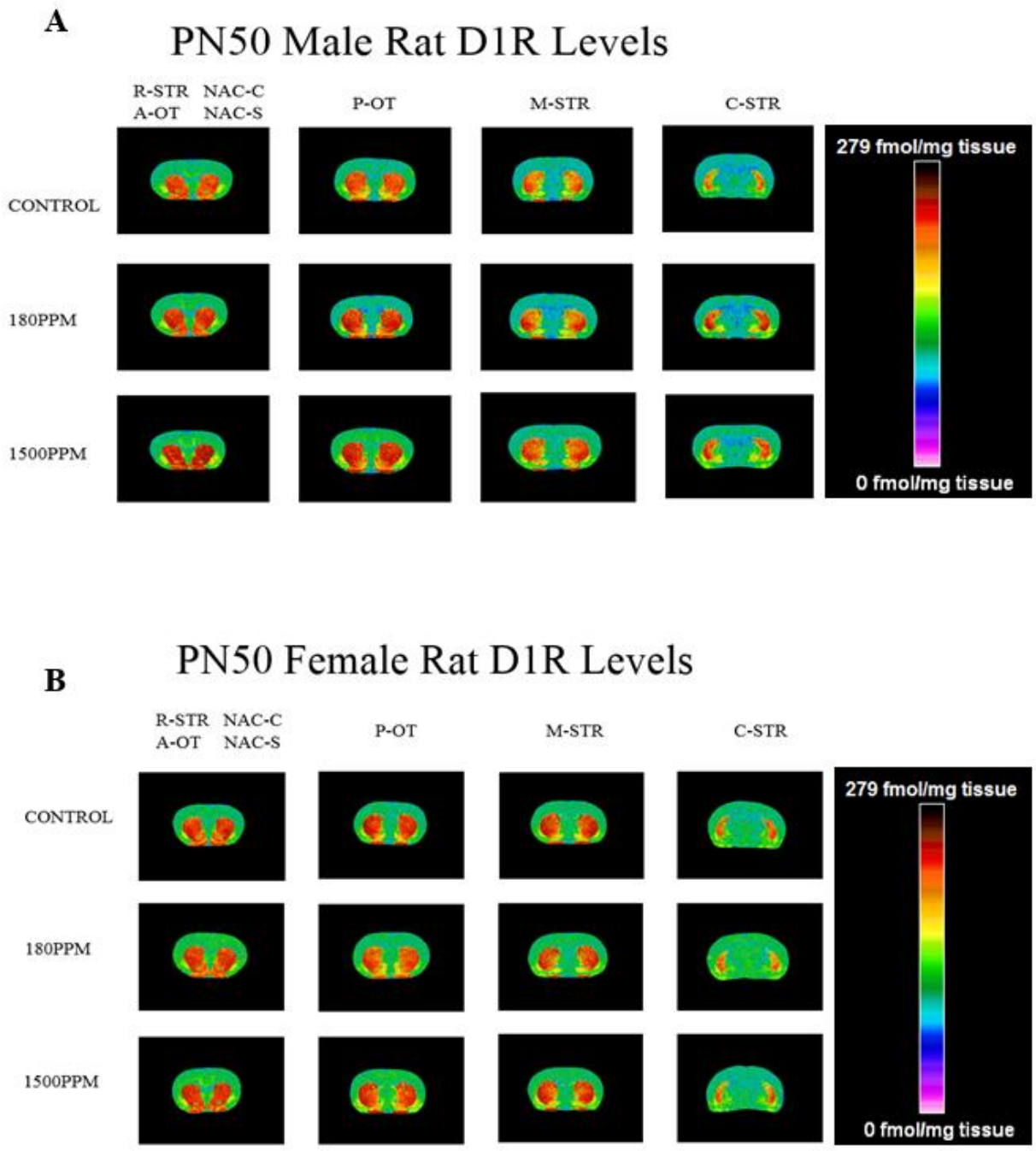


Figure 3.9. Representative autoradiograms illustrating [^{3}H]-SCH23390 binding in OT, NAC, and STR of control and Pb^{2+}-exposed male (A) and female (B) rats at PN50.

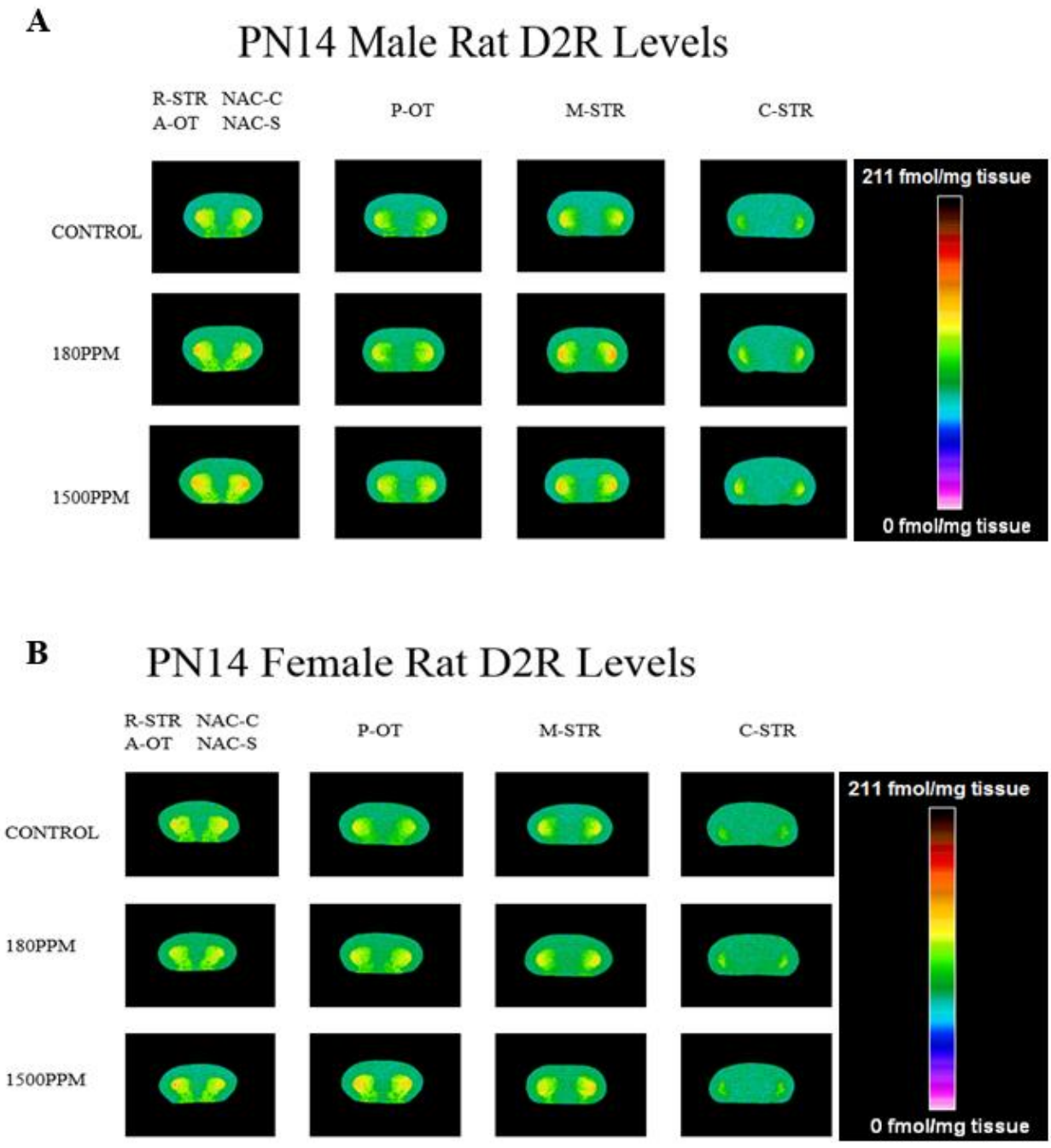


Figure 3.10. Representative autoradiograms illustrating [^{3}H]-Raclopride binding in OT, NAC, and STR of control and Pb^{2+}-exposed male (A) and female (B) rats at PN14.

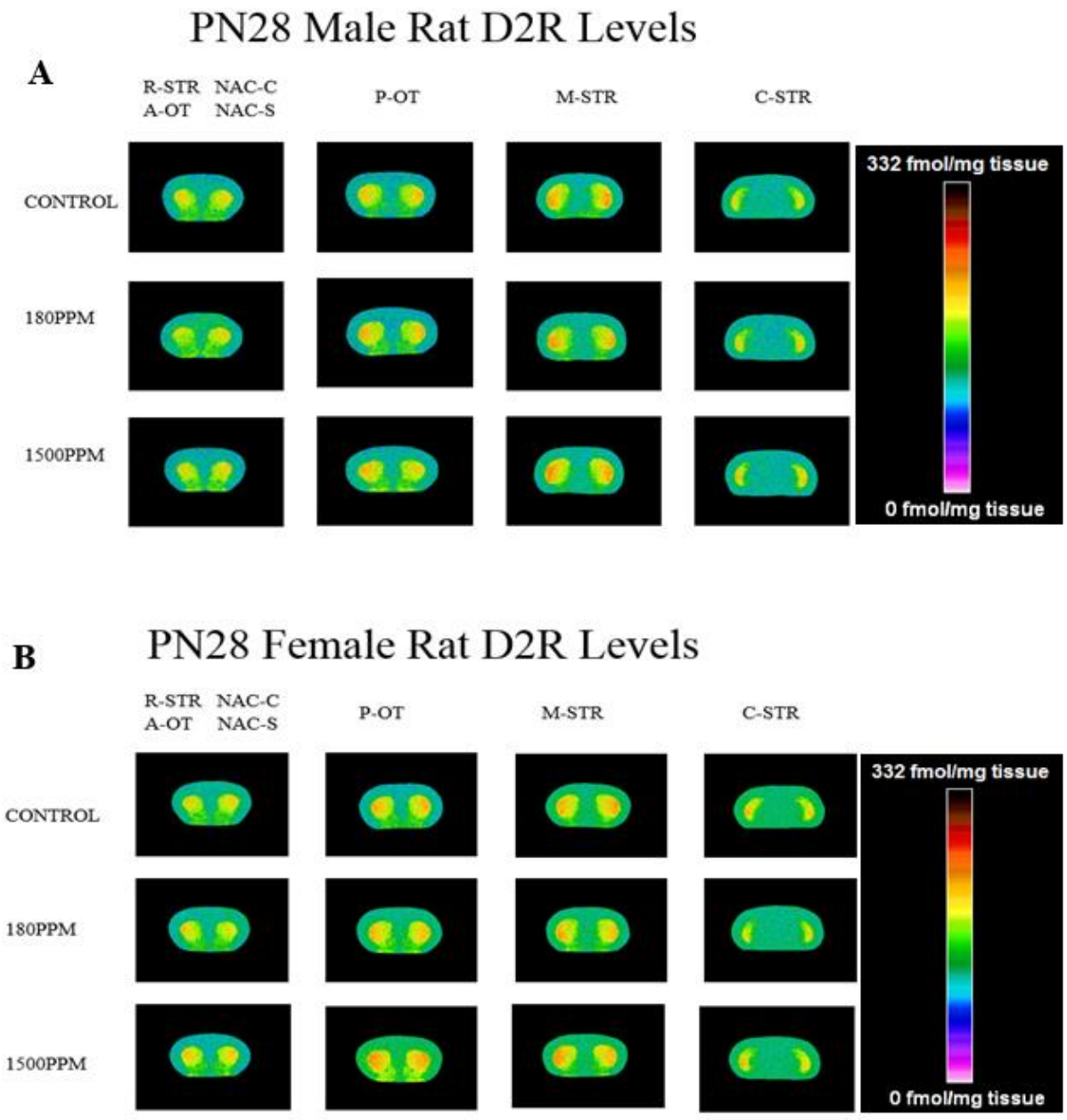


Figure 3.11. Representative autoradiograms illustrating [^{3}H]-Raclopride binding in OT, NAC, and STR of control and Pb^{2+}-exposed male (A) and female (B) rats at PN28.

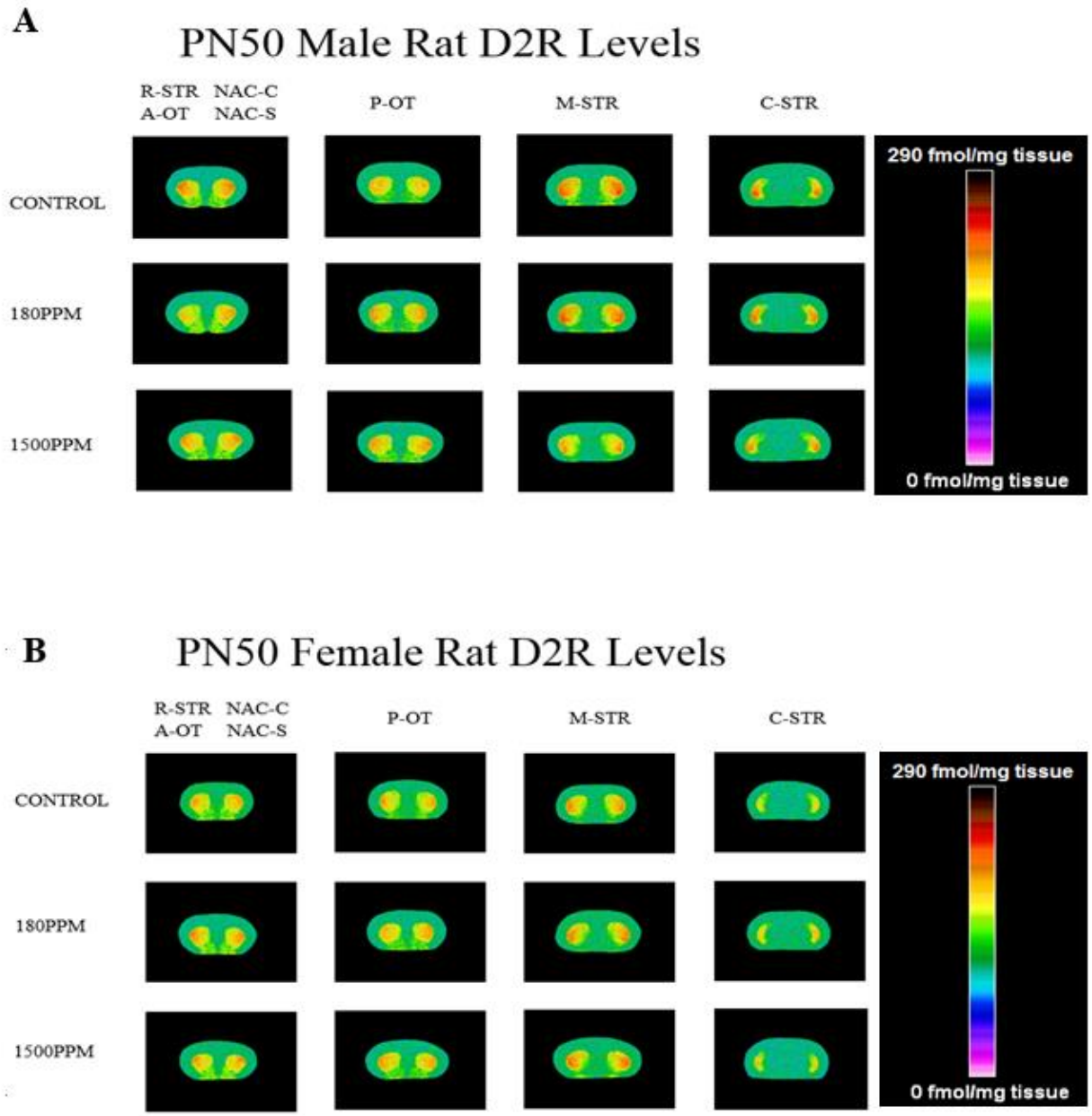


Figure 3.12. Representative autoradiograms illustrating [^{3}H]-Raclopride binding in OT, NAC, and STR of control and Pb^{2+}-exposed male (A) and female (B) rats at PN50.

Discussion

In the present study, we confirm that chronic Pb^{2+} exposure alters DA receptor levels in the OT, NAC, and STR. D1R levels were increased by 21% and 22% in the NAC and STR, respectively, in adolescent male rats exposed to 1500ppm PbAC diet versus control. A robust, dose-dependent increase in D1R levels was detected in the OT of adolescent male rats, with a 17% increase detected after chronic exposure to 180ppm PbAC diet and a 48% increase after chronic exposure to 1500ppm PbAC diet. A 41% increase in D1R levels was also detected in the OT of adolescent female rats exposed to 1500ppm PbAC diet. Together, these results demonstrate an increase in D1R levels in the mesolimbic and mesostriatal systems in the dorsal and ventral striatum. Our results are consistent with previous work by Gedeon (2001) demonstrating increases in D1R levels in the NAC of Pb^{2+}-exposed rats at PN90, PN120, and PN150 (Gedeon, Ramesh et al. 2001). However, the results of this study contrast with previous work demonstrating no change in (Ma, Chen et al. 1999) or a temporary decrease in (Pokora, Richfield et al. 1996) D1R levels in the NAC of rats after Pb^{2+} exposure. They also contrast previous work demonstrating no change in D1R levels in the STR of Pb^{2+}-exposed rats (Moresco, Dall'Olio et al. 1988, Pokora, Richfield et al. 1996, Ma, Chen et al. 1999).

To the best of our knowledge, this is the first study to describe significant increases in D1R in the OT of both male and female adolescent rats and the first study to describe a dose-dependent increase in D1R levels in the OT of adolescent male rats. Furthermore, our results demonstrate that D1R levels in the OT are markedly elevated by Pb^{2+} exposure relative to the STR and NAC, even at BLLs as low as 7µg/dl. Though the impact of Pb^{2+} toxicity on the OT has not been studied in depth, this region has been identified as a "trigger zone," a synaptic junction in the mesocorticolimbic system where the circuitry underlying reward function is first activated

(Ikemoto 2010). The OT also serves as a sensory integration site where the auditory, visual, gustatory, and olfactory cues that lead to a reward response first converge (Chiang and Strowbridge 2007, Wesson and Wilson 2010). As such, activation of OT by psychostimulant drugs may be a central component in activation of the circuitry underlying drug-related reward (Ikemoto 2010). Dysregulation of DAergic signaling systems in the OT may, therefore, play a significant role in the DAergic system dysregulation that underlies drug addiction (Volkow and Li 2005, Volkow, Wang et al. 2011, Volkow, Wang et al. 2011).

Studies by Ikemoto and colleagues have demonstrated that the OT is more sensitive to the psychostimulant effects of cocaine on locomotor activity and rearing than the STR and NAC (Ikemoto 2003, Ikemoto 2007). Rats have been demonstrated to learn to self-administer psychostimulants such as cocaine sooner and at much lower concentrations if injected into the OT versus the NAC and STR (Ikemoto 2003, Ikemoto 2010). These effects are abolished with co-administration of DA receptor antagonists (Ikemoto 2003, Ikemoto, Qin et al. 2005). Together with the work of Ikemoto, our results strongly suggest that Pb^{2+} exposure-induced D1R upregulation in the OT is a likely mechanism underlying the increased locomotor sensitization by cocaine in Pb^{2+}-exposed rats previously reported by our laboratory (Stansfield, Ruby et al. 2015, Stansfield KH 2015).

It should be noted that work by Ikemoto and colleagues also suggests a medio-lateral gradient for cocaine sensitivity in the NAC, STR, and OT, with the highest level of sensitivity in the medial posterior OT (Ikemoto, Qin et al. 2005, Ikemoto 2007, Ikemoto 2010). The current study did not have the power necessary to allow for statistical analysis of DA receptor levels in subregions of the STR, NAC, or OT. Furthermore, we did not have the power to allow for statistical comparisons across sex and age. Still the findings in our study do suggest that the

impact of chronic Pb^{2+} exposure on DA receptor levels may vary across age and sex for analysis of either D1R or D2R levels. Future work with larger sample sizes will be required to allow for subregional analysis of DA receptor level changes as well as comparisons across age and sex in exposed versus control animals.

Disruption of D1R and D2R signaling has been implicated in altered DAergic system function resulting from low-level Pb^{2+} exposure (Gedeon, Ramesh et al. 2001). Though we demonstrate increased D1R levels in animals, we did not evaluate the effects of increased D1R levels on phosphorylation of protein substrates downstream of D1R-mediated activation of the Camp/PKA/CREB pathway in the animals studied. Future studies should be undertaken to determine the effect of Pb^{2+} exposure on phosphorylation within the cAMP/PKA/CREB pathway as it may provide a mechanism through which Pb^{2+}-induced alterations in DA receptor levels may alter signaling in postsynaptic cells and ultimately lead to the enhanced sensitivity of our Pb^{2+}-exposed animals to cocaine's psychostimulant effects.

In the previous chapter, we demonstrated that Pb^{2+} exposure resulted in increased DA turnover in PN28 and PN50 male rats following chronic exposure to 180ppm and 1500ppm PbAC diet. Previous work has also demonstrated that DA turnover in these regions are sensitive to the neurotoxic effects of Pb^{2+} (Nation, Frye et al. 1989, Stansfield, Ruby et al. 2015). Our laboratory has not measured DA and DA turnover in the NAC or OT of the animals in this study. However, the robust increases in D1R levels we observed in the OT and NAC indicate DA turnover should be analyzed in these regions.

Even though we previously demonstrated significant increases in sensitization to cocaine's psychostimulant effects in both male and female rats at PN50, we detected no changes in DA or DA turnover in female rats in the previous chapter. This suggests that changes in DA

levels and turnover observed in Pb^{2+}-exposed animals may not underlie the increases in sensitivity to cocaine's psychostimulant effects we previously observed in both male and female rats. In the present study, very robust increases in D1R levels were detected in the OT of both female and male rats at PN28 following Pb^{2+} exposure. Given this region's high sensitivity to psychostimulants as well as the role of D1R in the modulation of phosphorylation in the postsynaptic cell, these results suggest that increased D1R in the OT may be a factor underlying the increased sensitization to cocaine we previously observed in both male and female rats. This hypothesis is strengthened by the dose-response effect observed in the OT of adolescent male rats as it demonstrates the sensitivity of this region to Pb^{2+} neurotoxicity. However, we cannot yet rule out other mechanisms through which Pb^{2+} exposure may induce a hyperactive DAergic state.

The results presented in this study support our hypothesis that chronic Pb^{2+} exposure increases sensitivity to the psychostimulant effects of cocaine by inducing a hyperactive DAergic state as reflected by increased D1R levels in the OT, NAC, and STR and DA turnover in the STR (Stansfield KH 2015). Here we demonstrate increases in D1R expression in young adolescent rats with BLLs as low as 7µg/dl. Together with the increased STR DA and DA turnover levels observed in Pb^{2+}-exposed male adolescent and young adult rats the previous chapter, these results suggest that dysregulation of DA synthesis, metabolism, and signaling may underlie increased locomotor activity we previously observed in Pb^{2+}-exposed animals.

References

Beaulieu, J. M. and R. R. Gainetdinov (2011). "The physiology, signaling, and pharmacology of dopamine receptors." Pharmacol Rev **63**(1): 182-217.

CDC, Centers for Disease Control and Prevention (2012, 7 Jun 2012). "CDC Response to Advisory Committee on Childhood Lead Poisoning Prevention Recommendations in "Low Level Lead Exposure Harms Children: A Renewed Call for Primary Prevention."." Retrieved 12/10/15, from http://www.cdc.gov/nceh/lead/ACCLPP/Final_Document_030712.pdf

CDC, Centers for Disease Control and Prevention (2016). "Number of Children Tested and Confirmed BLL's ≥10 µg/dL by State, Year, and BLL Group, Children < 72 Months Old."

Chiang, E. and B. W. Strowbridge (2007). "Diversity of neural signals mediated by multiple, burst-firing mechanisms in rat olfactory tubercle neurons." J Neurophysiol **98**(5): 2716-2728.

Cory-Slechta, D. A. (1995). "Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter system functions." Annu Rev Pharmacol Toxicol **35**: 391-415.

Cory-Slechta, D. A., K. M. Crofton, J. A. Foran, J. F. Ross, L. P. Sheets, B. Weiss and B. Mileson (2001). "Methods to identify and characterize developmental neurotoxicity for human health risk assessment. I: behavioral effects." Environ Health Perspect **109 Suppl 1**: 79-91.

Cory-Slechta, D. A. and D. V. Widzowski (1991). "Low level lead exposure increases sensitivity to the stimulus properties of dopamine D1 and D2 agonists." Brain Res **553**(1): 65-74.

Devi, C. B., G. H. Reddy, R. P. Prasanthi, C. S. Chetty and G. R. Reddy (2005). "Developmental lead exposure alters mitochondrial monoamine oxidase and synaptosomal catecholamine levels in rat brain." Int J Dev Neurosci **23**(4): 375-381.

Ensminger, M. E., J. C. Anthony and J. McCord (1997). "The inner city and drug use: initial findings from an epidemiological study." Drug Alcohol Depend **48**(3): 175-184.

Ensminger, M. E., H. S. Juon and K. E. Fothergill (2002). "Childhood and adolescent antecedents of substance use in adulthood." Addiction **97**(7): 833-844.

Fishbein, D. H., A. C. Todd, E. P. Ricketts and R. D. Semba (2008). "Relationship between lead exposure, cognitive function, and drug addiction: pilot study and research agenda." Environ Res **108**(3): 315-319.

Gedeon, Y., G. T. Ramesh, P. J. Wellman and A. L. Jadhav (2001). "Changes in mesocorticolimbic dopamine and D1/D2 receptor levels after low level lead exposure: a time course study." Toxicol Lett **123**(2-3): 217-226.

Ikemoto, S. (2002). "Ventral striatal anatomy of locomotor activity induced by cocaine, D-amphetamine, dopamine and D1/D2 agonists." Neuroscience **113**(4): 939-955.

Ikemoto, S. (2003). "Involvement of the olfactory tubercle in cocaine reward: intracranial self-administration studies." J Neurosci **23**(28): 9305-9311.

Ikemoto, S. (2007). "Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex." Brain Res Rev **56**(1): 27-78.

Ikemoto, S. (2010). "Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory." Neurosci Biobehav Rev **35**(2): 129-150.

Ikemoto, S., M. Qin and Z. H. Liu (2005). "The functional divide for primary reinforcement of D-amphetamine lies between the medial and lateral ventral striatum: is the division of the accumbens core, shell, and olfactory tubercle valid?" J Neurosci **25**(20): 5061-5065.

Jones, D. C. and G. W. Miller (2008). "The effects of environmental neurotoxicants on the dopaminergic system: A possible role in drug addiction." Biochem Pharmacol **76**(5): 569-581.

Koepp, M. J., R. N. Gunn, A. D. Lawrence, V. J. Cunningham, A. Dagher, T. Jones, D. J. Brooks, C. J. Bench and P. M. Grasby (1998). "Evidence for striatal dopamine release during a video game." Nature **393**(6682): 266-268.

Koob, G. F. and N. D. Volkow (2010). "Neurocircuitry of addiction." Neuropsychopharmacology **35**(1): 217-238.

Lasley, S. M. and J. D. Lane (1988). "Diminished regulation of mesolimbic dopaminergic activity in rat after chronic inorganic lead exposure." Toxicol Appl Pharmacol **95**(3): 474-483.

Leret, M. L., F. Garcia-Uceda and M. T. Antonio (2002). "Effects of maternal lead administration on monoaminergic, GABAergic and glutamatergic systems." Brain Res Bull **58**(5): 469-473.

Ma, T., H. H. Chen and I. K. Ho (1999). "Effects of chronic lead (Pb) exposure on neurobehavioral function and dopaminergic neurotransmitter receptors in rats." Toxicol Lett **105**(2): 111-121.

Miller, D. K., J. R. Nation and G. R. Bratton (2000). "Perinatal exposure to lead attenuates the conditioned reinforcing properties of cocaine in male rats." Pharmacol Biochem Behav **67**(1): 111-119.

Miller, D. K., J. R. Nation and G. R. Bratton (2001). "The effects of perinatal exposure to lead on the discriminative stimulus properties of cocaine and related drugs in rats." Psychopharmacology (Berl) **158**(2): 165-174.

Moresco, R. M., R. Dall'Olio, O. Gandolfi, S. Govoni, S. Di Giovine and M. Trabucchi (1988). "Lead neurotoxicity: a role for dopamine receptors." Toxicology **53**(2-3): 315-322.

Nairn, A. C., P. Svenningsson, A. Nishi, G. Fisone, J. A. Girault and P. Greengard (2004). "The role of DARPP-32 in the actions of drugs of abuse." Neuropharmacology **47 Suppl 1**: 14-23.

Nation, J. R., A. L. Cardon, H. M. Heard, R. Valles and G. R. Bratton (2003). "Perinatal lead exposure and relapse to drug-seeking behavior in the rat: a cocaine reinstatement study." Psychopharmacology (Berl) **168**(1-2): 236-243.

Nation, J. R., G. D. Frye, J. Von Stultz and G. R. Bratton (1989). "Effects of combined lead and cadmium exposure: changes in schedule-controlled responding and in dopamine, serotonin, and their metabolites." Behav Neurosci **103**(5): 1108-1114.

Nation, J. R., D. K. Miller and G. R. Bratton (2000). "Developmental lead exposure alters the stimulatory properties of cocaine at PND 30 and PND 90 in the rat." Neuropsychopharmacology **23**(4): 444-454.

Nation, J. R., K. R. Smith and G. R. Bratton (2004). "Early developmental lead exposure increases sensitivity to cocaine in a self-administration paradigm." Pharmacol Biochem Behav **77**(1): 127-135.

Nishi, A., G. L. Snyder and P. Greengard (1997). "Bidirectional regulation of DARPP-32 phosphorylation by dopamine." J Neurosci **17**(21): 8147-8155.

Paxinos, G. and C. Watson (1998). The Rat Brain in Stereotaxic Coordinates, Academic Press.

Pokora, M. J., E. K. Richfield and D. A. Cory-Slechta (1996). "Preferential vulnerability of nucleus accumbens dopamine binding sites to low-level lead exposure: time course of effects and interactions with chronic dopamine agonist treatments." J Neurochem **67**(4): 1540-1550.

Sanders, T., Y. Liu, V. Buchner and P. B. Tchounwou (2009). "Neurotoxic effects and biomarkers of lead exposure: a review." Rev Environ Health **24**(1): 15-45.

Stansfield KH, M. J., Guilarte TR. (2015). Early Life Lead Exposure and Sensitization to Cocaine: A Novel Pathway to Addiction. . Society of Toxicology. San Diego, CA.

Stansfield, K. H., K. N. Ruby, B. D. Soares, J. L. McGlothan, X. Liu and T. R. Guilarte (2015). "Early-life lead exposure recapitulates the selective loss of parvalbumin-positive GABAergic interneurons and subcortical dopamine system hyperactivity present in schizophrenia." Transl Psychiatry **5**: e522.

Surmeier, D. J., J. Ding, M. Day, Z. Wang and W. Shen (2007). "D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons." Trends Neurosci **30**(5): 228-235.

Szczerbak, G., P. Nowak, R. M. Kostrzewa and R. Brus (2007). "Maternal lead exposure produces long-term enhancement of dopaminergic reactivity in rat offspring." Neurochem Res **32**(10): 1791-1798.

Volkow, N. and T. K. Li (2005). "The neuroscience of addiction." Nat Neurosci **8**(11): 1429-1430.

Volkow, N. D., G. F. Koob and A. T. McLellan (2016). "Neurobiologic Advances from the Brain Disease Model of Addiction." N Engl J Med **374**(4): 363-371.

Volkow, N. D. and T. K. Li (2004). "Drug addiction: the neurobiology of behaviour gone awry." Nat Rev Neurosci **5**(12): 963-970.

Volkow, N. D., G. J. Wang and R. D. Baler (2011). "Reward, dopamine and the control of food intake: implications for obesity." Trends Cogn Sci **15**(1): 37-46.

Volkow, N. D., G. J. Wang, J. S. Fowler, D. Tomasi and F. Telang (2011). "Addiction: beyond dopamine reward circuitry." Proc Natl Acad Sci U S A **108**(37): 15037-15042.

Volkow, N. D. and R. A. Wise (2005). "How can drug addiction help us understand obesity?" Nat Neurosci **8**(5): 555-560.

Wesson, D. W. and D. A. Wilson (2010). "Smelling sounds: olfactory-auditory sensory convergence in the olfactory tubercle." J Neurosci **30**(8): 3013-3021.

Zuch, C. L., D. J. O'Mara and D. A. Cory-Slechta (1998). "Low-level lead exposure selectively enhances dopamine overflow in nucleus accumbens: an in vivo electrochemistry time course assessment." Toxicol Appl Pharmacol **150**(1): 174-185.

Chapter 4: Conclusion

Conclusion

Summary of Findings and Implications

The central finding of this dissertation is that chronic Pb^{2+} exposure induces changes in expression of proteins essential for DAergic transmission that may be suggestive of a hyperactive DAergic state characterized by increased DA levels and DA turnover in the STR and increased D1R levels in the OT, NAC and STR in male rats with BLL as low as 4µg/dl. The reinforcing properties of cocaine and other drugs of abuse stem from their ability to increase the magnitude and duration of elevated DA concentrations in the extracellular environment, surpassing the increases in DA elicited by natural reinforcers such as food (Volkow, Wang et al. 1997, Volkow and Li 2004, Volkow and Wise 2005, Koob and Volkow 2010). In Aim 1, we found that total STR DA levels were significantly higher in Pb^{2+}-exposed males versus control, both in adolescence and young adulthood, time points that correspond with a higher risk of drug use and onset of drug addiction (Volkow and Li 2004).

Addictive drugs activate brain regions associated with reward by eliciting rapid spikes in DA release and repeated exposure to the drug strengthens synaptic connections associated with learning and memory formation (Volkow, Koob et al. 2016). The transition from drug use to abuse begins with changes in the mesolimbic DAergic system that begin in the ventral striatum (OT and NAC), leading to the activation of other brain regions associated with reward (dorsal striatum, thalamus, and globus pallidus) (Volkow and Li 2004, Koob and Volkow 2010, Volkow, Wang et al. 2011, Volkow, Koob et al. 2016). Repeated exposure to drugs results in the strengthening of processes of the brain associated with reward and weakens those associated with inhibition control and decision making (Baler and Volkow 2006, Volkow, Koob et al. 2016). The central finding of this work is that chronic low-level Pb^{2+} exposure induces alterations consistent with a hyperactive dopaminergic state in the regions of the brain that are

most responsive to psychostimulants such as cocaine (Ikemoto 2003, Ikemoto 2007, Ikemoto 2010). Such Pb^{2+}-induced DAergic hyperactivity may underlie the increased sensitization to cocaine's psychostimulant effects that our laboratory previously observed in our animal model of low-level Pb^{2+} exposure (Stansfield, Ruby et al. 2015).

Drugs of addiction dysregulate DA release and reuptake, which can result in increased DA concentration and duration in the synapse (Koob and Volkow 2010). This, in turn, can alter DA-mediated synaptic plasticity, resulting in adaptations to DAergic circuits underlying reward and other processes associated with addiction (Volkow and Li 2004, Volkow, Koob et al. 2016). The increased DA turnover observed in the STR of Pb^{2+}-exposed animals versus control *in the absence of a reinforcer* suggests DA-mediated synaptic plasticity may also be altered in these animals in a manner similar to individuals after repeated exposure to drug of abuse and subsequent changes to neurocircuitry that predisposes them to addiction (Baler and Volkow 2006, Koob and Volkow 2010, Volkow, Koob et al. 2016).

DAergic system dysregulation is known to increase susceptibility to drug addiction onset and Pb^{2+} is known to alter DAergic transmission (Volkow, Wang et al. 2011, Volkow, Koob et al. 2016). The brain is particularly sensitive to the effects of drug-induced DA dysregulation in adolescence and young adulthood, as these periods coincide with increased neuroplasticity associated with the maturation of neurocircuitry underlying processes such as inhibition control (Koob and Volkow 2010, Volkow, Koob et al. 2016). Dysregulation of DA resulting from drug use in adolescence has been demonstrated to result in neuroadaptations that increase susceptibility to drug abuse in later stages of life (Volkow and Li 2004). It is possible that the Pb^{2+}-induced dysregulation of DA and DA turnover we observed in adolescent and young adult male rats could result in similar neuroadaptations and ultimately underlie the increased

sensitization to cocaine previously observed in our model for chronic Pb^{2+} exposure (Stansfield, Ruby et al. 2015).

This hypothesis is supported by the robust increases in D1R observed in the OT, NAC, and STR of adolescent Pb^{2+}-exposed male rats and in the OT of adolescent Pb^{2+}-exposed female rats. Of these regions, the OT has been demonstrated to be the most sensitive to cocaine (Ikemoto 2003, Ikemoto 2007, Ikemoto 2010). The D1R-mediated disinhibitory direct pathway underlies drug-related increases in locomotor function as well as other responses. It also undergoes long-term potentiation in response to increased DA associated with a natural reward or drugs of abuse such as cocaine. The D2R-mediated inhibitory indirect pathway, on the other hand, undergoes long-term depression in response to phasic release of DA or long-term potentiation in response to basal release of DA (Gruber and McDonald 2012). Together with the increases in DA and DA turnover observed in Aim 1, the increases in D1R observed in Aim 2 suggest that lead exposure may alter DA-modulated neurocircuitry in a manner that may increase responsiveness to cocaine's psychostimulant effects.

Drug addiction remains a public health concern and an improved understanding of the mechanisms underlying the transition from drug use to drug abuse may result in improved strategies for prevention (Volkow, Koob et al. 2016). Pb^{2+} has been demonstrated to alter DA release, DA turnover, DA receptors, DA synthesis, and uptake in regions that are sensitive to psychostimulant effects of cocaine (Nation, Frye et al. 1989, Cory-Slechta 1995, Zuch, O'Mara et al. 1998, Ikemoto 2003, Ikemoto 2007, Stansfield, Ruby et al. 2015). A number of studies have determined that early-life Pb^{2+} exposure increases the risk for cognitive and behavioral deficits later in life and an association between Pb^{2+} exposure and drug abuse has been suggested (Canfield, Henderson et al. 2003, Canfield, Kreher et al. 2003, Needleman 2004, Lanphear,

Hornung et al. 2005, Fishbein, Todd et al. 2008, Jones and Miller 2008, Jusko, Henderson et al. 2008).

NHANES data indicates that legislation has successfully decreased BLL of children under the age of 5 (Nevin 2007, CDC 2012, CDC 2016). Nevertheless, the effects of early-life Pb^{2+} exposure on cognitive and behavioral function can persist well after the exposure occurs and, as such, continue to affect populations that were exposed to Pb^{2+} as children in previous decades (Nevin 2000, Nevin 2007). The populations most at-risk for Pb^{2+} exposure are also the populations most at-risk for drug addiction, but the nature of the relationship between Pb^{2+} exposure and drug addiction has not yet been defined (Ensminger, Anthony et al. 1997, Ensminger, Juon et al. 2002, Cory-Slechta, Virgolini et al. 2004, Fishbein, Todd et al. 2008, Jones and Miller 2008). By demonstrating that Pb^{2+} induces a hyperactive DAergic state, our findings provide a neurobiological mechanism by which Pb^{2+} may act as a causal risk factor in the onset of drug addiction. Together with previous work by our laboratory and work by Nation et al., our findings expand upon our current understanding of how environmental pollutants can play a role in the onset of drug addiction (Miller, Nation et al. 2000, Nation, Smith et al. 2004, Jones and Miller 2008, Stansfield, Ruby et al. 2015).

Limitations and Directions for Future Research

One limitation of this study is the sample size used, as it did not allow us the power necessary to determine sex-, region-, and age-dependent effects on the biomarkers analyzed. Though our data suggest that differences occur across sex, brain regions, and age, we were unable to evaluate this statistically. Even so, the results of this work should be useful for conducting the power calculations necessary to determine the appropriate sample sizes to be used

in future studies evaluating sex-, region-, and age-dependent effects of Pb^{2+} on the DAergic system. Another key limitation is that we did not take estrous cycle into consideration when female brains were collected, as fluctuating estrogen levels can affect the endpoints we measured. Therefore, future work evaluating sex-dependent effects should also monitor the effects of Pb^{2+} at different stages of the female estrous cycle, as it may provide insights to how fluctuating hormones interact with Pb^{2+} exposure to affect the DAergic system.

In Chapter 2, we observed increased DA turnover in the STR of exposed male rats versus control at PN28 and PN50. D1R levels were significantly increased in the OT and NAC, demonstrating that the vulnerability of these regions to the neurotoxic effects of Pb^{2+}. As a result, we are currently in the process of collecting the tissue necessary to measure DA, DA metabolites, and DA turnover in these regions in addition to STR. Though the increased DOPAC/DA and HVA/DA ratios observed in exposed adolescent and young adult male rats indicate increased DA turnover in the STR, it is still unclear if DA release and reuptake were affected by Pb^{2+} exposure. We intend to measure DAT levels in OT, NAC, and STR in future work as a marker for DA reuptake. However, *in vivo* electrochemical analysis of evoked DA release and clearance should also be conducted in these regions as it will provide a more accurate assessment of whether Pb^{2+} increases the availability of DA following stimulation of DAergic neurons and, if so, provide further evidence to support a hyperactive DAergic system.

Though we demonstrated increased DA levels in Pb^{2+}-exposed male rats at PN28 and PN50, we found no change in pser40TH or TH, the rate-limiting enzyme in DA synthesis, in these animals. In contrast, increased pser40TH levels were detected in the STR of female rats exposed to 1500ppm PbAC diet at PN50, demonstrating that TH may be affected by Pb^{2+}. Though phosphorylation of TH indicates activation of this enzyme, it is not a direct measure of

TH activity. Future studies should, therefore, conduct a TH enzyme activity assay such as the assay described by Naoi (1988) to measure TH activity in the STR. This assay utilizes HPLC coupled with electrochemistry to detect L-DOPA generated by brain homogenate samples in the presence or absence of an amino acid decarboxylase inhibitor such as p-bromobenzyloxyamine (Naoi, Takahashi et al. 1988). Furthermore, the increased D1R levels observed in the OT of female rats and in the OT and NAC of male rats at PN28 indicate that these regions are vulnerable to Pb^{2+} toxicity and suggest that analysis of TH activity in these regions should also be undertaken.

In studies in Chapter 3, we observed increased D1R receptor levels in Pb^{2+}-exposed animals, but we did not evaluate DA receptor function. DA receptor function may be altered by Pb^{2+} exposure, resulting in compensatory upregulation of DA receptors. Given the robust increases in D1R levels observed in both male and female rats at PN28, future studies should be undertaken to determine the effect of Pb^{2+} exposure on D1R activation of the cyclic adenosine monophosphate (cAMP)-dependent pathway, which plays a significant role in regulating synaptic plasticity underlying strengthened reward circuits and weakened inhibitory control (Volkow, Koob et al. 2016).

One marker that should be studied further is phosphorylation of dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32) at its threonine 34 residue. DARPP-32, a protein expressed by medium spiny neurons, is necessary for the behavioral responses of cocaine (Halpain, Girault et al. 1990, Bjorklund and Dunnett 2007). Activation of D1R by DA or D1R agonists such as SKF81297 results in increased phosphorylation of the DARPP-32 threonine residue 34 (Thr34) (Halpain, Girault et al. 1990, Bjorklund and Dunnett 2007, Beaulieu and Gainetdinov 2011). Once phosphorylated at Thr34, DARPP-32 is activated and acts as a potent

protein phosphatase-1 (PP1) inhibitor. Inhibition of PP1, in turn, results in decreased dephosphorylation of a number of protein substrates (Halpain, Girault et al. 1990). To assess the impact of Pb^{2+} on D1R function, future studies should compare Thr34 phosphorylation levels of DARPP-32 in the OT, NAC, and STR as a marker for activation of signaling pathways downstream of DA receptors.

There are two isoforms of D2R, the long form (D2RL) and the short form (D2RS) (Usiello, Baik et al. 2000, Beaulieu and Gainetdinov 2011). D2RL is found postsynaptically, whereas D2RS is located presynaptically and plays a role in the regulation of DA synthesis and release (Lindgren, Xu et al. 2001, Centonze, Gubellini et al. 2004, Beaulieu and Gainetdinov 2011). Binding of DA or DA agonists to D2RS results in decreased release of DA and impaired autoreceptor function has been associated with altered DA transmission (Calipari, Sun et al. 2014). Binding of DA to D2RL, on the other hand, leads to the inhibition of adenylyl cyclase, which, in turn, leads to decreased activity of cAMP/protein kinase A (PKA) cascade activity and subsequent decreases in phosphorylation of PKA protein phosphates such CREB, NMDAR, AMPAR, and DARPP-32 (Surmeier, Ding et al. 2007, Beaulieu and Gainetdinov 2011). The D2R quantitative autoradiography conducted in Aim 2 does not distinguish between D2RS and D2RL levels. In Aim 1, we observed increases in DA in Pb^{2+}-exposed male rats at PN28 and PN50 in the absence of altered TH phosphorylation. As D2RS acts as an autoreceptor to regulate DA synthesis in presynaptic cells, future work should be undertaken to determine whether Pb^{2+} exposure has differential effects on D2RL and D2RS. Quantitative real-time PCR using methods similar to those described in Naneix (2013) can be implemented to measure D2RL and D2RS expression in the OT, NAC, and STR (Naneix, Marchand et al. 2013).

Preliminary subregional analysis of D1R and D2R levels in the OT, NAC, and STR (data not shown) indicated differences in magnitude and direction of Pb^{2+}-induced effects on D1R and D2R levels in neuroanatomical delineations corresponding with limbic, sensorimotor, and associative functions (Haber 2003, Voorn, Vanderschuren et al. 2004, Gruber and McDonald 2012). Subregional analyses of OT, NAC, and STR using a larger sample of animals may better elucidate functional relevance of Pb^{2+}-induced changes in DA receptor levels. Together with our current understanding of the neuroanatomical arrangement of DAergic circuitry associated with addiction, subregional analysis of D1R and D2R levels in the OT, NAC, and STR may allow us to better pin-point what behaviors and processes are most vulnerable to Pb^{2+} neurotoxicity.

In Chapter 3, using quantitative receptor autoradiography, we demonstrated that D1R and D2R levels were altered by chronic exposure to Pb^{2+}. Increased D2R levels were detected in the STR of PN14 male rats exposed to 180ppm PbAC diet, which resulted in BLL of 10µg/dl. We also observed robust increases in OT D1R levels in male and female rats at PN28 after exposure to 180ppm (BLL of 7µg/dl for males at PN28) and 1500ppm (BLL of 18µg/dl for males and 23µg/dl for females; BLL of 20µg/dl for combined sexes at PN28). Significant increases in D1R levels were also detected in the NAC and STR of male rats at PN28 following chronic Pb^{2+} exposure. The increased DA receptor levels observed in this aim, together with the increases in DA levels and DA turnover observed in the previous aim, support our hypothesis that Pb^{2+} exposure induces a hyperactive DAergic state that may ultimately result in increased sensitization to the effects of psychostimulants.

The extent of environmental Pb^{2+} exposure's impact on the prevalence of drug addiction in America today is unclear. Even so, in demonstrating a neurobiological mechanism through which Pb^{2+} may increase susceptibility for drug addiction later in life, we demonstrate yet

another long-term consequence of early-life Pb^{2+} exposure with far-reaching societal consequences. In light of recent events in Flint, Michigan (Hanna-Attisha and Kuehn 2016, Hanna-Attisha, LaChance et al. 2016), the results of this study highlight the importance of legislation and policy decisions designed to eliminate existing Pb^{2+} exposures. These results also highlight the need for legislation designed to eradicate the socioeconomic disparities associated with elevated BLL in children as these disparities may increase the risk for drug addiction as well as cognitive and behavioral deficits later in life.

References

Baler, R. D. and N. D. Volkow (2006). "Drug addiction: the neurobiology of disrupted self-control." Trends Mol Med **12**(12): 559-566.

Beaulieu, J. M. and R. R. Gainetdinov (2011). "The physiology, signaling, and pharmacology of dopamine receptors." Pharmacol Rev **63**(1): 182-217.

Bjorklund, A. and S. B. Dunnett (2007). "Dopamine neuron systems in the brain: an update." Trends Neurosci **30**(5): 194-202.

Calipari, E. S., H. Sun, K. Eldeeb, D. J. Luessen, X. Feng, A. C. Howlett, S. R. Jones and R. Chen (2014). "Amphetamine self-administration attenuates dopamine D2 autoreceptor function." Neuropsychopharmacology **39**(8): 1833-1842.

Canfield, R. L., C. R. Henderson, Jr., D. A. Cory-Slechta, C. Cox, T. A. Jusko and B. P. Lanphear (2003). "Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter." N Engl J Med **348**(16): 1517-1526.

Canfield, R. L., D. A. Kreher, C. Cornwell and C. R. Henderson, Jr. (2003). "Low-level lead exposure, executive functioning, and learning in early childhood." Child Neuropsychol **9**(1): 35-53.

CDC, Centers for Disease Control and Prevention. (2012, 7 Jun 2012). "CDC Response to Advisory Committee on Childhood Lead Poisoning Prevention Recommendations in "Low Level Lead Exposure Harms Children: A Renewed Call for Primary Prevention."." Retrieved 12/10/15, from http://www.cdc.gov/nceh/lead/ACCLPP/Final_Document_030712.pdf

CDC, Centers for Disease Control and Prevention. (2016). "Number of Children Tested and Confirmed BLL's ≥10 µg/dL by State, Year, and BLL Group, Children < 72 Months Old."

Centonze, D., P. Gubellini, A. Usiello, S. Rossi, A. Tscherter, E. Bracci, E. Erbs, N. Tognazzi, G. Bernardi, A. Pisani, P. Calabresi and E. Borrelli (2004). "Differential contribution of dopamine D2S and D2L receptors in the modulation of glutamate and GABA transmission in the striatum." Neuroscience **129**(1): 157-166.

Cory-Slechta, D. A. (1995). "Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter system functions." Annu Rev Pharmacol Toxicol **35**: 391-415.

Cory-Slechta, D. A., M. B. Virgolini, M. Thiruchelvam, D. D. Weston and M. R. Bauter (2004). "Maternal stress modulates the effects of developmental lead exposure." Environ Health Perspect **112**(6): 717-730.

Ensminger, M. E., J. C. Anthony and J. McCord (1997). "The inner city and drug use: initial findings from an epidemiological study." Drug Alcohol Depend **48**(3): 175-184.

Ensminger, M. E., H. S. Juon and K. E. Fothergill (2002). "Childhood and adolescent antecedents of substance use in adulthood." Addiction **97**(7): 833-844.

Fishbein, D. H., A. C. Todd, E. P. Ricketts and R. D. Semba (2008). "Relationship between lead exposure, cognitive function, and drug addiction: pilot study and research agenda." Environ Res **108**(3): 315-319.

Gruber, A. J. and R. J. McDonald (2012). "Context, emotion, and the strategic pursuit of goals: interactions among multiple brain systems controlling motivated behavior." Front Behav Neurosci **6**: 50.

Haber, S. N. (2003). "The primate basal ganglia: parallel and integrative networks." J Chem Neuroanat **26**(4): 317-330.

Halpain, S., J. A. Girault and P. Greengard (1990). "Activation of NMDA receptors induces dephosphorylation of DARPP-32 in rat striatal slices." Nature **343**(6256): 369-372.

Hanna-Attisha, M. and B. M. Kuehn (2016). "Pediatrician Sees Long Road Ahead for Flint After Lead Poisoning Crisis." JAMA **315**(10): 967-969.

Hanna-Attisha, M., J. LaChance, R. C. Sadler and A. Champney Schnepp (2016). "Elevated Blood Lead Levels in Children Associated With the Flint Drinking Water Crisis: A Spatial Analysis of Risk and Public Health Response." Am J Public Health **106**(2): 283-290.

Ikemoto, S. (2003). "Involvement of the olfactory tubercle in cocaine reward: intracranial self-administration studies." J Neurosci **23**(28): 9305-9311.

Ikemoto, S. (2007). "Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex." Brain Res Rev **56**(1): 27-78.

Ikemoto, S. (2010). "Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory." Neurosci Biobehav Rev **35**(2): 129-150.

Jones, D. C. and G. W. Miller (2008). "The effects of environmental neurotoxicants on the dopaminergic system: A possible role in drug addiction." Biochem Pharmacol **76**(5): 569-581.

Jusko, T. A., C. R. Henderson, B. P. Lanphear, D. A. Cory-Slechta, P. J. Parsons and R. L. Canfield (2008). "Blood lead concentrations < 10 microg/dL and child intelligence at 6 years of age." Environ Health Perspect **116**(2): 243-248.

Koob, G. F. and N. D. Volkow (2010). "Neurocircuitry of addiction." Neuropsychopharmacology **35**(1): 217-238.

Lanphear, B. P., R. Hornung, J. Khoury, K. Yolton, P. Baghurst, D. C. Bellinger, R. L. Canfield, K. N. Dietrich, R. Bornschein, T. Greene, S. J. Rothenberg, H. L. Needleman, L. Schnaas, G. Wasserman, J. Graziano and R. Roberts (2005). "Low-level environmental lead exposure and children's intellectual function: an international pooled analysis." Environ Health Perspect **113**(7): 894-899.

Lindgren, N., Z. Q. Xu, M. Herrera-Marschitz, J. Haycock, T. Hokfelt and G. Fisone (2001). "Dopamine D(2) receptors regulate tyrosine hydroxylase activity and phosphorylation at Ser40 in rat striatum." Eur J Neurosci **13**(4): 773-780.

Miller, D. K., J. R. Nation and G. R. Bratton (2000). "Perinatal exposure to lead attenuates the conditioned reinforcing properties of cocaine in male rats." Pharmacol Biochem Behav **67**(1): 111-119.

Naneix, F., A. R. Marchand, A. Pichon, J. R. Pape and E. Coutureau (2013). "Adolescent stimulation of D2 receptors alters the maturation of dopamine-dependent goal-directed behavior." Neuropsychopharmacology **38**(8): 1566-1574.

Naoi, M., T. Takahashi and T. Nagatsu (1988). "Simple assay procedure for tyrosine hydroxylase activity by high-performance liquid chromatography employing coulometric detection with minimal sample preparation." J Chromatogr **427**(2): 229-238.

Nation, J. R., G. D. Frye, J. Von Stultz and G. R. Bratton (1989). "Effects of combined lead and cadmium exposure: changes in schedule-controlled responding and in dopamine, serotonin, and their metabolites." Behav Neurosci **103**(5): 1108-1114.

Nation, J. R., K. R. Smith and G. R. Bratton (2004). "Early developmental lead exposure increases sensitivity to cocaine in a self-administration paradigm." Pharmacol Biochem Behav **77**(1): 127-135.

Needleman, H. L. (2004). "Low level lead exposure and the development of children." Southeast Asian J Trop Med Public Health **35**(2): 252-254.

Nevin, R. (2000). "How lead exposure relates to temporal changes in IQ, violent crime, and unwed pregnancy." Environ Res **83**(1): 1-22.

Nevin, R. (2007). "Understanding international crime trends: the legacy of preschool lead exposure." Environ Res **104**(3): 315-336.

Stansfield, K. H., K. N. Ruby, B. D. Soares, J. L. McGlothan, X. Liu and T. R. Guilarte (2015). "Early-life lead exposure recapitulates the selective loss of parvalbumin-positive GABAergic interneurons and subcortical dopamine system hyperactivity present in schizophrenia." Transl Psychiatry **5**: e522.

Surmeier, D. J., J. Ding, M. Day, Z. Wang and W. Shen (2007). "D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons." Trends Neurosci **30**(5): 228-235.

Usiello, A., J. H. Baik, F. Rouge-Pont, R. Picetti, A. Dierich, M. LeMeur, P. V. Piazza and E. Borrelli (2000). "Distinct functions of the two isoforms of dopamine D2 receptors." Nature **408**(6809): 199-203.

Volkow, N. D., G. F. Koob and A. T. McLellan (2016). "Neurobiologic Advances from the Brain Disease Model of Addiction." N Engl J Med **374**(4): 363-371.

Volkow, N. D. and T. K. Li (2004). "Drug addiction: the neurobiology of behaviour gone awry." Nat Rev Neurosci **5**(12): 963-970.

Volkow, N. D., G. J. Wang, M. W. Fischman, R. W. Foltin, J. S. Fowler, N. N. Abumrad, S. Vitkun, J. Logan, S. J. Gatley, N. Pappas, R. Hitzemann and C. E. Shea (1997). "Relationship between subjective effects of cocaine and dopamine transporter occupancy." Nature **386**(6627): 827-830.

Volkow, N. D., G. J. Wang, J. S. Fowler, D. Tomasi and F. Telang (2011). "Addiction: beyond dopamine reward circuitry." Proc Natl Acad Sci U S A **108**(37): 15037-15042.

Volkow, N. D. and R. A. Wise (2005). "How can drug addiction help us understand obesity?" Nat Neurosci **8**(5): 555-560.

Voorn, P., L. J. Vanderschuren, H. J. Groenewegen, T. W. Robbins and C. M. Pennartz (2004). "Putting a spin on the dorsal-ventral divide of the striatum." Trends Neurosci **27**(8): 468-474.

Zuch, C. L., D. J. O'Mara and D. A. Cory-Slechta (1998). "Low-level lead exposure selectively enhances dopamine overflow in nucleus accumbens: an in vivo electrochemistry time course assessment." Toxicol Appl Pharmacol **150**(1): 174-185.