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Molecular Neurobiology of Lead (Pb^{2+}): Effects on Synaptic Function

April P. Neal · Tomás R. Guilarte

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Abstract Lead (Pb^{2+}) is a ubiquitous environmental neurotoxicant that continues to threaten public health on a global scale. Epidemiological studies have demonstrated detrimental effects of Pb^{2+} on childhood IQ at very low levels of exposure. Recently, a mechanistic understanding of how Pb^{2+} affects brain development has begun to emerge. The cognitive effects of Pb^{2+} exposure are believed to be mediated through its selective inhibition of the *N-methyl-*D*-aspartate* receptor (NMDAR). Studies in animal models of developmental Pb^{2+} exposure exhibit altered NMDAR subunit ontogeny and disruption of NMDAR-dependent intracellular signaling. Additional studies have reported that Pb^{2+} exposure inhibits presynaptic calcium (Ca^{2+}) channels and affects presynaptic neurotransmission, but a mechanistic link between presynaptic and postsynaptic effects has been missing. Recent work has suggested that the presynaptic and postsynaptic effects of Pb^{2+} exposure are both due to inhibition of the NMDAR by Pb^{2+}, and that the presynaptic effects of Pb^{2+} may be mediated by disruption of NMDAR activity-dependent signaling of brain-derived neurotrophic factor (BDNF). These findings provide the basis for the first working model to describe the effects of Pb^{2+} exposure on synaptic function. Here, we review the neurotoxic effects of Pb^{2+} exposure and discuss the known effects of Pb^{2+} exposure in light of these recent findings.

A. P. Neal
Department of Pharmacology and Toxicology,
Michigan State University,
B307 Life Sciences Building,
East Lansing, MI 48824, USA
e-mail: Nealap@msu.edu

T. R. Guilarte (✉)
Department of Environmental Health Sciences,
Mailman School of Public Health, Columbia University,
600 Haven Avenue, B1-108,
New York, NY 10032, USA
e-mail: trguilarte@columbia.edu

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Neurotoxicity of Low-Level Pb^{2+} Exposure in Children

The neurological effects of Pb^{2+} have been a driving factor in reducing the level of human Pb^{2+} exposure from anthropogenic and environmental sources. The first report of the effects of Pb^{2+} on the cognitive abilities and social behavior in children was in 1943 [1], however, the full implications of these effects were not appreciated until decades later. Studies from the late 1970s to the early 1990s showed effects of Pb^{2+} on cognitive abilities at progressively lower exposures [2]. In 1991, the CDC lowered the definition of Pb^{2+} intoxication from 25 μg/dL blood lead level (BLL) to 10 μg/dL BLL, which is the current regulatory level, motivated by evidence that children with BLLs of at least 10 μg/dL had impaired intellectual function [3]. More recently, studies have shown that the dose–response of Pb^{2+} on IQ in children is non-linear, with lower exposures of Pb^{2+} resulting in a greater rate of IQ loss than at higher exposures [4–7]. These studies indicate that the majority of the estimated IQ loss in Pb^{2+}-exposed children occurs during the first 10 μg/dL of exposure, and suggest that Pb^{2+} may be a non-threshold neurotoxicant [4–8].

In addition to the cognitive deficits associated with Pb^{2+} exposure, children with elevated BLLs have behavioral deficits. Several studies have reported that school children with elevated BLLs are more likely to be disruptive in class, display anti-social behavior, and have attention problems [1, 9–12]. These behavioral effects appear to have an attention-deficit hyperactivity disorder (ADHD) phenotype; in fact, a recent study identified that childhood Pb^{2+} exposure was positively associated with ADHD diagnosis [13].

One of the most troubling aspects of Pb^{2+} effects in children is that the cognitive and behavioral effects of Pb^{2+} are irreversible. Chelation therapy, an intervention that is recommended for children with BLLs >45 μg/dL [14], can reduce the body burden of Pb^{2+}. However, even though the level of Pb^{2+} is reduced, chelation therapy does not remediate the cognitive or behavioral deficits associated with childhood Pb^{2+} exposure [15, 16]. This highlights the possibility that Pb^{2+} exposure induces long-lasting (or permanent) changes in the brain during a critical period of development in childhood.

Perhaps due to these long-lasting changes in the brain, children exposed to Pb^{2+} experience persistent cognitive and behavioral deficits long after the cessation of Pb^{2+} exposure [17]. Prenatal Pb^{2+} exposure has been associated with anti-social and delinquent behavior as adolescents [18], and individuals who had elevated BLLs as children are more likely to be arrested during adolescence [19] and as adults [20]. This predilection towards violent and anti-social behavior is believed by some to underlie trends in violent crime both in the USA and internationally [21–23]. Furthermore, some studies have shown that children who experience elevated BLLs are more likely to have reductions in brain volume as adults [24]. These changes could account for altered behavior in adults exposed to Pb^{2+} during childhood. Thus, developmental Pb^{2+} exposure in humans results in long-lasting effects on cognition and behavior even after cessation of exposure that may be mediated by Pb^{2+}-induced cellular changes in the brain.

Pb^{2+} Effects in the Brain

NMDA Receptor

The *N-methyl*-D-*aspartate* receptor (NMDAR) plays an essential role in hippocampus-mediated learning and memory, based on studies showing that intra-ventricular administration of an NMDAR antagonist (aminophosphonovaleric acid (APV)) in rats resulted in spatial learning impairments similar to those encountered with hippocampal lesions [25, 26]. Targeted knockout of the NMDAR in the hippocampus impairs spatial learning [27], lending further support to the role of the NMDAR in hippocampus-mediated learning processes. The major cellular mechanism within the hippocampus believed to be responsible for acquisition of new memories is long-term potentiation (LTP), a cellular phenomenon in which a long-lasting increase in synaptic efficacy follows brief, high-frequency stimulation [28, 29]. LTP is disrupted by inhibition of the NMDAR [28], and mice exhibiting hippocampal LTP deficits perform poorly in spatial memory tasks [28, 30]. Thus, impairment of the NMDAR has been shown to produce learning deficits on both the behavioral and cellular level.

The NMDAR is one of three main types of glutamatergic receptors in the mammalian brain [31] and is heavily expressed in the hippocampus and cerebral cortex [32, 33]. The NMDAR is composed of an obligatory NR1 subunit and one or more accessory subunits from the NR2 or NR3 families. The NR1 gene is alternatively spliced and specific splice variants of NR1 impart different pharmacological characteristics to the NMDAR [34]. The NR2 family consists of NR2A, NR2B, NR2C, and NR2D family members [32, 35, 36]. In the hippocampus, NR2A and NR2B are the most abundant NR2 family members. These two subunits exhibit differential developmental expression, with NR2B subunit expression levels high during fetal development and early postnatal life while NR2A subunit expression increases with postnatal maturation [32]. Besides exhibiting differential developmental expression, NR2A and NR2B subunits also have distinct intracellular protein associations [37] and signaling pathways [38–41], believed to be mediated through protein interactions with the C terminus. The different NR2 family members are linked to differential MAPK signaling [41], pro-death or pro-life signaling [38], and differential induction of nuclear gene expression [40]. Furthermore, NR2A-containing NMDARs (NR2A-NMDARs) are predominately located synaptically, while NR2B-containing NMDARs (NR2B-NMDARs) are expressed both synaptically and extrasynaptically [42]. Together, between NR1 splice variation and NR2 protein associations, NMDARs exhibit an exquisite degree of specialization and play essential roles in central synapses.

Pb^{2+} is a potent, non-competitive antagonist of the NMDAR [43–46], and has been shown to impair hippocampus-mediated learning in animal models of Pb^{2+} exposure [47–49]. It is believed to bind at the Zn^{2+} regulatory site of the NMDAR in a voltage-independent manner [50–52]. The NR2 subunits have different Zn^{2+} binding sites; the NR2A-NMDAR binds Zn^{2+} at a high-affinity site (nM affinity) while the NR2B-NMDAR binds Zn^{2+} with lower affinity (μM range) [45, 46, 53]. Recombinant NR2A- and NR2B-NMDARs containing mutated Zn^{2+} binding sites exhibit decreased affinity for Pb^{2+}. The Pb^{2+} IC_{50} for wild type NR2A-NMDARs was reported to be 1.3 μM, while it increased to 11.3 μM in mutant NR2A-NMDARs which exhibit reduced Zn^{2+} sensitivity. Similarly, the Pb^{2+} IC_{50} of wild type NR2B-NMDARs was 1.2 μM but increased to 6.9 μM in mutant NR2B-NMDARs [50]. Furthermore, this study observed evidence of competitive inhibition of Pb^{2+} with the NR2A-NMDAR Zn^{2+} binding site, but did not observe competitive inhibition with the NR2B-NMDAR Zn^{2+} site [50]. While several studies also observed evidence of competitive

inhibition of Pb^{2+} for the Zn^{2+} binding site, one study observed evidence of non-competitive inhibition. The work of Lasley and Gilbert used cortical preparations from adult rats to observed some evidence consistent with the above studies [54]. However, they also observed that in the presence of Zn^{2+}, the IC_{50} of Pb^{2+} was decreased and the inhibition curve was shifted to the left, indicating non-competitive inhibition [54]. Thus, some disagreement regarding the site of Pb^{2+} action remains.

Regardless of the above disagreement there is other evidence that may support the hypothesis that Pb^{2+} interacts with the Zn^{2+} binding site. Since Zn^{2+} binds with very high affinity (nanomolar) at a regulatory site on the NR2A subunit [53], but with lower affinity to the NR2B subunit [45], this may indicate a preferential sensitivity of NR2A-NMDARs for Pb^{2+} [50, 52]. In support of this hypothesis, electrophysiological studies with recombinant receptors demonstrate that Pb^{2+} more potently inhibits NR2A-NMDARs (IC_{50}=0.87 μM) than NR2B-NMDARs (IC_{50}= 1.21 μM) [51, 55] or the tri-heteromeric form, NR1/NR2A/NR2B-NMDAR (IC_{50}=6.1 μM) [55].

In addition to acting as an NMDAR antagonist, Pb^{2+} exposure also disrupts normal NMDAR ontogeny. Chronic developmental Pb^{2+} exposure results in decreased NR2A content in the hippocampus [56–59] and altered expression of NR1 splice variants [59–61]. In contrast, NR2B mRNA levels either remained unchanged or exhibited a slight increase in rats developmentally exposed to Pb^{2+} [56–59]. NR2B-specific radioligand binding is increased in the hippocampus and cerebral cortex of adults rats after developmental Pb^{2+} exposure [62]. Together, these data suggest that Pb^{2+} delays the developmental switch of increased NR2A incorporation with synapse maturation [62, 63]. Similar trends have also been observed in cultured neuron systems ([64] and Neal et al., unpublished data) and suggest that Pb^{2+} exposure may cause lasting changes in NMDAR subunit composition and expression. We hypothesize that long-term inhibition of the NMDAR by Pb^{2+} results in altered NMDAR targeting and expression. In support of this hypothesis, other paradigms of NMDAR inhibition have shown increased NR2B and decreased NR2A surface expression in response to decreased NMDAR activity [65, 66], which is very similar to what is observed during chronic or prolonged Pb^{2+} exposure.

Changes in NMDAR subunit composition can result in altered NMDAR-dependent signaling. As described above, many signaling pathways are dependent on NMDAR subunit composition and/or localization. Thus, Pb^{2+}-induced alterations in NMDAR subunit composition could result in changes in downstream signaling. In support of this hypothesis, chronic developmental Pb^{2+} exposure results in altered MAPK signaling [67], calcium/calmodulin kinase II (CaMKII) activity [68], and altered cyclic AMP response element binding protein (CREB) phosphorylation status and binding affinity [62, 69]. 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 [70, 71]. Altered IEG expression in animals exposed to Pb^{2+} has been observed [72], indicating that altered CREB activity due to Pb^{2+}-mediated disruption of NMDAR signaling may result in impaired cellular learning and memory processes.

Presynaptic Function

Chronic developmental Pb^{2+} exposure also results in impaired neurotransmission. In vivo, rats chronically exposed to low levels of Pb^{2+} have reduced Ca^{2+}-dependent glutamate and γ-aminobutyric acid (GABA) release in the hippocampus [73–75]. In vitro, Pb^{2+} exposure impairs excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) in cultured hippocampal neurons [76] and brain slices [75]. 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 Pb^{2+} exposure.

A recent study from our laboratory has shown that prolonged (5-day) Pb^{2+} exposure in cultured hippocampal neurons resulted in altered presynaptic protein expression and deficits in vesicular neurotransmitter release [77]. 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 [75, 76]. Vesicular release in Pb^{2+}-exposed neurons was significantly slower than under control conditions, as determined by live-imaging studies using the synaptic vesicle dye FM 1–43 [77]. These studies revealed that Pb^{2+}-exposed neurons exhibited a specific loss of fast-releasing sites. Deficits in vesicular release were likely due to a reduction in presynaptic proteins involved in the release process, and not a reduction in the total vesicle pool, since there was no significant difference in FM 1–43 loading between control and Pb^{2+}-treated neurons [77]. These findings are consistent with electrophysiological studies in which the effects of Pb^{2+} on IPSCs and EPSCs in cultured hippocampal neurons were not due to vesicle pool size, as treatment with 4-aminopyridine, an agent which forces exocytosis of the vesicle pool, abolished the effects of Pb^{2+} on postsynaptic currents [76]. Thus, similar effects of Pb^{2+} on neurotransmission have been observed in a range of studies using different techniques.

Several hypotheses for the effects of Pb^{2+} on neurotransmission have been suggested. One hypothesis is that Pb^{2+} interacts with presynaptic intracellular targets and has the ability to modulate presynaptic neurotransmission. Pb^{2+} has been shown to interact with synaptotagmin I (Syt) in vitro [78]. Syt is a Ca^{2+}-sensing protein found in neurotransmitter vesicles and is responsible for promoting vesicular fusion in the presence of Ca^{2+} signaling [79]. Pb^{2+} bound Syt with 1000-fold higher affinity than Ca^{2+}, which may prevent detection of Ca^{2+} signaling essential to neurotransmission [78]. Although Pb^{2+} exposure did not affect Syt protein expression in cultured hippocampal neurons [77], it is possible that Pb^{2+} may interfere with the Ca^{2+}-sensing ability of Syt in neurons, thus masking the cellular signal for Ca^{2+}-dependent vesicular release.

Pb^{2+} interactions with Syt may be related to the ability of Pb^{2+} to mimic Ca^{2+}. Pb^{2+} has an ionic radius of 1.2 Å, which is similar to the ionic radius of Ca^{2+} (0.99 Å) [80, 81]. The positive charges and high electronegativity (2.33 on the Pauling scale) of Pb^{2+} may allow it to interact with the same residues on Ca^{2+} binding sites that interact with Ca^{2+} ions [81]. Pb^{2+} has been shown to interact with several neuronal intracellular Ca^{2+}-binding proteins in addition to Syt (described above), such as the Ca^{2+}-binding protein calmodulin (CaM) [80, 82, 83], the CaM/Ca^{2+}-dependent phosphatase calcineurin [84], CaMKII [68], and protein kinase C [85–88], suggesting that Ca^{2+} mimicry may be a common characteristic of Pb^{2+} toxicity [89–91]. Thus, the ability of Pb^{2+} to mimic Ca^{2+} may interfere with normal synaptic signaling events.

Another hypothesis regarding the disruption of neurotransmission is that Pb^{2+} may interfere with Ca^{2+} signals by inhibiting Ca^{2+} channels [75, 76, 92]. Neurotransmission relies on the influx of Ca^{2+} from P/Q-, N-, and to some extent R-type voltage-gated Ca^{2+} channels (VGCCs) [93]. Pb^{2+} has been shown to inhibit VGCCs in recombinant systems with high affinity [92]. Furthermore, removal of extracellular Ca^{2+} resulted in identical effects on IPSC frequency as Pb^{2+} exposure, suggesting that the Pb^{2+}-induced inhibition of IPSC frequency is via reduction of Ca^{2+} influx through VGCCs [75]. Inhibition of presynaptic VGCCs may prevent the necessary rise in internal Ca^{2+} required for fast, Ca^{2+}-dependent vesicular release, thus interfering with neurotransmission.

Inhibition of presynaptic VGCCs by Pb^{2+} is not the only mechanism underlying the effects of chronic Pb^{2+} exposure on neurotransmitter release. Exposure to APV, an NMDAR antagonist that does not inhibit presynaptic VGCCs, resulted in nearly identical effects on presynaptic and postsynaptic proteins as Pb^{2+} exposure (Neal et al., unpublished data and [77]). This suggests that NMDAR inhibition independent of VGCC inhibition can mediate presynaptic changes during Pb^{2+} exposure.

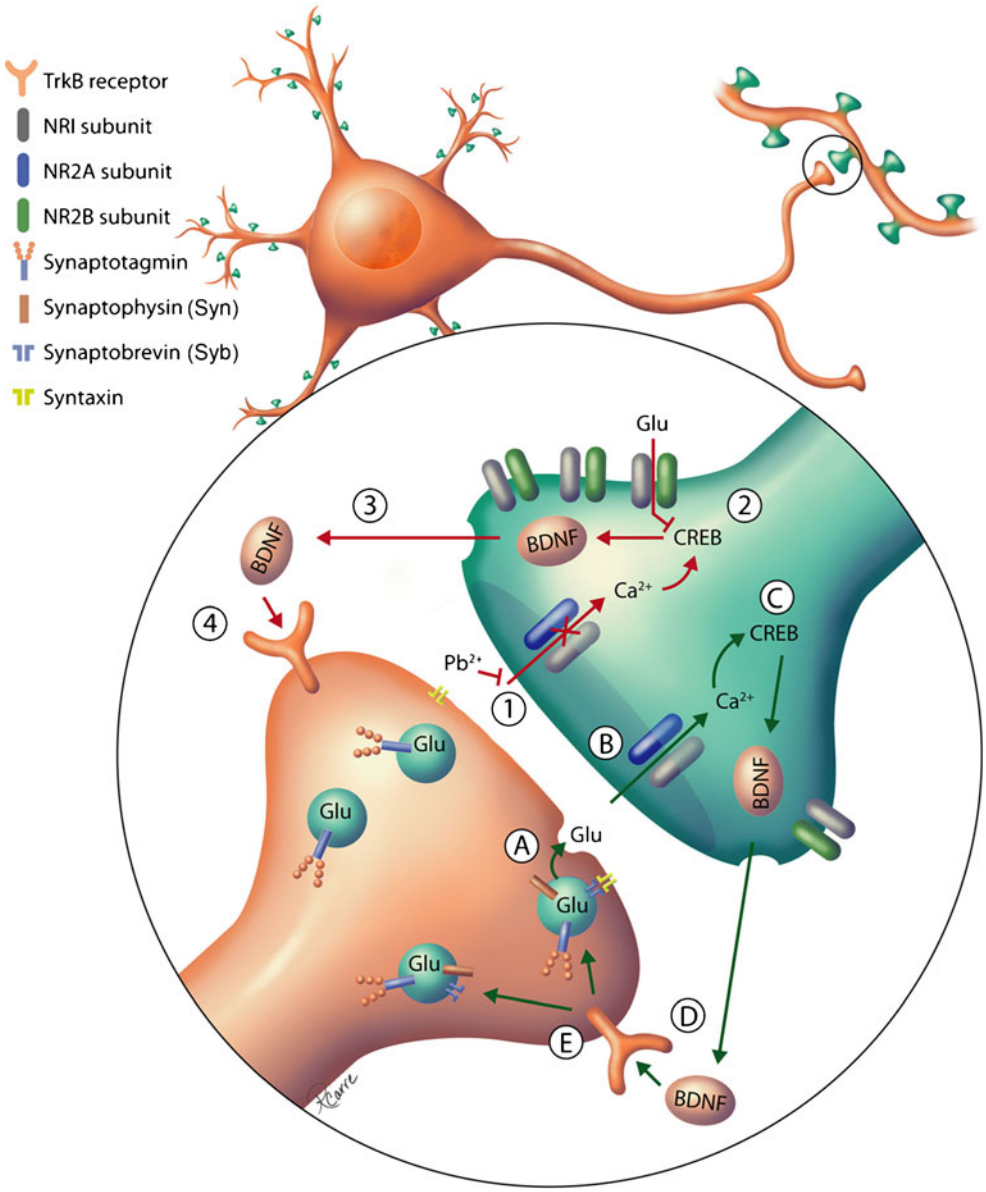
Disruption of NMDAR Activity-Dependent BDNF Signaling by Pb^{2+}

As an alternative hypothesis for the presynaptic effects of Pb^{2+} exposure, we have advanced the possibility that inhibition of synaptic NMDARs by Pb^{2+} results in altered NMDAR-dependent retrograde signaling, particularly of brain-derived neurotrophic factor (BDNF). We demonstrated that incubating hippocampal neurons with exogenous BDNF for the last 24 h of prolonged (5-day) Pb^{2+} exposure resulted in complete recovery of Pb^{2+}-induced changes in presynaptic protein levels and vesicular neurotransmitter release [77]. This indicates that disruption of NMDAR-dependent retrograde signaling of BDNF may be impaired during Pb^{2+} exposure.

In developing neurons, the stabilization of functional presynaptic release sites is controlled by retrograde signals from the postsynaptic side [94–96]. One of these retrograde signals, BDNF, has been implicated in axon morphology, synaptic connectivity, and synaptic ultrastructure [97]. NMDAR activation can result in the generation and release of BDNF [98–100], which can be released from both axon and dendrite [101]. BDNF has been shown in live-imaging studies to be secreted postsynaptically from hippocampal neuron cultures when stimulated by NMDA [102]. This NMDAR-dependent release of BDNF may be essential to the generation or un-masking of presynaptic neurotransmitter release sites [98]. Interestingly, BDNF signaling can stimulate further glutamate release by increasing glutamate release probability [103], resulting in a positive feedback cycle.

In addition to exhibiting activity-dependent release, BDNF exhibits activity-dependent gene transcription. The BDNF gene is unique in that it contains eight different 5′ non-coding exons with separate promoters and one coding 3′ exon (exon IX) [104]. Differential transcription of promoters results in distinct splice variant transcripts, but all give rise to an identical BDNF protein. In particular, synaptic activity-dependent BDNF upregulation occurs after stimulation with depolarizing conditions, kainate treatment, and NMDAR activation [100, 105–108]. BDNF activity-dependent gene expression in hippocampal neurons has been linked to NR2A-NMDAR activation, and may be inhibited by NR2B-NMDAR activity [109].

BDNF signaling results in changes in gene expression of both pre- and postsynaptic proteins. Exogenous BDNF can increase the expression of Syn, Syt, and Syb in hippocampal slices [110], and may enhance NR2A but not NR2B subunit expression [111, 112]. Conversely, BDNF knockout mice exhibit reduced expression of the NR2A but not the NR2B subunit [113] and reduced Syn and Syb expression [114]. Therefore, it appears that NR2A-NMDARs may be preferentially linked to BDNF activating pathways and that



Scheme 1 Working model of molecular mechanism(s) by which chronic Pb^{2+} exposure disrupts synapse formation and plasticity in developing hippocampal neurons. *Green arrows* indicate normal processes; *red arrows* indicate Pb^{2+}-impaired processes. In normal glutamatergic synapses, glutamatergic vesicles undergo Ca^{2+}-dependent fusion with the plasma membrane in response to presynaptic signals (**a**). This process is mediated by the interaction with the v-SNARE Synaptobrevin and the t-SNAREs Syntaxin or SNAP-25. As a result of vesicular fusion, glutamate (*Glu*) is released from presynaptic terminals, resulting in NMDAR activation (**b**). Ca^{2+} enters the postsynaptic cell through synaptic NMDARs, activating downstream intracellular pathways. Under normal conditions, the predominant NMDARs at mature synapses are NR2A-NMDARs, which are linked to CREB phosphorylation (**c**). Activity-dependent CREB activation results in transcription of BDNF, which is transported and released in an activity-dependent retrograde fashion. Secreted BDNF interacts with the tropomyosin-related kinase receptor B (*TrkB*) (**d**). In the presynaptic neuron, BDNF activation of TrkB increases the number of docked vesicles and enhances vesicular release, as well as increases the incorporation of Syn and Syb into synaptic vesicles (**e**). During chronic Pb^{2+} exposure, however, Ca^{2+} influx through synaptic NMDAR is inhibited [1]. This causes a reduction in NR2A-NMDARs while NR2B-NMDARs are increased, possibly at extrasynaptic sites. As a result of reduced NR2A-NMDAR activation, NR2B-NMDARs are operational and are coupled to a CREB shut-off pathway. Reduced CREB signaling during Pb^{2+} exposure may result in decreased BDNF protein levels [2]. BDNF secretion is impaired during Pb^{2+} exposure [3], resulting in reduced activation of presynaptic TrkB receptors by BDNF [4]. Without positive feedback, there is reduced incorporation of synaptic vesicle proteins Syn and Syb and impaired vesicular release that can be rescued by exogenous addition of BDNF

BDNF can modulate presynaptic plasticity through changes in protein and/or gene expression.

Pb^{2+} exposure during hippocampal neuron synaptogenesis decreases the number of synapses expressing NR2A-NMDARs (Neal et al., unpublished data), suggesting that the production and release of BDNF may be impaired. Indeed, Pb^{2+}-exposed hippocampal neurons exhibit reduced proBDNF expression and BDNF release [77]. The functional outcome of disrupting BDNF signaling on NR2A and NR2B subunit expression as seen in BDNF knockout mice is similar to the phenotype observed in Pb^{2+}-exposed hippocampal cultures (Neal et al., unpublished data) and in the hippocampus of developing animals exposed to Pb^{2+} [57, 62]. Furthermore, BDNF knockout animals exhibit deficits in vesicular release that can be alleviated by exogenous BDNF [114] in a similar manner to what has been observed with Pb^{2+} exposure [77]. Together, these data strongly suggest a role for the disruption of NMDAR activity-dependent BDNF signaling in the mechanism of Pb^{2+} toxicity in synapses. We have summarized the effects of Pb^{2+} on this signaling pathway in a working model of Pb^{2+} molecular neurotoxicity in Scheme 1.

Discussion

Until now, the postsynaptic and presynaptic effects of Pb^{2+} exposure have been hypothesized to occur through different mechanisms. Studies in animal models and hippocampal cultures have revealed that Pb^{2+} exposure results in altered NMDAR mRNA and protein expression, delaying or preventing the developmental switch from NR2B-NMDARs to NR2A-NMDARs [56, 57, 59, 62]. It has been hypothesized that these postsynaptic effects are due to dampened NMDAR activity, as Pb^{2+} is a potent NMDAR antagonist [43, 50, 52, 55] and activity-dependent alterations in NMDAR subunit trafficking and expression have been reported in other models of NMDAR inhibition [66, 115–117]. In addition to these postsynaptic effects, other studies have reported that both glutamatergic and GABAergic neurotransmission are impaired after Pb^{2+} exposure [73, 76]. These effects, for lack of a mechanistic link between the presynaptic and postsynaptic zones, have been postulated to be a result of inhibition of presynaptic Ca^{2+} channels. While Pb^{2+} does inhibit Ca^{2+} channels [92], loss of NMDAR-dependent retrograde signaling may be a viable alternate mechanism underlying the presynaptic effects of Pb^{2+} exposure [77].

This working model may provide insight to possible remediation of Pb^{2+} neurotoxicity. Children exposed to Pb^{2+} exhibit cognitive and behavioral deficits long after the cessation of elevated exposure [17] that are unresponsive to chelation therapy [15, 16]. In the laboratory setting, rats exposed to Pb^{2+} from conception to weaning exhibit impaired learning ability in adulthood [48]. However, placing Pb^{2+}-exposed rats in an enriched environment completely mitigates the effects of Pb^{2+} [49]. A key finding from the latter study is that BDNF gene expression levels are increased in the enriched, Pb^{2+}-exposed rats, suggesting that the reversal of learning deficits by the enriched environment may be due to upregulation of BDNF [49]. Thus, not only does our model suggest a mechanistic role for impaired retrograde signaling in Pb^{2+} toxicity mediated by NMDAR inhibition, it also suggests that the effects of Pb^{2+} exposure are at least partially reversible with interventions that result in increased BDNF levels.

NMDAR activity-dependent retrograde signaling appears to be a global mechanism governing central synapse development [98, 118, 119]. Thus, it is not surprising that other exposure paradigms involving NMDAR inhibition result in similar presynaptic and postsynaptic effects as observed with Pb^{2+} exposure. For example, ethanol, another developmental neurotoxicant that inhibits the NMDAR [120, 121], can cause similar effects on NMDAR targeting that have been observed with Pb^{2+} exposure. Neurons exposed to ethanol in chronic or intermittent exposure paradigms exhibit altered synaptic NMDAR targeting, with increased levels of NR2B-NDMARs [122, 123], suggesting a specific increase in NR2B-NMDARs similar to what has been observed with Pb^{2+} exposure in both cell and animal models ([62] and Neal et al., unpublished data). Furthermore, several other groups have reported effects on presynaptic neurotransmission after ethanol exposure [124]. Thus, it may be that disruption of NMDAR activity-dependent retrograde signaling is a general mechanism underlying neurodevelopmental disorders with an environmental etiology.

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