

Mechanisms of Heavy Metal Neurotoxicity: Lead and Manganese

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Abstract

Human exposure to heavy metals is a global public health problem. Heavy metals which cause neurological toxicity, such as lead (Pb²⁺) and manganese (Mn), are of particular concern due to the long-lasting and possibly irreversible nature of their effects. Pb²⁺ exposure in childhood can result in cognitive and behavioral deficits in children. These effects are long-lasting and persist into adulthood even after Pb²⁺ exposure has been reduced or eliminated. While Mn is an essential element of the human diet and serves many cellular functions in the human body, elevated Mn levels can result in a Parkinson's disease (PD)-like syndrome and developmental Mn exposure can adversely affect childhood neurological development. Due to the ubiquitous presence of both metals, reducing human exposure to toxic levels of Mn and Pb²⁺ remains a world-wide public health challenge. In this review we summarize the toxicokinetics of Pb²⁺ and Mn, describe their neurotoxic mechanisms, and discuss common themes in heavy metal toxicology.

Keywords: Lead; Manganese; Presynaptic; Neurotoxicity; Development; Brain

Introduction

It has been estimated that 1 billion people in the world suffer from some form of disability [1]. Of the top 20 health conditions resulting in disability, one quarter are neurological [1]. Furthermore, it has recently been estimated that the global prevalence of intellectual disability may be as high as 1% [2]. Additionally, mental disability prevalence rates were twice as high in developing countries as found in developed countries. Environmental factors, such as maternal and child health care, immunizations, and environmental pollution, can influence the prevalence of mental disability [2]. Thus, poorer health quality and higher contamination levels of pollutants in developing countries may contribute to the higher prevalence rates.

A prime agent implicated in cognitive and neurological deficits is environmental exposure to heavy metals. Heavy metal exposure can occur through contaminated air, food, water, or in hazardous occupations. While the levels of heavy metal contamination of the environment have decreased in recent decades in the developed world, the developing world experiences high levels of metal pollution. In particular, Asian and African countries have high levels of metal contamination, especially in urban environments [3,4]. This contamination largely derives from anthropogenic sources, such as the combustion of leaded gasoline or unregulated industrial emissions. There is also a significant problem with metal contamination from mining in developing countries, which results in elevated metal levels in water and air [5,6]. Another major source of metal contamination in developing countries is the practice of electronic waste recycling. Electronic waste, which is composed of used or broken computers, mobile phones, and other electronic devices contains valuable metals such as copper and gold. This waste is exported from developed countries for disposal in developing countries, where few regulations are in place regarding safe disposal [7]. Unfortunately, primitive and unsafe methods are used for the extraction of the precious metals, resulting in contamination of the local environment of highly toxic metals such as mercury and lead [8,9]. Due to the toxic nature of many of the chemicals and metals found in electronic waste, this pollution may have lasting detrimental effects on the neurodevelopment of children [10].

The heavy metals lead (Pb^{2+}) and manganese (Mn) have both been shown to induce cognitive and behavioral deficits in adults and children with elevated levels of exposure [11-14]. While both metals can result in distinct neurological effects, with different brain targets and modes of action, they share a key similarity in that they both disrupt presynaptic neurotransmission. The aims of this review are to summarize the toxicokinetics of Pb^{2+} and Mn, describe their neurotoxic mechanisms, and discuss common themes in heavy metal toxicology.

Lead (Pb²⁺)

Pb²⁺ exposure has received world-wide attention due to its ability to cause behavioral and cognitive deficits in exposed children. The dose-response of Pb²⁺ effects on the intelligence quotient (IQ) of children is non-linear, with lower exposures of Pb²⁺ resulting in a greater rate of IQ loss than at higher exposures [13,15,16]. These studies indicate that the majority of the estimated IQ loss in Pb²⁺-exposed children occurs during the first 10 µg/dL of exposure, and suggest that Pb²⁺ may be a non-threshold neurotoxicant [13,15,16]. Due to these effects, there have been global initiatives to reduce the use of Pb²⁺, but despite these efforts Pb²⁺ exposure remains a widespread problem [17,18].

Pb²⁺ Exposure

In the United States, Pb²⁺ was once commonly added to petrolbased fuel as an antiknock agent to improve engine efficiency. However, major concerns regarding Pb²⁺ exposure and its adverse effect on child neurological function [19,20] ultimately resulted in the reduction and eventual ban of leaded fuel. Analysis of population blood lead levels (BLLs) show that as Pb²⁺ was removed from gasoline, BLLs dropped

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significantly [21-24]. In a meta-analysis of 17 studies from 5 continents, the average BLL after removal of Pb²⁺ from gasoline was estimated to be 3 μ g/dL [25]. This value is close to the current US population average BLL [26] and levels estimated in isolated populations [27], and may indicate background Pb²⁺ exposure from sources other than emissions from combustion of leaded gasoline. Alternative airborne sources of Pb²⁺ exposure include primary and secondary smelters and piston-engine aircraft, which still use Pb²⁺-containing gasoline [28].

As a result of evidence that Pb^{2+} in paint could cause neurological deficits when ingested by children, Pb^{2+} was removed from paint in Europe in 1922 and in the United States in 1978 [29,30]. However, banning the sale of leaded paint did not remove the threat of Pb^{2+} contamination in homes already containing leaded paint. Many homes in the United States still contain leaded paint [30,31], especially in city centers [32]. Chipped and peeling leaded paint constitutes a major source of current childhood Pb^{2+} exposure, as the desiccated paint can easily disintegrate at friction surfaces to form Pb^{2+} dust [33,34]. Pb^{2+} dust can also be formed from the combustion of leaded fuels; previous emissions of leaded fuel resulted in a massive dispersion of Pb^{2+} dust in the environment, especially along roadways [35]. Particulate Pb^{2+} is characteristically fine (2-10 μ M) [35], does not degrade, and continues to be a major source of human exposure [32,36].

Drinking water can be another source of environmental Pb²⁺ exposure. Leaching of Pb²⁺ into drinking water occurs from outdated fixtures and solders containing Pb²⁺. The significance of Pb²⁺ leaching into drinking water was emphasized during the 2001 Washington, DC, "Lead in drinking water crisis", when leaching of Pb²⁺ from pipes into drinking water rapidly increased the amount of Pb²⁺ contamination, resulting in a 9.6 fold increase in the incidence of elevated blood Pb²⁺ levels in children [37]. This unfortunate incident highlights the role contaminated drinking water can play in overall childhood Pb²⁺ exposure.

Due to the success of environmental interventions regarding Pb²⁺, childhood Pb2+ exposure in the US has decreased since the 1970's. The most recent evidence indicates that contemporary childhood BLLs in the US are on the order of 1.9 μ g/dL while the percentage of children with elevated BLLs (above 10 µg/dL) has dropped to 1.4% [26]. Peak BLLs occur when children are roughly 2 years of age [13,16,38,39]. Significant decreases in BLLs have been observed in this age group as well: average BLLs have decreased to 2.1 µg/dL and the percentage of children in this age group with elevated BLLs dropped to 2.4% [26]. Thus, the contemporary exposure levels for children in the US are generally under 3 μ g/dL, and are approaching the levels of Pb²⁺ exposure measured in geographically isolated populations [27]. However, while the average BLLs in the US have decreased, there are still at-risk populations with higher than average BLLs. Children of lower social economic status (SES) or racial minority status are still at higher risk of Pb²⁺ poisoning [26] and some regions in the US have higher prevalence rates of elevated BLLs in children [40].

Pb²⁺ Toxicokinetics

The main routes of exposure for Pb^{2+} are inhalation and ingestion. Inhalation exposure to Pb^{2+} is a much more efficient route of absorption than ingestion, with an estimated absorption efficiency ratio of 10 to 1 in the lung compared to the GI tract [41,42]. Due to the reduction in use of leaded gasoline, inhalation exposure in developed countries is generally limited to people who live near smelters and to workers in occupational settings [43,44]. The deposition of inhaled inorganic Pb^{2+} is dependent on particle size and composition. Larger particles Page 2 of 13

(> 2.5 μ m) are deposited in the upper airways and can be cleared via mucociliary clearance. Particles < 1 μ m penetrate to alveoli and are subsequently absorbed by phagocytosis. Particles cleared by mucociliary clearance can be subsequently ingested, contributing to Pb²⁺ exposure via ingestion [45].

Absorption of Pb²⁺ from the intestine is mediated by both passive and facilitated diffusion, although passive diffusion plays a minor role in total absorption [46]. Studies on the intestinal absorption of Pb2+ have consistently reported evidence of carrier-mediated transport [47,48], but the identity of the transporter or transporters is still a matter of debate. Some evidence supports the hypothesis that divalent metal transporter 1 (DMT1) is responsible for transporting Pb2+. DMT1 is a metal ion transporter that can transport metals such as Pb²⁺, Cd^{2+} , and Zn^{2+} in addition to its physiological substrate, iron (Fe) [49]. Over expression of DMT1 in an intestinal cell culture model (CaCo-2) resulted in increased Pb2+ transport, but knockdown of the transporter did not abolish Pb2+ transfer [49]. Furthermore, a recent study established that Pb2+ is absorbed both in the duodenum, which exhibits high levels of DMT1, as well as the ileum which exhibits low expression of DMT1 [50,51]. Thus, while DMT1 likely plays a role in Pb²⁺ uptake from the GI tract, it is apparent that other carrier proteins exist. One such candidate is the calcium (Ca²⁺) binding protein calbindin, which is responsible for basolateral Ca²⁺ transfer in enterocytes and has been shown to bind both Pb^{2+} and Ca^{2+} with similar affinity (5 μ M) [52,53]. Although never shown experimentally, hypothetically calbindin may basolaterally transport Pb2+ as well as Ca2+.

In blood, Pb²⁺ is primarily bound to protein. Up to 40% of blood Pb2+ (BPb) is bound to serum albumin, and the remaining BPb is bound to sulfhydryl- or thiol-containing ligands [54]. Work with the radiotracer 203-Pb in rats demonstrated that Pb2+ is taken up into the brain most likely as a free ion (PbOH⁺) or complexed with small molecular weight ligands. PbOH⁺ most likely crosses the blood brain barrier (BBB) through passive diffusion [55,56], but could also be transported through cation transporters [55]. DMT1 is highly expressed in the striatum, cortex, hippocampus, and cerebellum [57] and may facilitate Pb2+ transfer across the BBB [58]. Brain efflux is likely mediated through ATP-dependent Ca2+ pumps [56,59]. Within the brain, there is substantial debate regarding Pb2+ distribution; some studies have reported that Pb2+ preferentially accumulates in specific brain regions, such as the hippocampus [60]. However, other studies using different methodologies did not observe any differences in regional brain accumulation of Pb²⁺ [61].

About 94% of the human Pb^{2+} body burden is found in bone in adults, but only 73% in children. Pb^{2+} readily displaces Ca^{2+} in the bone matrix by cation-exchanges processes [62]. Recycling of Pb^{2+} between bone and blood occurs continuously; if recycling between blood and bone compartments could be eliminated the half-life of Pb^{2+} in blood would drop from 40 days to about 10 days [63]. Metabolic balance studies indicate Pb^{2+} is predominately excreted through feces, with urinary excretion playing a secondary role. Trace amounts of Pb^{2+} can also be excreted through hair, sweat, breast milk, and nails [64-66].

Child Susceptibility to Pb2+

Children are more susceptible to the effects of Pb^{2+} than adults for a number of reasons. First, children with hand to mouth behavior are at particular risk of elevated Pb^{2+} exposure due to the ingestion of Pb^{2+} dust [30]. Additionally, the BBB is immature during fetal development [67], which may contribute to greater accumulation of Pb^{2+} in the developing brain. Another factor is that children have a higher basal uptake of Pb²⁺ than adults. Adult human absorption of Pb²⁺ is around 10% [68] while infant absorption of Pb²⁺ is about 26.2% [66]. Radiolabel studies using ²¹⁰Pb in rhesus monkeys demonstrated that young monkeys cleared less Pb²⁺, absorbed more Pb²⁺, and may have increased Pb²⁺ absorption from blood to soft tissues relative to adult animals [64]. In particular, the brains of young animals absorbed eight times the amount of Pb²⁺ compared to adult animals [69].

Partly due to increased absorption, children have a higher burden of mobile Pb^{2+} stores. As discussed above, children store less Pb^{2+} in bone, resulting in a higher BPb burdun. Furthermore, bone turnover in children due to skeletal growth results in a constant leaching of Pb^{2+} into the blood stream, causing continuous endogenous exposure [70]. Infants with low Pb^{2+} exposure actually have a higher excretion rate of Pb^{2+} than is accounted for by dietary intake, suggesting that Pb^{2+} stored in bone during fetal development and then mobilized by skeletal growth may contribute to a source of postnatal exposure [66,71].

Modifying factors in human Pb²⁺ exposure

Dietary factors can significantly impact Pb^{2+} absorption. Children deficient in Fe or Ca^{2+} are more likely to have elevated BLLs [72-75]. Supporting this observation, Fe-deficient animals retained five times the administered Pb^{2+} than animals with normal Fe levels [76]. One plausible mechanism for Fe-induced dietary alterations in Pb^{2+} absorption is through regulation of DMT1. DMT1 is regulated at the mRNA level by Fe through the iron response element [77], thus, iron-deficient diets increase the levels of DMT1 and concomitantly increase Pb^{2+} absorption [52,78]. Furthermore, DMT1 is found in brain endothelial cells of the BBB, so upregulation of DMT1 by Fe deficiency may also increase transport into the brain.

Neurotoxic effects of Pb²⁺: Results from Epidemiological Studies

The neurological effects of Pb²⁺ in exposed children have been a driving factor in reducing the level of Pb²⁺ in the environment. In 1991 the United States Centers for Disease Control and Prevention (CDC) lowered the definition of Pb²⁺ intoxication to 10 μ g/dL BLL (the current regulatory level) motivated by the evidence from several studies that children with BLL of at least 10 μ g/dL had impaired intellectual function [79]. More recently, studies have shown that the dose-response of Pb²⁺ on IQ in children is non-linear, with lower exposures of Pb²⁺ resulting in a greater rate of IQ loss than at higher exposures [13,15,16,80]. These data clearly demonstrate that the majority of the estimated IQ loss in Pb²⁺-exposed children occurs during the first 10 μ g/dL, and many studies have suggested a lack of a threshold for the effects of Pb²⁺ on IQ [13,15,16].

A large, internationally-pooled analysis of Pb^{2+} -exposed children estimated that children with BLLs of 10 µg/dL experience a deficit of about 6.2 IQ points relative to children with estimated BLLs of 1 µg/ dL [13]. This is comparable to the deficit of 7.4 IQ points observed in children with BLLs of 10 µg/dL in another large study [16]. On an individual level, a decrease in IQ of 6-7 points would be difficult to detect. However, the effect of a population decrease in IQ of this magnitude is quite significant. By shifting the normal distribution of IQ scores lower, the number of children with impaired intelligence would increase significantly while the number of exceptionally gifted children would decrease [29]. Several researchers have studied this effect from an economical standpoint and suggest that the monetary cost of such an effect may total over 40 billion dollars for one age group alone. Over a 20-year period, one generation, this loss may amount to nearly 800 billion dollars [29,81]. In addition to the cognitive deficits associated with Pb^{2+} exposure, children with elevated BLLs experience behavioral deficits. School children with elevated BLLs are more likely to act out in class, display antisocial behavior, and have trouble paying attention [82-84]. Cumulative childhood Pb^{2+} exposure was associated with a higher incidence in behavioral problems in 8-year-old children [83,85]. These behavioral effects appear to have a phenotype similar to attentiondeficit hyperactivity disorder (ADHD). Furthermore, recent studies have identified that childhood Pb^{2+} exposure is positivity associated with ADHD diagnosis [84,86].

The cognitive and behavioral deficits of Pb^{2+} -exposed children persist even after the cessation of Pb^{2+} exposure [87], and chelation therapy is unable to remediate the effect of Pb^{2+} on cognition [88-90]. Prenatal and/or childhood Pb^{2+} exposure was associated with anti-social and delinquent behavior as adolescents [91], an increased likelihood be an adjudicated delinquent [92] or to be arrested as an adult [93]. Furthermore, childhood Pb^{2+} exposure may predict adult cognitive function [94]. Children who experience elevated Pb^{2+} levels are more likely to have decreased brain volume in adulthood in specific brain regions [95]. These changes could account for altered behavior and cognition in adults exposed to Pb^{2+} as children. Thus, developmental Pb^{2+} exposure in humans results in long-lasting effects on cognition and behavior even after cessation of exposure.

Possible Mechanism of Pb²⁺ Neurotoxicity: Results from experimental animal studies

It is believed that Pb²⁺ targets the learning and memory processes of the brain by inhibiting the N-methyl-D-aspartate receptor (NMDAR), which is essential for hippocampus-mediated learning and memory [96,97]. The NMDAR is essential for learning spatial navigation tasks in animal models [96], and animals which have been developmentally exposed to Pb²⁺ exhibit similar learning deficits as those with absent or impaired NMDARs [96-98].

The NMDAR is composed of an obligatory NR1 subunit and one or more accessory subunits from the NR2 and NR3 families. In the hippocampus, NR2A and NR2B are the most abundant NR2 family members. Pb^{2+} is a potent, non-competitive antagonist of the NMDAR [14,99-102]. Evidence suggests that Pb^{2+} binds the Zn^{2+} regulatory site of the NMDAR in a voltage-independent manner [103-105]. Since Zn^{2+} binds with high affinity at a regulatory site on the NR2A subunit [106], but with lower affinity to the NR2B subunit [107], this suggests a preferential sensitivity of NR2A-NMDARs for Pb^{2+} [103,105]. In support of this hypothesis, electrophysiological studies on recombinant receptors demonstrate that Pb^{2+} more potently inhibits NR2A-NMDARs than NR2B-NMDARs [104,108] or the tri-heteromeric form, NR1/NR2A/NR2B-NMDAR [108].

In addition to acting as an NMDAR antagonist, Pb²⁺ exposure also disrupts normal NMDAR ontogeny. Chronic developmental Pb²⁺ exposure results in decreased NR2A content in the hippocampus [109-112] and altered expression of NR1 splice variants [112-114]. In contrast, NR2B mRNA levels either remained unchanged or slightly increased in rats developmentally exposed to Pb²⁺ [109-112,115]. Together, these data suggest that Pb²⁺ delays the normal developmental switch of increased NR2A incorporation in NMDARs with synapse maturation [14,115]. Similar trends have also been observed in cultured neuron systems [116,117] and suggest that Pb²⁺ exposure may cause lasting changes in NMDAR subunit composition and expression.

In addition to hippocampal changes in NMDAR subunit

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expression and ontogeny, Pb2+ may alter the cellular distribution of NMDAR populations. We have shown that Pb2+ exposure during synaptic development in hippocampal cultures reduces the levels of synaptic NR2A-NMDARs with a concomitant increase in extrasynaptic NR2B-NMDARs [116]. This is significant because the NR2 family members are linked to differential MAPK signaling [118], pro-death or pro-life signaling [119], and differential induction of nuclear gene expression [120]. In particular, NR2A-NMDAR activation is linked to cell survival pathways and cyclic AMP response element binding protein (CREB) activation while NR2B-NMDAR activation is linked to cell death pathways and CREB shutoff [120]. Thus, changes in synaptic localization of NMDARs by Pb2+ could alter downstream NMDAR-mediated signaling. Supporting this hypothesis, chronic developmental Pb²⁺ exposure results in altered MAPK signaling [121], calcium/calmodulin kinase II (CaMKII) activity [122], and altered CREB phosphorylation and binding affinity [115,123]. CREB is a transcription factor for many immediate early genes (IEGs), which play an essential role in memory consolidation and are expressed as a result of NMDAR activity [124]. Altered IEG expression in animals exposed to Pb2+ has been observed [125], indicating that altered CREB activity due to Pb2+-mediated disruption of NMDAR signaling may result in impaired learning and memory processes.

Pb²⁺ exposure can cause deficits in neurotransmission. Rats chronically exposed to low levels of Pb²⁺ have reduced Ca²⁺-dependent glutamate and γ -aminobutyric acid (GABA) release in the hippocampus [126-128], which indicates presynaptic neuron dysfunction during Pb²⁺ exposure. In cultured hippocampal neurons [129] and in brain slices [128], Pb²⁺ exposure impairs excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs). EPSCs and IPSCs are dependent upon neurotransmitter release from the presynaptic neuron, thus, reductions in EPSCs and IPSCs indicate a deficit in neurotransmission in both the glutamatergic and GABAergic systems as a result of Pb²⁺ exposure.

A recent study from our laboratory has shown that Pb^{2+} exposure in cultured hippocampal neurons during synaptic development resulted in altered presynaptic protein expression and deficits in vesicular neurotransmitter release [130]. Pb^{2+} exposure reduced the expression of key presynaptic proteins involved in vesicular release, such as synaptophysin (Syn) and synaptobrevin (Syb). Reductions of vesicular release proteins were associated with both glutamatergic and GABAergic synapses, consistent with electrophysiological observations regarding EPSC and IPSC generation during Pb^{2+} exposure [128,129]. Vesicular release in Pb^{2+} -exposed neurons was significantly impaired relative to control conditions as determined by live-imaging studies using the synaptic vesicle dye FM 1-43 [130]. Together, animal and cell culture studies indicate a role for Pb^{2+} in presynaptic dysfunction which results in reduced neurotransmission [131].

One molecular mechanism by which Pb^{2+} may disrupt neurotransmission is by inhibiting neuronal voltage-gated calcium (Ca²⁺) channels (VGCCs) [132]. Removal of extracellular Ca²⁺ from hippocampal slice cultures resulted in identical effects on IPSC frequency as Pb^{2+} exposure, suggesting that the Pb^{2+} -induced inhibition of IPSC frequency occurred via reduction of Ca²⁺ influx through VGCCs [128]. Inhibition of presynaptic VGCCs may prevent the necessary rise in internal Ca²⁺ required for fast, Ca²⁺-dependent vesicular release, thus interfering with neurotransmission. However, the effects of Pb^{2+} we observed on presynaptic protein expression were dependent on NMDAR activity, based on comparison studies with the specific NMDAR antagonist aminophosphonovaleric acid (APV, Page 4 of 13

which does not inhibit VGCCs) which resulted in similar effects as Pb²⁺ exposure [130]. Thus, while Pb²⁺ inhibits VGCCs, which may result in impaired neurotransmission, VGCC inhibition by Pb²⁺ is not exclusively responsible for the presynaptic effects of Pb²⁺ and long-term NMDAR inhibition plays an important role in these effects.

An emerging theme in the mechanism of Pb²⁺ neurotoxicity is the disruption of intracellular Ca2+ dynamics. Inhibition of either VGCCs or NMDARs by Pb2+ would result in a significant reduction of Ca2+ entry into the cell. This is important because Ca²⁺ signaling is essential for synaptic development and plasticity [133,134] and perturbation of these processes can lead to neurological disease states [134,135]. One key Ca2+-dependent pathway involved in synaptic development and neurotransmitter release is brain-derived neurotrophic factor (BDNF) signaling [136-139]. BDNF is a trans-synaptic signaling molecule that is released from both axons and dendrites [139]. We have recently shown that BDNF levels are reduced in Pb2+-exposed cultures and that exogenous BDNF supplementation during Pb2+ exposure can fully mitigate the effects of Pb2+ on presynaptic function and protein expression [130]. Furthermore, BDNF expression and release are dependent on Ca2+ signaling, and both NMDAR- and VGCC-dependent Ca²⁺ pathways have been implicated in BDNF neurotransmission [139-141]. Interestingly, NMDAR-dependent release of BDNF may play a greater role in dendritic BDNF release rather than axonic BDNF [139]. This would support our hypothesis that NMDAR-dependent release of BDNF is disrupted during Pb²⁺ exposure [130,131], since the majority of NMDARs are postsynaptically located [142].

Regardless of whether Ca^{2+} disruption occurs via block of NMDAR or VGCC (or both), BDNF expression and release are impaired during Pb²⁺ exposure, which has effects on synaptic development [130] and may cause long-term impairment of hippocampal function *in vivo*. Interestingly in an animal study investigating the effects of environmental enrichment on Pb²⁺ exposure, animals exposed to Pb²⁺ but living in an enriched environment did not exhibit the deficits in spatial learning tasks usually observed in rats chronically exposed to Pb²⁺ [143]. In fact, Pb²⁺-exposed rats living in an enriched environment performed equally as rats which were not exposed to Pb²⁺. Furthermore, the Pb²⁺-exposed rats living in enriched environments exhibited elevated mRNA levels of BDNF relative to Pb²⁺-exposed rats living in normal conditions. This indicates that BDNF may be implicated *in vivo* in the effects of Pb²⁺ on learning and memory.

To summarize, Pb²⁺ remains a neurotoxiciant of concern due to its ubiquitious environmental presence and the absence of "safe" levels of exposure. Pb²⁺ exposure can cause both behavioral and cognitive deficits in children at very low (<10 µg/dL blood lead) levels of exposure. Recent progress has been made in the understanding of the cellular mechanism of Pb²⁺ toxicity, but further work is needed to address intervention and/or remediation strategies.

Manganese (Mn)

Manganese, an essential element of the human diet, is a naturally occurring component of the earth's crust. After iron, Mn is the second most abundant heavy metal. Unlike Pb²⁺, which has no known physiological role, Mn has many beneficial roles in human physiology [144]. The adequate daily intake of Mn has been set by the National Academy of Sciences (NAS) at 2.3 mg/day for men and 1.8 mg/day for women [145]. Dietary Mn is sufficient to maintain adequate Mn homeostasis and Mn deficiencies in humans are exceedingly rare [144]. However, elevated Mn levels can cause human neurotoxicity. Notably, workers exposed to high airborne Mn levels are at elevated risk of

developing a Parkinson's disease (PD)-like neurological disorder known as manganism [146], and recently adverse effects of exposure to elevated Mn in drinking water have been observed in children [11,147-149].

Mn Exposure and Toxicokinetics

While Mn can exist in 11 different oxidation states, Mn(II) and Mn(III) are the most biologically relevant [150-152]. The primary route for non-occupational exposures to Mn is ingestion. Three to five percent of ingested Mn is absorbed by the gut. Overt toxicity from ingestion is rare due to the tight regulation of Mn homeostasis coordinated through absorption and biliary excretion [144]. Biliary excretion is the predominate route of Mn excretion, but a fraction of Mn is reabsorbed in the gut, establishing an entero-hepatic loop [153]. Human Mn exposure can also occur through Mn contamination of drinking water and from Mn-containing agriculture agents, such as the fungicide Maneb [154]. There is significant concern regarding increased human exposure to Mn through use of the Mn-containing fuel additive methycyclopentadienyl manganese tricarbonyl (MMT) [155-157], and regions using MMT have elevated air Mn levels, particularly near roads with heavy traffic [158,159].

In contrast to the relatively minor routes of exposure listed above, exposure to airborne Mn in occupational settings is believed to be the cause of the majority of human Mn toxicity. In particular, miners, welders, smelters, workers of ferro-alloy plants, and drycell battery workers are at higher risk for Mn-related toxicity [160-165]. Airborne Mn is readily absorbed from the lung. As with Pb²⁺, pulmonary absorption of Mn is much higher than GI absorption [166] and pulmonary absorption of Mn likely occurs through Ca²⁺ channels [150,167]. Mn inhaled through the nose can access the olfactory bulb [168-170], which may be a direct route of Mn exposure to the brain.

The majority (80%) of Mn in blood (BMn) is bound to albumin, globulin, or transferrin [151]. A small fraction of BMn is present as Mn-citrate. From the blood, Mn crosses the blood brain barrier by facilitated diffusion or crosses cell membranes using DMT1-, Zrt-like/ Irt-like 8 (ZIP8), or transferrin-mediated mechanisms. Similar to Pb2+, Mn(II) may be transported by DMT1 both across the intestinal wall and across the BBB [170-172], although substantial debate exists regarding the contribution of DMT1 in brain Mn import [150]. Stronger evidence exists for transport of Mn into brain via transferrin (Tf). Mn(III) tightly binds Tf, forming a Mn-Tf complex [173]. Mn(II) may be oxidized to Mn(III) for subsequent loading onto Tf by ceruloplasmin (Cp), a protein which facilitates the oxidation of Fe(II) to Fe(III) [174]. However, recent evidence suggests that Cp does not oxidize Mn(II) and instead Mn(II) may either auto-oxidize in plasma or be oxidized by another pro-oxidant before binding Tf [175]. The Mn-Tf complex binds transferrin receptors (TfRs) and is subsequently endocytosed by brain microvascular endothelial cells. Within the endothelial cell, Mn dissociates from Tf by endosomal acidification and is transferred to the abluminal cell surface for release into the extracellular environment within the brain (for review, see [152]). While a role for Mn transport via ZIP8 has been proposed based on studies in cell culture models [152,176], physiological evidence of ZIP8 transport of Mn into the brain has yet to be established. There also may be a role for a storeoperated Ca²⁺ channel for Mn brain import [150]. Mn may also cross the choroid plexus into cerebrospinal fluid (CSF) and thus gain access to brain tissues, particularly at high BMn levels [177,178]. Once across the blood-brain or blood-CSF barriers, Mn is predominately found as Mn-citrate [151] and accumulates inside of neurons, oligodendrocytes, and astrocytes, likely via DMT-1 dependent mechanisms [152,172,179]. Brain efflux of Mn is likely mediated by diffusion [150]. Since several carrier-mediated import pathways exist while the only known efflux pathway is diffusion, Mn has the potential to be retained in the brain for an extended period.

Effects of Mn exposure in humans: Results from Epidemiological studies

Exposure to high levels of Mn can result in manganism, an extrapyramidal neurological disease characterized by rigidity, a mask-like expression, action tremor, bradykinesia, gait disturbances, memory and cognitive dysfunction, micrographia, and mood disorder [180-183]. The symptoms of manganism are strikingly similar to that of Parkinson's disease (PD); however, manganism usually presents with marked differences from PD, such as insensitivity to levodopa (L-DOPA) administration and differences both in disease progression [12] and in symptoms [146]. While insensitivity to L-DOPA is generally considered a key difference between manganism and PD [12], some patients with manganism responded positively to L-DOPA therapy in a minority of studies [180,184,185]. However, it is possible that the cases which responded to L-DOPA may have had underlying PD etiology and that the effects of Mn are secondary to or compounded by those of PD [12].

The extra-pyramidal effects of Mn are thought to be mediated by Mn-induced neurotoxicity in the globus pallidus and other basal ganglia structures of the human brain [12,186]. The chemical characteristics of Mn lend an advantage to non-invasive measurement techniques; since Mn is a paramagnetic metal molecular imaging technique such as T1-weighted magnetic resonance imaging (T1-MRI), have allowed researchers to determine the distribution of Mn non-invasively in humans [12]. In humans occupationally exposed to airborne Mn, the metal accumulates in the basal ganglia [12,162]. Using single-photon emission computed tomography (SPECT) and positron emission tomography (PET), it has been shown that elevated brain Mn can result in deficits in the dopaminergic system in exposed humans [12]. However, the results from studies on Mn-intoxicated humans need to be interpreted with care because several confounding factors, such as underlying PD, may influence the results.

Although the majority of our understanding of Mn neurotoxicity relates to adult exposures, there has been increasing evidence that Mn may have developmental neurotoxic effects in children. Recent epidemiological studies have shown that children who drink water from wells with elevated Mn levels exhibit both cognitive and behavioral deficits [11,147,148]. School children exhibited decreased IQ with increasing groundwater Mn exposure (Mn well water range 0.1-2700 µg/L, geometric mean=20 µg/L), with a decrease of 6.2 IQ points between the median of the lowest $(1 \mu g/L)$ and highest (216 µg/L) Mn exposure quintiles [11]. Furthermore, school children who ingested well water with elevated Mn levels displayed more hyperactive behavior, aggressive behavior, and deficits in attention in school [147]. Children exposed to Mn from living in close proximity to a Mn alloy plant exhibited elevated hair Mn levels that were negatively associated with both full scale and verbal IQ scores [149]. In a longitudinal study of early-life Mn exposure examining neurodevelopment endpoints at 12 and 36 months of age, it was observed that high Mn exposure was negatively associated with neurodevelopment score [187]. Together these data suggest that excess exposure to Mn, like Pb²⁺, is a developmental neurotoxicant with both behavioral and cognitive effects in exposed children.

Susceptibility factors in Mn toxicity

Similar to Pb²⁺ exposure, children and infants are more susceptible to Mn poisoning than adults. Neonates in particular exhibit high Mn absorption rates, up to 40% of ingested Mn by some estimates [188], compared to roughly 3% absorption in adults [166]. Infants and especially neonates are further susceptible due to diminished biliary excretion [189], which is the major route of Mn excretion in humans [144]. An important source of exposure for this group is infant formula, particularly soy-based formula, which can contain 100 times the amount of Mn as human breast milk [144]. Children may be furthermore at risk for greater combined exposure from airborne Mn and ingested Mn based on physiology-based pharmacokinetics (PBPK) modeling incorporating the increased breathing rates, lower body masses, and increased GI absorption of children [190]. Based on this study, children may easily exceed the recommended dietary intake of Mn through a combination of airborne and dietary sources.

Deficits in biliary excretion as a result of liver injury or disease can also result in elevated Mn levels in blood [191] and in the basal ganglia [184,192,193]. Patients with elevated BMn due to liver disease exhibit motor deficits consistent with manganism, such as tremor, rigidity, and gait disturbances [184,194]. One postmortem study observed that patients with liver failure who exhibited parkinsonian symptoms had 4.7 fold higher brain Mn levels compared to patients with liver failure that had normal brain function [193]. If patients with compromised liver function receive a liver transplant, BMn levels decrease and in rare cases the neurological symptoms are reversed or lessened [195,196].

Similar to Pb²⁺ toxicity, dietary factors can influence Mn toxicity. Fe-deficient individuals exhibit higher Mn absorption likely due to upregulated DMT1 in the gut and in cells of the BBB [170,172]. Upregulation of DMT1 in the olfactory bulb due to iron deficiency has also been shown to increase Mn accumulation of Mn in the basal ganglia of rats [170].

Finally, patients receiving parenteral nutrition (PN) can experience elevated Mn levels [197,182], sometimes accompanied by parkinsonian movement disorders [198,199]. The elevated Mn levels are likely due to the fact that PN solutions without Mn supplementation still contain 7.3 μ g/L of Mn as a contaminant [200]. Further Mn supplementation of PN solutions can result in an added 5.0-7.5 μ g/kg body weight of Mn [144]. Under normal conditions only a fraction of ingested Mn is absorbed, but since PN bypasses the GI system patients who receive PN are likely to develop elevated BMn levels. Mn accumulation in the brain of patients on PN can be detected before clinical symptoms present [201], and if PN is removed Mn is cleared from the brain and BMn levels diminish [198,201].

Possible Mechanism of Mn Neurotoxicity: Results from laboratory studies

Within the substantia nigra (SN), globus pallidus (GP), and striatum (STR), Mn accumulates in neurons, astrocytes, and oligodendrocytes [152,172]. Intracellular Mn accumulates within the mitochondria, where it disrupts ATP synthesis [202]. The Ca²⁺ uniporter sequesters Mn in the mitochrondria, however, no known mitochondria export process exists, resulting in rapid Mn accumulation [203]. Until recently it was believed that Mn disrupted ATP synthesis by inhibiting the F₁/ F₀ ATP synthase [202] or complex 1 (NADH dehydrogenase) of the mitochondrial respiration chain [204]. However, a recent study revealed that Mn inhibits ATP synthesis at two sites in brain mitochondria, either complex II (succinate dehydrogenase) or the glutamate/aspartate

exchanger, depending on the mitochondrial energy source [205]. Disruption of ATP synthesis leads to decreased intracellular ATP levels and increased oxidative stress [206,207], which may contribute to Mn cellular toxicity [208]. Further contributing to intracellular oxidative stress is the ability of Mn to oxidize dopamine (DA) to reactive quinone species (for review, see [209]). Between increased free radical generation via disrupted mitochondrial respiration and the oxidation of DA to reactive species, Mn exposure results in a decrease in the levels of free thiol and hydroxyl groups in cellular antioxidant proteins [209]. The increase in oxidative species combined with a decreased reductive capacity can result in dendritic degeneration [206] and cytotoxicity in culture systems [210].

The sensitivity of the dopaminergic system to Mn is an active area of investigation. Studies in nematode [211], cell culture [212], rodent [177,213,214], and non-human primate [215] models of Mn toxicity all demonstrate specific deficits in the dopaminergic system caused by Mn exposure. In contrast, the glutamatergic and GABA-ergic systems of the brain remain relatively unaffected by Mn exposure [216], and Mn(II) is more toxic to DA-producing cells than non-DA producing cells *in vitro* [212]. A recent study in *C. elegans* suggests that extracellular, not intracellular, DA is converted to the reactive species. This reactive DA species is taken up by the dopamine transporter (DAT1), thus resulting in dopaminergic neurotoxicity [211]. The findings of this study need to be confirmed in other model systems, but may indicate a basis for the enhanced sensitivity of the dopaminergic system to Mn(II).

Interestingly, the different species of Mn have different potencies in the cellular effects described above. Mn(III) is taken up by cells more efficiently than Mn(II) [217,218]. Furthermore, Mn(III) has a higher reduction potential than Mn(II), is a more potent oxidizer of DA than Mn(II), and is more cytotoxic than Mn(II) [217-220]. However, no difference was observed in the disruption of ATP synthesis between studies using Mn(II) or Mn(III) compounds [218]. The in vivo effects of Mn(II) and Mn(III) were compared in a rat study [221]. Adult female Sprague Dawley rats were injected intraperitoneally with either Mn(II)-chloride or Mn(III)-pyrophosphate and the effect of Mn species on brain Mn accumulation and effects were examined. Even with comparable BMn levels, Mn(III) exhibited greater accumulation in the brain, suggesting that either the uptake of Mn into the brain or retention of Mn in the brain may be dependent on oxidation state [221]. However, these differences could also be explained by the difference in solubility between Mn(II)-chloride or Mn(III)-pyrophosphate because Mn(III)-pyrophosphate has low solubility in biological media. Furthermore, this study did not observe regional differences in brain Mn accumulation, unlike studies in non-human primates [215] and Mn levels observed in occupationally-exposed humans [12] which demonstrate a clear tendency of Mn to accumulate in basal ganglia structures. This highlights the challenges in finding appropriate disease models of Mn. Studies in rodents are limited by the fact that rodents are less sensitive to Mn than are humans or non-human primates [12,222]. Rodents do not accumulate Mn in the same brain regions as humans or non-human primates [223]. Furthermore, rodent models of Mn toxicity do not develop analogous behavioral deficits as observed in humans or non-human primates chronically exposed to Mn [12].

For reasons described above, non-human primates remain the most relevant animal model of Mn intoxication for the human condition. In non-human primates, *in vivo* imaging studies have found that Mn accumulates preferentially in the caudate-putamen (CP), SN, and GP [215]. These findings have been supported in studies showing increased Mn content in the STR, GP, and SN of non-human primates

using graphite furnace atomic absorption spectroscopy [177] and highresolution inductively-coupled plasma mass spectrometry (ICPMS) [224]. In the largest study of non-human primates chronically exposed to Mn, it was observed that DAT and dopamine receptor levels were unchanged in STR of Mn-exposed animals relative to controls. However, Mn-exposed animals had an altered response to amphetamine, which is a DAT substrate [225]. That is, Mn exposure resulted in a marked impairment of in vivo dopamine release in the STR of Mn-exposed non-human primates [224,225]. Several other studies have indicated that Mn can interact with DAT, although the exact mechanism is unclear [226-228]. An altered response to DAT ligands caused by Mn may indicate presynaptic deficits in the nigrostriatal system [224,225], which may explain the intractability of Mn-exposed subjects with parkinsonism to L-DOPA treatment. If there is reduced DA availability at the synapse due to impaired DA release or altered reuptake, then supplementation with L-DOPA would be ineffective at alleviating the movement disorders associated with Mn toxicity. Furthermore, the fact that the glutamatergic and GABAergic systems of non-human primates chronically exposed to Mn are unaffected in the presence of behavioral deficits suggests that the behavioral effects of Mn in non-human primates are related to the changes in the dopaminergic system [215,216]. Thus, even though cell culture and animal model systems of Mn toxicity suggest that the DA system has a special sensitivity to Mn, in humans and non-human primates this may not mean that Mn exposure causes DA neuron degeneration (as occurs in PD) but instead results in DA neuron dysfunction [12]. In support of this hypothesis, it was recently observed that welders can be asymptomatic for manganism but still exhibit a small increase in the United Parkinsons Disease Rating Scale (UPDRS) and exhibit dysfunctional L-DOPA uptake in the caudate measured by PET. This indicates presynaptic nigrostriatal deficits can precede overt symptoms of Mn-induced movement abnormalities [229] and may be an early neurochemical marker of dopaminergic dysfunction. Importantly, the pattern of L-DOPA uptake measured by PET in the caudate and putamen of welders was completely opposite to the pattern observed in idiopathic Parkinson's disease patients. That is, in welders there was a significant decrease of L-DOPA uptake in the caudate with no change in the putamen, while idiopathic PD changes exhibit a change in the putamen and not in the caudate. This PET data shows that the pattern of change between welders with a subtle but significant increase in the UPDRS is distinctly different from patients with idiopathic Parkinson's disease.

The effects of Mn on behavior and cognitive abilities in children may be related to effects on the dopaminergic system during development. In rodents, exposure to Mn during early postnatal development resulted in behavioral deficits reminiscent of hyperactivity as well as impaired performance on cognitive tests [230]. These neurological deficits were accompanied by altered DAT and DA receptor levels in the prefrontal cortex, nucleus accumbens, and dorsal striatum [230]. In monkey infants fed soy-based formula with or without supplemental Mn (1000 µg/L), the Mn-exposed animals exhibited a reduced response to the DA receptor agonist apomorphine, altered social interactions, and poorer learning rates in cognitive assessments [231]. Mn exposure in developing organisms may have lasting changes in the brain; rats exposed to Mn only during the pre-weaning time period exhibited altered DA receptor levels, altered response to DA agonists, and increased astrocyte activation in adulthood, even though the levels of Mn in blood and brain decreased [214]. These findings, especially the reduced response to DA receptor agonists, are consistent with what was observed in adult non-human primates [224], and indicates that Page 7 of 13

deficits in DA neurotransmission during early development may result in lasting behavioral and cognitive deficits.

Conclusions

We have reviewed the neurotoxicology of two common environmental neurotoxicants, Pb^{2+} and Mn. While Mn is an essential element of the human diet and has many beneficial uses in the human body, elevated Mn levels can result in a PD-like syndrome and developmental Mn exposure can adversely affect childhood neurological development. In contrast, Pb^{2+} has no known physiological function and all known effects of Pb^{2+} are detrimental to humans. Like Mn, Pb^{2+} exposure in childhood can result in cognitive and behavioral deficits in children. These effects are long lasting and persist into adulthood even after Pb^{2+} exposure has been reduced or eliminated.

It is important to emphasize that one of the common links between Pb²⁺ and Mn neurotoxicity is presynaptic dysfunction. Pb²⁺ appears to interfere with glutamatergic neurotransmission and may disrupt trans-synaptic signaling critical to synaptic development [126,127,130,131,232]. Mn appears to interfere with dopaminergic synaptic transmission, possibly by impairing presynaptic DA release [215,224,225,233]. The developmental effects of either metal on cognition and behavior in children may be linked to this common theme of toxicity. The developing brain is particularly sensitive to agents that disrupt synaptic activity [234-236], as synaptic development depends critically on feedback signaling between neurons [232,237]. Furthermore, presynaptic dysfunction has been identified in many neurological disorders and diseases, including dementia, autism, bipolar disorder, Down syndrome, and schizophrenia (for review, see [134]). Interestingly, Pb²⁺ and/or Mn exposure has been linked to schizophrenia, dementia, PD, autism, and hyperactivity disorders as potential risk factors for disease etiology [86,238-244]. It is possible that presynaptic dysfunction may account for many of the chronic effects of Pb²⁺ and/or Mn exposure and increase susceptibility for neurological diseases which exhibit environmental etiology.

A common susceptibility factor for both Pb2+ and Mn toxicity is Fe deficiency. Fe-deficient diets can result in increased heavy metal uptake through increased DMT1 levels [78], which results in elevated BPb and BMn. This is significant particularly in developing countries. Developing countries tend to have higher environmental levels of Pb2+ and Mn, resulting in higher human exposure levels. Developing countries also have much higher rates of Fe deficiency than developed countries [245]. The WHO has estimated that 1.3% of the global disability burden stems from Fe deficiency, and that 40% of the burden occurs in Asia and another 25% occurs in Africa [245]. These same regions experience elevated levels of heavy metal contamination [246], resulting in a potentially devastating combination for metal toxicity. Indeed, a recent study in Pakistan showed a significant, dosedependent correlation between mild and severe anemia and BPb in children [247]. Thus, children in the developing world are at particular risk of experiencing metal toxicity, due to combined dietary deficits and elevated metal exposure.

Generally humans are not exposed to a single heavy metal, but instead are exposed to heterogeneous metal mixtures. The effect of human exposure to mixtures of heavy metals is currently an active area of research. Some parts of the world, such as northern Mexico [248] and Bangladesh [249-251], exhibit extremely high levels of as in the water table. Furthermore, co-exposure to high levels of Pb²⁺ and Mn also occurs. A recent study observed that combined exposure to Mn and As in Bangladeshi children was significantly associated with poorer

performance on cognitive tests, although an interaction between the two metals was not supported statistically [148]. While an interaction was not observed in the Bangladesh study, in a study of children in Mexico the combined exposure to Pb^{2+} and Mn resulted in greater effects on cognitive performance than Pb^{2+} exposure alone [252]. This suggests that exposure to multiple metals may result in greater developmental deficits than to single metals and emphasizes the need to understand the toxicology of complex mixtures.

In conclusion, widespread exposure to Pb^{2+} and Mn continue to cause neurological deficits and disease. Heavy metal pollution is a global public health challenge, with a disproportionate burden laid upon developing nations. The developing world, with increased heavy metal contamination and higher prevalence of dietary deficiencies, is at particular risk for metal toxicity. The irreversible nature of the effects of Pb²⁺ and Mn on neurodevelopment strongly supports environmental intervention in regions where children are exposed to these metals via polluted air or ground water.

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