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RELATIONSHIPS BETWEEN LEAD-INDUCED LEARNING IMPAIRMENTS AND CHANGES IN DOPAMINERGIC, CHOLINERGIC, AND GLUTAMATERGIC NEUROTRANSMITTER SYSTEM FUNCTIONS

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ABSTRACT

Behavioral consequences of low-level lead (Pb) exposure include impairments in learning processes and in Fixed-Interval schedule-controlled operant behavior. Although the neurobiological bases of these effects remain undetermined, current evidence suggests that inhibitory effects of Pb on the NMDA receptor complex may play a preferential role in the learning deficits. In contrast, alterations in dopaminergic systems, consistent with a decrease in dopamine availability, appear to be related to the changes in Fixed-Interval performance. Hypocholinergic function has also been described, but its relationship to the behavioral changes is not yet known. Explication of these relationships will require more efforts involving direct rather than correlative methods. The answers are critical for understanding risks associated with exposure and for the development of behavioral or chemical therapeutic strategies for dealing with lead neurotoxicity.

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INTRODUCTION AND SCOPE

Although it has long been recognized that high-level exposure to lead (Pb) can produce permanent mental retardation and other cognitive deficits, the neurobiological bases of these effects, and the nature of the dose-effect curves describing them, continue to engender controversy. Of the various behavioral effects of Pb, the cognitive impairments attributed to it have clearly aroused the greatest public health concern, because of mounting evidence for their occurrence at extremely low (in fact, environmental) levels of exposure. With respect to neurobiological mechanisms, the focus has shifted to neuropharmacological substrates, inasmuch as neuropathological and morphological sequelae are observed at far higher exposure levels than those currently associated with cognitive deficits.

This chapter explores the possible involvement in lead-induced learning impairments of three neurotransmitter systems, namely the dopaminergic (DA), cholinergic (ACh), and glutamatergic (GLU) systems, all known to play key roles in cognitive processes under normal conditions. The chapter begins by explaining the ongoing nature of the problem of Pb neurotoxicity. Because efforts to identify neurobiological substrates should depend upon a precise documentation and characterization of the behavioral effects, a summary of the evidence supporting direct effects of Pb per se on cognitive functions, the behavioral mechanisms of these effects, and the associated exposure levels is next presented. This summary is followed by evidence linking each of the three neurotransmitter systems to Pb-induced learning deficits, as well as discussions of the identification of neuropharmacological mechanisms, research gaps, and future research needs.

WHY LEAD REMAINS A PUBLIC HEALTH PROBLEM TODAY

The realization that exposure to Pb could give rise to an array of toxic manifestations, including those related to the nervous system, came even with the first uses of this metal by humans. As early as the second century B.C., some of the most common signs of overexposure were described in the *Alexipharmaca* by the Greek physician and poet Nicander. The Romans made even greater use of Pb than did the Greeks, and were also well aware of its toxicity. Pliny, for example, described attempts of workers to avoid Pb dusts by placing their faces in loose bags.

The most extensive problems with Pb, however, have occurred in more modern history, from its use in paints and gasoline and, to a lesser extent, in products such as batteries. Removal of Pb-based paints, either intentionally or unintentionally, and atmospheric dispersal of Pb from automobile exhaust have

resulted in a ubiquitous incorporation of this toxicant into dusts, soils, foods, and water supplies, resulting in widespread exposure of the general population.

Increasing concern and awareness of Pb as a significant public health problem, particularly for pediatric populations, eventually prompted a decline in the use of Pb, but has not eliminated the problems. The residual accumulation in dust and soil continues to sustain lifetime exposures of the general population. Elimination of Pb from dust and soil is not economically feasible, given the extent of the problem. Furthermore, it is now known that the levels of Pb associated with deleterious consequences are far lower than ever previously realized. Pb concentrations in blood (PbBs) of 80 μg/dl were considered potentially adverse for children 40 years ago, but the corresponding value today is 10–15 μg/dl, levels that are not uncommon in the United States, especially in some urban areas.

Pb also remains a public health problem because strategies for decreasing the body burden of Pb, such as chelation therapy, are currently controversial. Evidence indicating that some chelating agents can mobilize Pb from bone and redistribute it to soft-tissue target organs, including brain, has been reported (1). Moreover, at present, an insufficient basis exists to support the contention that chelation treatments actually reverse Pb-induced behavioral manifestations (2).

COGNITIVE IMPAIRMENTS AND LEAD EXPOSURE

Human Developmental Studies

As a result of their greater vulnerability to Pb, as indicated by lower effect levels and/or increased magnitude of effects, much of the recent work elaborating and defining the behavioral toxicity of Pb in humans has concentrated on developmental exposures of children. Early case reports described permanent mental retardation as a residual effect of acute high-level Pb exposure (3, 4), setting the stage for a subsequent experimental focus on cognitive functions. Subsequent cross-sectional epidemiological studies, considered collectively, pointed to decreased intelligence quotient (IQ) scores (on the order of 4–6 points) and other psychometric alterations in children with PbBs of only 30–40 μg/dl (5–7).

The most compelling human evidence for lead-induced changes in cognitive functions, however, derives from prospective longitudinal epidemiological studies, carried out in several countries, which were initiated to redress the methodological limitations of the cross-sectional studies (8). Many of these longitudinal studies share common experimental design features, including pre- or perinatal subject recruitment, longitudinal assessment of PbBs beginning antenatally or at birth, the use of similar well-standardized, validated

instruments for determination of cognitive function, and assessments of such functions in infancy, late preschool age, and, where possible, during the school-age years. The study populations also exhibit differences, many of which have proven instructive rather than limiting, including the degree to which various covariates of IQ (e.g. parental IQ, socioeconomic status) are present, the extent of Pb exposure, and sample sizes.

Although some inconsistencies were evident in reported effects measured during infancy or early preschool in these studies, later preschool and school-age assessments have more consistently revealed significant inverse associations between PbBs and IQ scores, with Pb-related decrements in IQ scores ranging from approximately 3 to 7 points (8). The PbBs associated with such effects have differed as a function of the particular cohort. In a Boston-based cohort (6), the mean PbB for the group was only 7.0 μg/dl, and an inverse relationship between IQ score and PbB occurred over a range of approximately 4–14 μg/dl. In a Cincinnati-based cohort where socioeconomic status was much lower, decrements of 7 IQ points were found at PbBs >20 μg/dl (9).

Toxicokinetic factors have been cited as the basis for the absence of comparable effects in two of the prospective studies (10). The Cleveland cohort (11) comes from a population base comprising 50% alcoholic pregnancies. Interactions between Pb and alcohol appear to produce an altered metabolism of Pb (12). Moreover, both Pb and alcohol affect nutritional status (8), a variable likely to impact the measures of developmental outcome used in these studies. The Sydney cohort (13) utilized both capillary and venous puncture blood sampling. Capillary sampling methods may entail substantial contamination and, in fact, PbBs measured this way were 30% higher than those obtained by venous puncture. This raises obvious concerns about misclassification of the Pb exposure of participants, even though additional subjects were recruited to attempt to minimize this problem.

Occupational Studies

Lead-related changes in cognitive function are not restricted to developmental exposures. Several, although not all, studies of occupationally Pb-exposed populations report deficits in learning and/or memory processes (14), again in the context of changes in standardized test scores. Studies relying on a more selective use of neuropsychological tests to characterize the behavioral nature of these deficits have reported that the primary effect of PbBs of 40 μg/dl and above was a general slowing of sensory-motor reaction time, and mild impairment of attention, verbal memory, and linguistic processing (15, 16).

Experimental Animal Studies

Given the nonspecific nature of IQ changes, experimental animal studies have been particularly crucial to a confirmation of Pb-induced changes in learning.

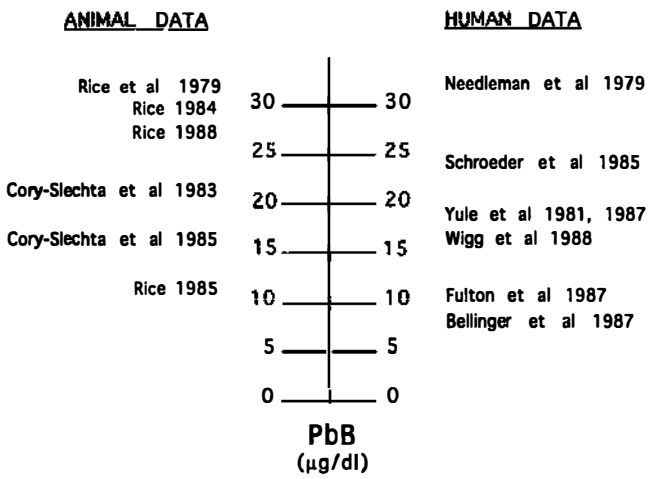

Figure 1 Blood lead levels (PbBs) at which behavioral effects are reported in experimental animal studies (*left*) and in human exposure studies (*right*). Adapted from Cory-Slechta (76) based on (18, 19, 29, 128–137).

They have provided a direct measurement of this behavioral process per se, and have done so in the absence of the covariates (e.g. socioeconomic status, parental IQ) known to affect IQ scores in human studies. Pb-related cognitive deficits have now been reported, both in rodents and in nonhuman primates, on various behavioral baselines, such as repeated learning, Fixed-Interval schedule-controlled behavior, concurrent schedule transitions, and acquisition as well as reversal discrimination learning (17–21). Moreover, the PbB levels at which such deficits are observed has steadily declined over the past 15 years (Figure 1) and are now at comparable values in human pediatric populations and experimental animal studies. Furthermore, these PbBs are not thresholds of effect, but simply the lowest exposure levels that have as yet been studied.

Several studies show that these deficits represent direct effects of Pb on learning, and do not arise indirectly from changes in other behavioral functions. For example, Bushnell & Bowman (21) demonstrated that a change from a food-based to a non-food-based reinforcing stimulus (reward) did not abolish a Pb-induced reversal learning deficit in primates, eliminating a motivational basis for these effects. Similarly, in another case, food intake levels were uniform in control and Pb-exposed monkeys even though the latter exhibited deficits in learning set formation (22). Cohn et al (17) utilized a multiple schedule of repeated acquisition and performance in which learning was required only in the repeated acquisition component while performance of an already learned response was required in the performance component. Both

components, however, required intact sensory, motor, and motivational function. Lead-exposed rats exhibited accuracy impairments only in the repeated acquisition component of the schedule, consistent with direct effects of Pb on learning processes as distinct from changes in sensory, motor, or motivational function.

Behavioral Mechanisms

While both the human and experimental animal studies provide compelling evidence of Pb-associated deficits in learning processes, the underlying behavioral mechanisms contributing to this impairment are as yet unclear. Some experimental animal studies suggest response perseveration (repetitive responding) as the basis (17, 20, 23–25). Pb exposure produces repeated iterations of a previously correct response, both in reversal learning and repeated acquisition paradigms.

Few corresponding efforts have as yet been undertaken in the human studies, but perseverative behavior was recently reported on the California Verbal Learning Test for Children and the Wisconsin Card Sorting Test in a subset of children from the Boston prospective study cohort (26). The authors deemed this effect surprising in light of the very low and restricted range of PbBs at 10 years of age, and the relatively small numbers of subjects on which these data were available.

Increased distractibility has also been cited as the basis of Pb-associated cognitive impairments (20, 27–30). In some regards, both perseverative behavior and distractibility, although seemingly opposite effects, may be considered attention deficits. Behavioral paradigms that can specifically distinguish between the contributions of these two proposed bases of learning impairments have yet to be systematically evaluated in either human or experimental animal studies.

Alterations in the efficacy of conditioned and/or unconditioned reinforcement, and/or inability to perceive changes in reinforcement magnitude, may be another behavioral mechanism of Pb-induced learning impairments, one that perhaps explains both perseveration and distractibility. The sustained occurrence of either perseveration or distractibility might suggest that the reinforcement contingencies (i.e. reward) for these inappropriate behaviors are more reinforcing (or less aversive) than are the consequences for the responses mandated by the learning paradigm. Alternatively, the magnitude of reinforcement may be insufficient under conditions of Pb exposure to sustain behavioral functions requiring learning or transition of behavior (25), processes that clearly require greater "mental effort," i.e. entail a greater response cost, than does performance of an already learned response. The fact that Pb exposure exerts more pronounced effects on acquisition behaviors, and on difficult as opposed to relatively simple discriminations, supports this possibility (31).

Of course Pb exposure induces behavioral impairments other than learning deficits, including changes in reaction time, as noted in pediatric populations (7), auditory and visual system deficits, both in humans and experimental animals (32), and motor function disturbances such as postural disequilibrium in children (33). While Pb does exert direct effects on learning, as demonstrated in experimental animal studies, it is important to remember that these additional behavioral impairments may contribute to cognitive problems in other contexts.

CHANGES IN NEUROTRANSMITTER SYSTEMS AND THEIR POTENTIAL ROLES IN LEAD-INDUCED LEARNING IMPAIRMENTS

Difficulties Involved in Identifying Neurobiological Substrates

Considerable difficulties are encountered in defining the underlying neuropharmacological substrates of Pb-induced learning impairments. One difficulty arises from the as-yet-incomplete understanding of the behavioral mechanism(s) of this deficit. The more precisely defined the behavioral aberration, the greater the specificity with which studies of cellular and molecular mechanisms of action can be directed. Further efforts will clearly be necessary to determine the precise contributions of changes in attention and/or subtle modifications of reinforcement efficacy to Pb-induced learning impairments.

Linking in vitro or in vivo biochemical or cellular effects of Pb to its behavioral impairments presents a particular challenge to defining mechanisms. In vitro effects must first be validated in vivo to verify their occurrence under normal physiological conditions. Even then, questions remain as to whether biochemical or cellular effects are of sufficient biological strength and relevance to even serve as mechanisms of behavioral toxicity. If so, then demonstrating a causal relationship between such effects and learning impairments requires evidence of a direct nature. Too often, such attempts have been based purely on correlations. Studies directly evaluating these relationships will ultimately be necessary to confirm the neurobiological substrates of Pb-related learning impairments. Further complicating this process is the possibility that multiple mechanisms may be involved—not an unlikely possibility, given the wide spectrum of biological effects of Pb (34).

Any search for underlying substrates of Pb-induced learning impairments must also consider that such changes occur in response both to developmental and occupational Pb exposures. It is possible that similar mechanisms of action underlie the behavioral manifestations in both cases, in which case morphological, developmentally based mechanisms could not provide adequate explanations, since occupational exposures obviously occur well beyond periods

of early development. Alternatively, different mechanisms may underlie occupational and developmental Pb effects. Furthermore, for developmental exposures, the mechanism(s) of toxicity may be related very specifically to the period(s) during which such exposure occurs. This underscores the importance of understanding normal nervous system development, how its impairment relates to behavioral disturbances, and how Pb alters these ontogenetic processes.

In spite of the various difficulties entailed in establishing relationships between Pb-induced changes in neurotransmitter functions and learning impairments, some preliminary understanding can be established for dopaminergic, cholinergic, and glutamatergic systems, as described here and summarized in Table 1. Although these relationships are still more suggestive than conclusive, the accumulated findings allow specific hypotheses to be developed that can be used to explore these possible connections further.

Changes in Dopaminergic Systems

Although not featured prominently in this context, dopamine systems are increasingly implicated in aspects of cognitive functions. For example, the importance of the nucleus accumbens, dopamine, and both D1 and D2 receptors to conditioned reinforcement has been described (35, 36). Lesions of dopamine neurons impair behavior in various types of learning and cognitive tasks (37–39). Similarly, Parkinson's disease, a disease associated with striatal dopamine depletion, is also sometimes accompanied by difficulties in cognitive functions (40).

BIOCHEMICAL/CELLULAR EFFECTS Several lines of evidence point to an effect of Pb on dopamine systems consistent with an impaired regulation of dopamine synthesis and release, which may be presynaptic in nature. The evidence includes decreased synaptosomal release of dopamine (41) and decreased synaptic transmission in peripheral nerve (42). Moreover, the ability of a dopamine agonist to prevent the increase in dopamine content produced by gamma-butyrolactone (GBL) was attenuated in nucleus accumbens (NAC) (43) and caudate-putamen (44) at PbBs of 32–36 μg/dl, effects shown not to result from altered autoreceptor regulation of tyrosine hydroxylase activity. These effects were accompanied by decreased levels of dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in both mesolimbic and nigrostriatal regions. The authors concluded that autoreceptor-mediated regulation of dopamine synthesis was impaired by Pb, and that a Pb-related decrease in dopamine release could occur either via vesicular storage changes or decreased synaptic transmission. In addition, decreased dopamine turnover occurs in response to developmental Pb exposures (45, 46).

Pb treatment is frequently reported to exert differential, often opposing

Table 1 Summary of lead-induced changes relative to control[a]

	Dopaminergic	Glutamatergic	Cholinergic
Kinetics[b]	↓ DA release, availability	↓ Glutamine synthetase ↓ NMDA activity ↓ MK-801 binding	↓ ACh release ↓ ACh function
Receptor subtypes involved[c]	D2 > D1	NMDA > quisqualate, NMDA > kainate	M2?
Brain regions[d]	NAc ≠ Str	hippocampus > cerebral cortex	Cortex, hippocampus, septum, midbrain, striatum
Behavioral and drug discrimination sensitivity[e]	↑ D1 ↑ D2 ↓ cocaine, d-amphetamine	↓ MK-801 ↑ NMDA	↑ Oxotremorine — Arecholine
Accuracy of repeated learning[f]	— D1 agonist — D2 agonist — AMPT	↓ MK-801 effect ↑ NMDA effect	
Fixed-interval response rate[g]	↓ D1 agonist ↑ D2 agonist	—MK-801 —NMDA	— Arecholine
Developmental susceptibility[h]		Young > Old	

[a] ↑, increased; ↓, decreased; ≠, not equal to; >, greater than; —, no differential effect
[b] References: 46–51, 100–109, 121–122
[c] References: 55, 57, 103, 106, 122
[d] References: 55–60, 62, 106, 121–122
[e] References: 52–53, 61, 69–74, 99a
[f] References: 86, 91, 112
[f] References: 89, 99a
[f] References: 106

effects on nigrostriatal and mesolimbic dopamine systems. For example, D2 binding is increased in striatum but decreased in NAC following Pb exposure (47, 48). In another study, the number of D2 sites was increased by Pb in NAC and concomitantly decreased in striatum following low-level postnatal exposures (49). Similar regional differences in dopamine synthesis, turnover, and uptake have been described after Pb exposure (50–52). Increased D2 sensitivity following either postweaning (53) or postnatal Pb exposure (54) has been found to be accompanied by increased D2-binding sites in nucleus accumbens, but not in striatum (49, 55). Such regional differences between striatum and nucleus accumbens in response to Pb exposure should not be unexpected, given reported differences between the regions in aspects of function such as the rates of dopamine synthesis and utilization (56), regulation of dopamine synthesis by autoreceptors (57, 58), and regulation of dopamine uptake (59).

Some indications of differential sensitivity of the two major subtypes of dopamine receptors, D1 and D2, to Pb exposure have also been noted. At least two studies now suggest an enhanced sensitivity of D2 dopamine receptors to alterations by Pb (47, 49). How this differential vulnerability relates to the D1–D2 receptor interactions that have been described repeatedly is not yet known.

The differential effects of Pb often noted in the two terminal dopamine projection areas, and in D1 and D2 receptor sites, certainly augment the difficulties of identifying the biochemical cascade of events that ultimately results in a net decrease in dopamine availability. Obviously, a better understanding of the basis of dopaminergic function in mesolimbic as compared to nigrostriatal tracts will assist such efforts.

Also consistent with a contention of decreased dopamine release and availability are the findings of both D2 (quinpirole) and D1 (SKF38393) supersensitivity in postweaning Pb-exposed rats assessed using drug discrimination procedures, effects occurring at PbBs of about 30 μg/dl (53). A later study showed the D2 agonist quinpirole to be mediated presynaptically rather than postsynaptically (60). A net effect of Pb of decreased dopamine release or availability would deprive dopamine receptors of endogenous neurotransmitter and result eventually in supersensitivity at both presynaptic and postsynaptic dopamine receptor sites. This interpretation is also congruent with the findings of Levin et al (61) that prolonged administration of L-dopa could reverse an impairment of delayed match-to-sample behavior in Pb-treated monkeys, suggesting dopaminergic deficiency as the basis of this behavioral deficit.

Pb exposure produces subsensitivity to the effects of both d-amphetamine and cocaine, two compounds that share dopamine reuptake blockade properties. Altered d-amphetamine sensitivity has been found in Pb-treated animals on a wide variety of behavioral baselines (62–65). This subsensitivity is further confirmed by drug discrimination studies showing that higher doses of d-am-

phetamine were required by Pb-exposed rats to sustain a behavioral discrimination between injections of d-amphetamine and saline (66). Similarly, an attenuated increase in motor activity has been noted following administration of 10 mg/kg cocaine to Pb-treated rats (67).

This attenuated behavioral response to d-amphetamine and cocaine may seem contradictory to the D1 and D2 supersensitivity described after Pb treatment by Cory-Slechta & Widzowski (53). If an important component of the effects of both d-amphetamine and cocaine, however, derives from their dopamine reuptake blockade effects, then decreased dopamine release, as the postulated net effect of Pb, could reduce the amount of dopamine available for reuptake, thus minimizing the behavioral basis of d-amphetamine's and cocaine's effects. Moreover, a blockade of dopamine reuptake could increase dopamine availability at autoreceptor sites, producing additional declines in dopamine release, and perpetuating this postulated cascade of effects. On the other hand, decreased dopamine release might also be expected ultimately to increase the sensitivity of reuptake sites.

The specific contribution of the developmental period of Pb exposure to dopaminergic system alterations remains to be determined. Many of the studies relevant to this issue have used a single developmental exposure period and Pb concentration, and assessed dopaminergic changes at only one time point. Recent work demonstrating complex time- and region-specific changes in dopamine binding after postnatal Pb exposure in rats underscores the critical need to explore the parameters of developmental period, Pb concentration, and the time course of effects in far greater detail (49).

ROLE OF DOPAMINERGIC ALTERATIONS IN LEAD-INDUCED LEARNING IMPAIRMENTS As noted above, perseverative deficits have been described in some studies as the basis of Pb-induced learning impairments (17, 26, 68). Similarly, excitotoxic lesions of NAC, a terminal DA projection area, increased resistance to extinction and impaired response-switching strategies of rats in a series of discrimination reversals (37). Perseverative responding after NAC and frontal cortex lesions have been observed on several other behavioral baselines as well (69–71). Such findings could suggest a preferential involvement of mesolimbic dopamine systems in the effects of Pb (72).

Similarities of behavioral outcome from mesolimbic lesions and Pb exposure, while suggestive, do not provide direct evidence for the involvement of dopamine system changes in Pb-induced learning impairments. Cohn & Cory-Slechta (73) adopted the approach that dopamine system involvement in Pb-induced learning impairments would be indicated by differential changes in learning accuracy in control and Pb-exposed rats following administration of dopaminergic compounds. However, while the D1 agonist SKF38393, the D2/D3 agonist quinpirole, and the catecholamine depleter alpha-methyl-para-

tyrosine all decreased accuracy in the learning component of a multiple repeated acquisition and performance schedule, no differential effects were found related to Pb treatment. As evaluated by this approach, then, dopamine system changes do not appear to contribute to Pb-induced learning impairments.

A role for dopaminergic system disturbances in some aspects of Pb-related behavioral toxicity was certainly suggested by the report of Levin et al (61), which stated that among various classes of pharmacological compounds, only chronic L-Dopa was able to reverse an apparently permanent deficit in delayed spatial alteration in Pb-treated monkeys. Unfortunately, the exact nature of this behavioral deficit remains unclear. That it was not a memory dysfunction is indicated by the evidence of changes in accuracy at even the shortest delay values imposed between responses, where memory requirements would be negligible. Also, Pb exposure has been reported both to impair and to improve spatial delayed alternation performance (23, 68, 74, 75).

Dopaminergic system changes may, instead, play a role in the altered response rates on Fixed-Interval (FI) schedules of reinforcement that have repeatedly been reported in Pb-exposed animals (31, 76), assuming that such an involvement is suggested by differential effects of dopaminergic compounds in control and Pb-treated animals on FI response rates. Carter & Leander (77) found differential effects on FI response rates in Pb-treated pigeons of d-amphetamine and of the norepinephrine uptake inhibitor protriptyline, but not of pentobarbital, the peripheral sympathomimetic hydoxyamphetamine, the GABA antagonist picrotoxin, the glycine antagonist strychnine, the nonspecific convulsant pentylenetetrazol, the direct serotonin agonist quipazine, the indirect serotonin agonist 1-5-HTP, the muscarinic cholinergic antagonist scopolamine, the acetylcholinesterase inhibitor physostigmine, or the nicotinic agonist nicotine. Similarly, Cory-Slechta & Pokora (DA Cory-Slechta & MJ Pokora, unpublished data) have noted differential effects on FI overall response rates in Pb-treated rats of the D2 agonist quinpirole, and the D1 agonists SKF38393 and SKF82958, but not of the mu agonist morphine, the glutamate agonist *N*-methyl-D-aspartate (NMDA), the noncompetitive NMDA antagonist MK-801, or the muscarinic agonist arecholine. Taken together, these findings suggest a preferential involvement of central catecholaminergic systems in the effects of Pb on FI performance.

Changes in Glutamatergic Systems

The importance of glutamatergic systems, particularly the NMDA receptor complex, to learning and memory processes has been confirmed from numerous studies demonstrating that noncompetitive NMDA antagonists, such as dizocilpine (MK-801) and phencyclidine (PCP), impair performance on a variety of learning and memory tasks (78–85). Moreover, administration of NMDA itself, which acts at the glutamate site of the NMDA receptor complex,

has been reported to facilitate behavior or even to reverse deficits in some behaviors (86, 87). In addition, the NMDA receptor complex has been associated with the long-term potentiation (LTP) of excitatory synaptic transmission, a potential neurobiological substrate of learning and memory processes.

BIOCHEMICAL/CELLULAR EFFECTS Both in vivo and in vitro evidence indicates effects of Pb on glutamatergic systems at extremely low PbBs. Sierra et al (88) described reductions of 30–40% in glutamine synthetase activity in pregnant guinea pigs and their offspring at PbBs of only 13 μg/dl. Similar effects were found in vitro, with reductions of 17–52% at levels of 0.25–1.0 μM Pb (89). A decrease in glutamine synthetase activity in homogenates of mixed glial primary cultures, with a maximum inhibition of 70% of control at a Pb concentration of 2.5 μM, has also been reported (90). Since glutamine synthetase is the enzyme that catalyzes the formation of glutamine from glutamate, such effects could alter glutamate kinetics.

In vitro Pb exposure also inhibits NMDA-evoked whole-neuron and single-channel currents in glutamate synapses derived from rat hippocampal pyramidal neurons in a concentration-dependent manner with an IC_{50} of 10 μM Pb (91). These effects were shown to be specific to NMDA receptors, since much higher Pb levels were required to perturb quisqualate or kainate receptors. The frequency of NMDA-activated channel openings also decreased, an effect found to be only slowly reversible. These effects did not appear to be a function of interactions of Pb with Ca^{2+}, with the Mg^{2+}-binding site, or with either the glycine- or glutamate-binding sites. The inhibition of MK-801 binding by Pb suggested Pb's action, instead, at the Zn-binding site, since Zn^{2+} has been shown to be the most potent blocker of MK-801 binding among several divalent cations. Subsequent studies (92) indicated more pronounced effects of Pb in young than in older cultured neurons, and that Pb's effects on the NMDA receptor complex were exerted outside the membrane electric field, unlike those of Zn, suggesting a noncompetitive NMDA and glycine site antagonism by Pb. A similar mechanism was proposed by Uteshev et al (93), who also reported that Pb exposure decreased aspartate and glycine activation of the NMDA channel in acutely dissociated hippocampal CA1- and CA3-neurons from adult rats.

Pb exposure also inhibits the binding of the noncompetitive antagonist MK-801 to its site within the channel, an effect noted in hippocampus of adult rats in one study (91). Guilarte & Miceli (94) subsequently reported that Pb was a more potent inhibitor of MK-801 binding than was either Zn^{2+} or Mg^{2+}, and that such effects were age dependent, since the IC_{50} for this effect was lower in neonatal than in adult rat cortical membranes. Additional experiments indicated that Pb acted in the same way as Zn^{2+} in inhibiting MK-801 binding, and that the IC_{50} value for hippocampal binding was lower than that for cerebral

cortex, consistent with a regional heterogeneity of these effects. Although in vivo experiments from that study were also stated to suggest a preferential inhibition of MK-801 binding in neonatal as compared to adult rats, the lower brain Pb concentrations of the adult rats negates any valid comparisons.

Considered collectively, these biochemical studies are consonant with an inhibition of NMDA receptor complex activity. From a functional perspective, it has also been reported that Pb exposure inhibits long-term potentiation, a possible cellular substrate of learning and memory processes. These effects have generally been noted at relatively high exposure levels, however: in one study by Altmann et al, at in vitro Pb concentrations of 10–20 μM in the CA1 region of hippocampus (95). In a later study, these authors did provide evidence for such an effect in hippocampal slices from animals exposed in vivo with resulting PbBs of only 14 μg/dl, but only in animals whose exposure was continuous from gestation through the duration of the experiment (96). While Hori et al (97) also found that Pb exposure inhibited LTP in vitro at 5–10 μM, they noted that these effects were not mediated via the NMDA receptor complex, since these same Pb concentrations had no effect on responses to ionophoretically applied NMDA, leaving questions about the mechanisms of Pb-based changes in LTP. Other evidence for a functional counterpart of Pb-induced glutamatergic system changes comes from a study describing an attenuated response of Pb-exposed rats to the seizure-inducing properties of NMDA at PbBs of 43 μg/dl, with potentiated responses occurring at much higher PbBs (98).

ROLE OF GLUTAMATERGIC ALTERATIONS IN LEAD-INDUCED LEARNING IMPAIRMENTS Several lines of evidence support an involvement of NMDA receptor complex function in Pb-induced learning deficits. Pb decreases sensitivity to the stimulus properties of MK-801, as indicated by increased ED_{50} values in a drug discrimination paradigm (99, 99a). Furthermore, unlike dopaminergic compounds (73), glutamatergic compounds do induce differential changes in learning accuracy in control and Pb-exposed rats, one possible indication of their involvement in Pb-induced learning impairments. These changes consist of an attenuation of the accuracy-impairing properties of acute MK-801 administration (79), and a potentiation of the accuracy-impairing properties of NMDA (100). Moreover, acute MK-801 administration and Pb exposure both impair learning accuracy primarily through perseverative errors (78,80).

These combined findings—i.e. subsensitivity to both the stimulus properties and the accuracy-impairing effects of MK-801, coupled with supersensitivity to the accuracy-impairing effects of NMDA—are consistent with the biochemical studies reporting inhibition of MK-801 binding and decreased NMDA receptor complex activity. Specifically, under conditions of Pb exposure, MK-801 cannot readily access its binding site; thus its behavioral properties are

attenuated. Additionally, a decline in NMDA receptor complex activity might eventually produce supersensitivity at the glutamate-binding site, which must be occupied for NMDA receptor complex activity.

CAVEATS REGARDING THE ROLE OF GLUTAMATERGIC SYSTEM CHANGES IN LEAD-INDUCED LEARNING IMPAIRMENTS Before any definitive conclusions can be drawn, however, it is important to recognize that the behavioral properties of glutamatergic compounds on learning are not invariant. NMDA agonists, for example, do not uniformly enhance cognitive function. Systemically administered NMDA has been reported to impair acquisition of a passive avoidance response (101) as well as to decrease accuracy in a repeated learning baseline (100). Nor do NMDA antagonists consistently impair cognitive functions. While NMDA antagonists did impair performance in a step-through dark avoidance paradigm in one study, they also facilitated retention in a step-down passive avoidance paradigm (102). Such reports clearly underscore the importance of the behavioral paradigm itself in modulating the effects of NMDA agonists and antagonists, a facet that must be considered in studies relating the role of neurotransmitter systems to Pb-induced learning impairments or to any other behavioral manifestations.

Changes in Cholinergic Systems

An integral role for cholinergic systems in learning/memory processes is widely accepted. The cognitive deficits and dementias associated with Alzheimer's disease are frequently attributed to a loss of cholinergic function in cortex and hippocampus (103), and numerous studies have demonstrated that anticholinergics, as well as various lesions of the cholinergic system, can impair performance on cognitive and attention-based tasks (104–106).

BIOCHEMICAL/CELLULAR EFFECTS Although Pb exposure produces numerous changes in cholinergic system function (107, 108), many of these effects have been inconsistently detected, or are of opposite direction in different studies. One relatively uniform finding, however, both in vivo and in vitro, is a Pb-induced decline in evoked acetylcholine release and diminished cholinergic function. Shih & Hanin (109), for example, reported decreases of in vivo acetylcholine turnover rate of 35–54% in cortex, hippocampus, midbrain, and striatum in rats exposed to Pb lactationally. Although some undernutrition may have contributed to those effects, Bielarczyk et al (110) recently reported a selective 30–40% reduction of choline acetyltransferase activity in septum and hippocampus after gestational and postnatal Pb exposures associated with PbBs of approximately 20 μg/dl. The authors speculated that such a septal cholinergic hypofunction produced by Pb could result in an upregulation of postsynaptic sites, possibly M2 sites in the hippocampus. Few reports yet exist related

to postsynaptic cholinergic changes. One of these reports observed a decline rather than an increase in muscarinic receptor binding in a presumably postsynaptic site, i.e. visual cortex, in adult rats that were exposed postnatally to peak PbBs of 50–60 μg/dl (111). These levels had returned to control values by the time muscarinic receptor binding was evaluated.

ROLE OF CHOLINERGIC ALTERATIONS IN LEAD-INDUCED LEARNING IMPAIRMENTS Despite an obvious potential for cholinergic hypofunction to be involved in Pb-induced learning impairments, little experimental attention has yet focused on this possibility. One study reported that physostigmine improved passive avoidance performance, increased the rate of spontaneous alternation, and lowered open field activity scores of Pb-treated but not control rats, and that scopolamine administration also resulted in differential effects related to Pb treatment, at least for activity. These effects, however, were noted at PbBs (ranging from 331 to 1297 μg/dl) with neither environmental nor occupational relevance, and in the presence of both maternal weight loss and pup body weight loss, raising serious questions about their relevance to cognitive impairments related to low-level exposures (112).

More recently, DA Cory-Slechta (submitted) observed significant alterations in cholinergic sensitivity following Pb treatment. Rats were initially trained to discriminate the stimulus properties of the muscarinic agonist arecholine from saline, but no Pb-related differences in sensitivity to arecholine were found. Oxotremorine, however, substituted completely for arecholine and produced lower ED_{50} values in rats with PbBs of 15–25 μg/dl. Thus, Pb-treated rats were supersensitive to oxotremorine, providing evidence for functionally significant cholinergic system changes at PbBs just above those of current environmental concern (10–15 μg/dl).

The somewhat surprising demonstration that Pb exposure would produce supersensitivity to only one of two muscarinic agonists may be clarified by studies delineating the various subtypes of muscarinic cholinergic receptors now known, M1–M5 (113). At least in hippocampus, cortex, and striatum (114–116), M2 muscarinic receptors appear to be the predominant presynaptic cholinergic receptor subtype, such that M2 agonism decreases acetylcholine release. Oxotremorine has been reported to bind 3–10-fold more potently to m2 (protein) systems than to either of two m4 (protein) systems (117). In contrast, arecholine was significantly more efficacious in binding to the two m4 systems than to the m2 system. These findings suggest that oxotremorine may be a preferential M2 agonist.

Pb-induced supersensitivity to oxotremorine could then be indicative of presynaptic muscarinic supersensitivity, a finding in concert with the biochemical studies reporting cholinergic hypofunction with Pb. Acetylcholine deprivation would be expected to upregulate both pre- and postsynaptic cholinergic

receptors eventually, since both would be deprived of their endogenous ligand. Possible specific site(s) for these effects include at least hippocampus, cortex, and striatum, i.e. areas where M2 presynaptic function has been described (114–116). As yet, this potential scenario has not been systematically explored, and at least one finding of decreased rather than increased muscarinic binding has been reported (111).

CONCLUSIONS AND FUTURE DIRECTIONS

When considered as a whole, the current evidence, as shown in Table 1, implicates glutamatergic system disturbances in lead-induced learning impairments. Reports of decreased NMDA receptor complex activity and inhibition of MK-801 binding coincide with the patterns of glutamate sensitivity that have been observed: namely, subsensitivity to MK-801 and enhanced sensitivity to NMDA. Finally, glutamatergic compounds evoke differential impairments of learning accuracy in Pb-treated animals relative to controls. In contrast, dopaminergic compounds produce differential FI response rate changes in control and Pb-exposed animals, but no differential accuracy changes in a learning paradigm, suggesting a preferential involvement of this system in FI alterations, but no obvious role in learning impairments. There is as yet virtually no evidence from which to determine whether cholinergic system dysfunction contributes to Pb-induced learning impairments, but reports of cholinergic hypofunction certainly lend credence to such a possibility.

The pace to a more complete resolution of such questions can be accelerated by research endeavors in several areas. One must be the study of the specific behavioral nature of lead-induced learning impairments and of the behavioral mechanisms contributing to such effects. In conjunction with microdialysis and microinjection techniques, such studies would provide critical information relating to the roles of various neurotransmitter systems and different brain regions in behavioral manifestations. Moreover, if carried out in conjunction with new technologies for selectively activating or turning off specific populations of receptors (e.g. EEDQ, antisense), such studies would permit highly defined assessments of the role of specific receptor subtypes as well.

The strategies adopted to date to examine the potential involvement of neurotransmitter system dysfunctions in lead-induced learning impairments have been indirect, based on the contention that an involvement of any neurotransmitter system would be indicated by differential changes in learning accuracy in Pb-exposed and control rats following administration of class-specific compounds. Other, more direct assessments are clearly warranted.

It might be presumed that comparing the nature of Pb-induced changes in a particular learning paradigm to those produced by specific brain lesions would provide direct information not only on possible regional involvement,

but also of the primary neurotransmitter systems operative in these regions. Such a strategy was utilized by Munoz et al (118, 119), who reported some but not total similarities between the effects of Pb and hippocampal or amygdaloid lesions on radial arm maze learning deficits. Given Pb's wide spectrum of biological properties (34), however, it is unlikely that Pb exposure would, in fact, exert selective effects in any one brain region. Although prior studies have suggested preferential accumulation of Pb in regions such as hippocampus, more recent endeavors show a homogenous distribution and suggest that reports of regional accumulation resulted from the use of dry rather than wet tissue weights in Pb analyses (120). It may be, therefore, that system rather than regional lesions would prove instructive.

A more direct approach to linking Pb-induced neuropharmacological and learning changes is to generate in non-Pb-exposed, normal animals the same pattern of neuropharmacological changes that result from Pb exposure, and then to determine whether this pattern engenders similar profiles of learning impairments. In other words, would MK-801 subsensitivity/NMDA supersensitivity in control (normal) animals engender a profile of learning impairments akin to that produced by Pb exposure? A complementary approach is to determine whether the reversal of neuropharmacological changes in Pb-treated animals also reverses Pb-induced learning impairments.

With respect to the latter strategy, one must be cognizant of the fact that Pb body burdens cannot be rapidly reversed, and termination of external exposure may result in mobilization of bone Pb stores into soft-tissue organs such as brain, counteracting the effects of any treatments designed to reverse neuropharmacological changes. Moreover, it is likely that chronic rather than acute neuropharmacological regimens would be necessary to reverse Pb-related neurotransmitter system changes or to produce corresponding effects in normal animals. Altered neurotransmitter system function normally occurs only in response to chronic perturbation of that system, in which case acute pharmacological probes would not provide an adequate assessment of the involvement of a particular neurotransmitter system in behavioral manifestations. Nevertheless, these approaches may prove promising, and can also be explored regionally by coupling them with microinjection and microdialysis procedures.

Although neurotransmitter systems are discussed as if they were independent entities, it is becoming increasingly clear that these systems interact and even regulate aspects of each other's functions. One of the difficulties then is defining the specific contribution of any given neurotransmitter system to Pb-induced learning impairments, and ascertaining whether that system's effects are exerted directly or indirectly.

Numerous studies document interactions of dopaminergic and glutamatergic neurotransmitter systems, although the exact nature of the interactions are not

yet agreed upon (121, 122). Similar interactions occur between dopaminergic and cholinergic systems (123, 124), and glutamatergic and cholinergic systems (125, 126). The existence of these documented interactions raises possibilities such as: (*a*) ostensibly dopaminergic effects of Pb being an indirect result of direct effects on glutamatergic systems, (*b*) presumed glutamatergic system effects arising indirectly as a result of primary effects on dopamine systems, or (*c*) a combination of these two. Distinguishing among these various alternatives will not be a simple undertaking.

A simple comparison of levels of Pb exposure at which effects on various neurotransmitter systems occur may not necessarily resolve such issues. For example, Cory-Slechta & Widzowski (53) reported that exposure levels of 50 ppm produced both D1 and D2 dopamine supersensitivity. MK-801 subsensitivity (99a) was noted only at 150 ppm, suggesting that glutamatergic effects were secondary to changes in dopamine systems. However, oxotremorine supersensitivity (DA Cory-Slechta, unpublished data) was observed in response to a 50 ppm exposure level, whereas similar but nonsignificant changes were noted at 150 ppm. This type of biphasic dose-effect function is often described for aspects of Pb neurotoxicity (127) and underscores the difficulties with approaches based solely on effective exposure levels.

The administration of selective antagonists of various neurotransmitter systems might provide a more direct and compelling approach to the problem of neurotransmitter interactions. For example, the ability of dopamine antagonists to block glutamatergic sensitivity changes in Pb-treated rats would be consistent with a primary dopaminergic mechanism of such effects. Further, intracerebral injections of antagonists would allow the identification of brain regions important to neurotransmitter system changes. While these strategies have yet to be employed, they carry promise for unraveling the nature of sometimes complex interactions.

Finally, the various effects shown in Table 1 have been discussed as if developmental period of exposure were not a consideration. It is clear that further efforts addressing susceptibility at different stages of the life cycle—including young, adult, and advanced stages—are necessary, given the possibilities that different mechanisms are operative at different stages of maturity or that multiple mechanisms may be operative with developmental exposures.

Any conclusions arrived at now must necessarily be tentative, as many research gaps remain. It is only by enhancing our understanding of the behavioral consequences of Pb exposure and their neuropharmacological substrates, however, that more sensitive and specific measures of toxicity can be established, that improved neurochemical and behavioral therapeutic strategies can be devised, and that the actual risks of exposure can be fully grasped.

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