0022-3565/80/2143-0642$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 214, No. 3
Printed in U.S.A.

Alterations in the Biochemical Properties of Central Dopamine Synapses following Chronic Postnatal $PbCO_3$ Exposure[1]

LEON C. WINCE,[2] CAROL A. DONOVAN and ALBERT J. AZZARO

Department of Pharmacology and Toxicology and Department of Neurology, West Virginia University Medical Center, Morgantown, West Virginia

Accepted for publication June 9, 1980

ABSTRACT

Wince, Leon C., Carol A. Donovan and Albert J. Azzaro: *Alterations in the biochemical properties of central dopamine synapses following chronic postnatal $PbCO_3$ exposure.* J. Pharmacol. Exp. Ther. **214:** 642–650, 1980.

Behavioral studies on spontaneous and stimulant-induced locomotor activity after chronic low-level lead (Pb) exposure prompted us to investigate the pre- and postjunctional biochemical properties at central dopamine (DA) synapses. Rat pups were chronically exposed from birth to 40 ppm of Pb and tested behaviorally and biochemically at 25 to 35 days of age. Pb-exposed rat pups demonstrated locomotor hyperactivity, but the locomotor response to *d*-amphetamine SO_4 (0.5–2.0 mg/kg) or apomorphine HCl (0.5–5.0 mg/kg) was attenuated. Correlative biochemical studies in rat brain tissue demonstrated associated pre- and postjunctional changes at central DA synapses. These biochemical changes included 1) diminished KC1-evoked release of exogenous [^{3}H]DA from brain slices, and 2) suppression of the DA receptor-mediated activation of adenylate cyclase in neostriatal homogenates. No changes were observed in the synthesis, accumulation or *d*-amphetamine-induced release of radiolabeled DA in brain tissue from these animals. These results are discussed in relation to the possible mechanisms associated with Pb-evoked neurochemical changes as well as the role that each of these effects may play in the Pb-induced alterations of locomotor activity.

The nervous system is particularly vulnerable to the toxic effects of lead (Pb). In this regard, clinical (*e.g.*, ataxia, stupor, convulsions and coma) and morphological (hemorrhage, edema and necrosis) encephalopathy is the prominent feature in the immature nervous system, whereas peripheral nerve toxicity (segmental demyelination and axonal degeneration) is more common in the adult (for review see Goyer and Rhyne, 1973).

Because Pb is highly toxic to the developing central nervous system (CNS), increasing concern over the rise in the Pb content in the environment has been expressed (Cannon and Bowles, 1962; Chow, 1970). These concerns seem appropriate since more recent clinical studies have shown that prolonged exposure of children to so called "subclinical" concentrations of Pb may be associated with behavioral disorders, learning disabilities and mental retardation (de la Burde and Choate, 1975; Lin-Fu, 1972; David *et al.*, 1972, 1976; Landrigan *et al.*, 1975; Beattie *et al.*, 1975). Moreover, animal studies have shown that chronic perinatal low-level Pb exposure elicits alterations of both learned and spontaneous behavioral patterns (Silbergeld and Goldberg, 1973; Sauerhoff and Michaelson, 1973; Snowdon, 1973; Sobotka and Cook, 1974; Carson *et al.*, 1974; Sobotka *et al.*, 1975; Reiter *et al.*, 1975; Brown, 1975; Driscoll and Stegner, 1976; Winneke *et al.*, 1977) in the absence of the typical outward signs of Pb-induced neurological toxicity (Michaelson and Sauerhoff, 1974; Silbergeld and Goldberg, 1975).

In addition to the above mentioned behavioral abnormalities, chronic Pb exposure also alters the behavioral responses to pharmacological agents. Chronic postnatal exposure of neonates to low concentrations of Pb salts causes an attenuation of the locomotor stimulatory properties of *d*-amphetamine (Silbergeld and Goldberg, 1974; Sobotka and Cook, 1974; Wince and Azzaro, 1977) and apomorphine (Wince and Azzaro, 1977) when the recorded activity is expressed as a percentage of the ongoing or base line activity. These studies demonstrate that amphetamine or apomorphine are still capable of enhancing locomotor activity but in a manner that is nonproportional to the elevated basal level of motor activity seen after chronic postnatal lead exposure (Silbergeld and Goldberg, 1973; Sauerhoff and Michaelson, 1973). This finding is curious since animals made hyperactive by other methods (*e.g.*, raphe lesion or *p*-chlorophenylalanine administration) demonstrate *d*-amphetamine-induced increases in locomotion proportional to the enhanced basal level of spontaneous activity (Neill *et al.*, 1972; Mabry and Campbell, 1973; Jacobs *et al.*, 1975) and therefore suggests that chronic postnatal Pb exposure elicits central neurochemical changes that allow for an altered *d*-amphetamine or apomorphine behavioral response.

Since both *d*-amphetamine and apomorphine are believed to produce locomotor stimulation through neuropharmacological interactions at central dopamine (DA) synapses (Anden *et al.*, 1967; Ernst, 1967; Hollister *et al.*, 1974; Roberts *et al.*, 1975; Pijnenburg *et al.*, 1975; Kelly *et al.*, 1975), the possibility exists for an action of Pb on some aspect of central DA metabolism.

Received for publication September 17, 1979.
[1] This work was supported in part by U.S. Public Health Service Grant 5-T01-GM00076-18, DOE Contract EY-77-C-21-8087 and the West Virginia University Medical Corporation.
[2] Present address: Department of Pharmacology & Toxicology, School of Pharmacy, University of Kansas, Lawrence, KS 66045.

In this regard, earlier studies were unsuccessful in demonstrating a change in either the steady-state levels of DA (Golter and Michaelson, 1975; Silbergeld and Goldberg, 1975; Sobotka *et al.*, 1975) or the rate of DA turnover (Michaelson *et al.*, 1974). However, Silbergeld and Goldberg (1975) reported a small (about 20%) but significant reduction in [^{3}H]DA accumulation by brain nerve ending particles (synaptosomes) prepared from chronically Pb-exposed developing mice. This latter finding suggests the possibility of more subtle Pb-induced alterations in one or more of the biochemical properties of the DA synapse.

The present series of experiments were performed to more fully explore the possible interaction of Pb with a number of pre- and postjunctional biochemical properties of the central DA synapse. In this study, rats were chronically exposed to low concentrations of $PbCO_3$ which produce no typical outward signs (weakness, ataxia and paraplegia, etc.) of neurological toxicity (Pentschew and Garro, 1966; Michaelson, 1974). However, all litters displayed alterations in spontaneous and pharmacologically induced locomotor responses. A preliminary report of this work has been presented in abstract form (Wince & Azzaro, 1978).

Methods

Pregnant Sprague-Dawley rats (17 days gestation) were caged individually in the medical center animal facility and given food and water *ad libitum*. After parturition, experimental dams were given a powdered laboratory chow containing 4% $PbCO_3$ (31,016 ppm) and control dams were provided unadulterated powdered laboratory chow (Sauerhoff and Michaelson, 1973). At 5 days of age, all litters were normalized to eight pups per dam. Control dams were pair-fed daily with respect to the amount of chow consumed by experimental dams during the previous 24-hr period (Michaelson and Sauerhoff, 1974). This procedure minimized the differences in weight gain between experimental and control litters. Lead-exposed neonates were weaned to a diet of 40 ppm of Pb. This weaned exposure level was chosen since maternal milk from experimental dams exposed to a diet consisting of 4% $PbCO_3$ contains 40 to 50 ppm of Pb (Pentschew and Garro, 1966). By using this method of exposure, rat pups were chronically exposed to low levels of lead through 35 days of age. All behavioral or biochemical studies were performed in 25- to 35-day-old rat pups.

Atomic absorption analysis of whole-brain Pb levels. Rat pups were decapitated, their brain removed, weighed and rinsed with glass distilled water. The tissue was liquified in HNO_3 by a modification of the methods of Murphy *et al.* (1973) and Parker *et al.* (1967) as follows: 10 ml of concentrated redistilled HNO_3 was added to each tissue sample and heated to a gentle boil under a water cooled reflux column for 2.5 to 3 hr. The liquified tissue samples were concentrated to approximately 0.5 ml by using an all glass flash evaporator (Buchler Instruments) under reduced pressure. The concentrated sample was transferred, the reaction flask was rinsed 2 to 3 times with 0.5-ml aliquots of 1% HNO_3 and the washings were combined with the concentrated sample. The final volume of each sample was adjusted to 2.0 ml with 1% HNO_3. Blank values were obtained by repeating this procedure in the absence of tissue.

The quantity of Pb in each sample was determined by using a Perkin-Elmer (model 305B) atomic absorption spectrophotometer (λ = 2170.0Å). Each day, lead standards (certified atomic absorption standard, Fisher Scientific Co., Pittsburgh, PA) were prepared in 1% HNO_3 and used to establish a standard curve.

The quantity of Pb (micrograms per milliliter) in each sample was determined from the standard curve. This value was corrected for volume and background Pb content (blank). The final quantity of Pb (micrograms) was normalized to tissue wet weights.

All glassware used throughout these studies was thoroughly scrubbed in hot, soapy tap water, rinsed with glass distilled water, soaked overnight in glass distilled water and oven dried before use. This procedure was effective in maintaining low background values.

Spontaneous and drug-induced locomotor activity. The spontaneous locomotor activity and the locomotor stimulation effects of *d*-amphetamine sulfate (0.5–2.0 mg/kg, i.p.) or apomorphine hydrochloride (0.5–5.0 mg/kg i.p.) were determined by using a Columbus Instruments selective activity meter (model 2S, Columbus Instruments, Columbus, OH). This instrument uses radio frequency electromagnetic proximity sensors (six equally spaced) to detect the horizontal movement of an animal confined to its surface (Taylor and Sulser, 1973). The activity meter was adjusted to a sensitivity setting of 9.9 and a tuning setting of 80% of the maximum needle deflection.

Rat pups were randomly selected from approximately 100 litters over a 2-year period. The testing was performed in a room isolated from all major laboratory traffic and noise. In performing the study, each animal was placed in a recording environment consisting of a plastic cage (17″ × 18.5″ × 6.5″) positioned over the six electromagnetic proximity sensors. The bottom of the plastic cage was cut away to maximize the contact between the animal and the recording surface. The animal was allowed a 30-min acclimation period before testing. After acclimation, each animal was administered saline, *d*-amphetamine or apomorphine by i.p. injection (0.2 ml/100 g b.wt.). The doses of *d*-amphetamine or apomorphine were carefully chosen to avoid vertical (rearing) or stereotyping (gnawing and grooming, etc.) activity. The locomotor activity of each animal was monitored for a maximum of 120 min. Cumulative total locomotor activity was recorded in 5-min intervals by an attached Sodeco print-out recorder. Each animal was tested once and then destroyed. All rat pups were of an average of 30 days.

Synthesis of DA in synaptosomal-rich fractions of rat forebrain. Lead exposed and pair-fed control rat pups were decapitated, their brains quickly removed and placed into ice-cold physiological buffer (Besson *et al.*, 1969). The composition of the buffer was as follows (millimoles per liter): NaCl, 126.45; KCl, 2.40; $CaCl_2$, 0.97; $MgCl_2$, 0.83; Na_2SO_4, 0.50; KH_2PO_4, 0.50; $NaHCO_3$, 27.62; and glucose, 5.88. A forebrain section was prepared by making a coronal cut from the rostral border of the superior colliculi to the optic chiasm. Forebrains were blotted, weighed and homogenized in 10 volumes of ice-cold 0.32 M sucrose. A crude synaptosomal (P_2) fraction was prepared as described by Gray and Whittaker (1962). The P_2 pellet was resuspended in 2.5 volumes of ice-cold physiologic buffer (0.4 g of original tissue weight per ml).

A 200-μl aliquot of the resuspended P_2 fraction was assayed for the capacity to synthesize DA by using the method described by Kuczenski and Segal (1974) with only minor modifications. The ionic composition of the physiological medium was as above. The incubation was performed for 30 min (37°C) in the presence of 20 μM [1-^{14}C]tyrosine (New England Nuclear, Boston, MA, 53.6 mCi/mmol) and the production of $^{14}CO_2$ was monitored. The reaction was linear for 60 min and proportional to homgenate protein. Nonspecific evolution of $^{14}CO_2$ was determined in the presence of 2 mM 3-iodotyrosine (Sigma Chemical Company, St. Louis, MO), a specific inhibitor of tyrosine hydroxylase (Udenfriend *et al.*, 1965) and routinely subtracted from all experimental values. The $^{14}CO_2$ was assayed by liquid scintillation spectrometry.

Release of [^{3}H]DA by *d*-amphetamine and KCl. Rat pup forebrain slices were prepared with a mechanical tissue chopper at 0.3-mm intervals, rotated 90° and resliced as before (Ziance *et al.*, 1972). Release of [^{3}H]DA was examined by using methods previously described (Azzaro and Rutledge, 1973). Briefly, forebrain slices were incubated at 37°C in the presence of 0.8 μM [^{3}H]DA for 15 min. After extensive washing of the tissue slices with physiological medium (Besson *et al.*, 1969), the tissue was equally distributed among several incubation tubes containing 0.1 μM to 0.1 mM *d*-amphetamine or 2.4 to 45 mM KCl. The tissue was incubated for 10 min (37°C) and the amount of [^{3}H]DA released from the tissue into the medium was isolated by ion-exchange (Dowex 50W-X4; 200–400 mesh; Na^+ form) chromatography and assayed by liquid scintillation spectrometry.

The protein content of each tissue sample was determined by the biuret method (Layne, 1957). The [^{3}H]DA in both tissue and medium fractions was adjusted to the tissue protein. The results are expressed as [^{3}H]DA in the medium as a percentage of the total [^{3}H]DA in tissue and medium (Azzaro and Rutledge 1973).

In some experiments, the amphetamine-induced release of newly-synthesized DA was examined as above except that the tissue was

incubated with 20 μM [^{3}H]tyrosine (New England Nuclear; 0.25 Ci/mmol) and allowed to form [^{3}H]DA for a period of 40 min (37°C; 95% O_2-5% CO_2). Medium and tissue [^{3}H]DA was separated from [^{3}H]tyrosine, [^{3}H]norepinephrine and [^{3}H]deaminated catabolites of DA or norepinephrine by cation-exchange chromatography (Dowex 50W-X4; 200–400 mesh; H^+ form). Briefly, medium and tissue extracts were acidified (pH = 2.0) and passed over a 60 × 5 mm Dowex 50W-X4 cation-exchange column. [^{3}H]Deaminated catabolites were washed through the column with 20 ml of glass distilled water. [^{3}H]Tyrosine, [^{3}H]norepinephrine and [^{3}H]DA were sequentially eluted with 0.1 M sodium phosphate (pH = 6.5), 1 N and 2 N HCl, respectively. The prior addition of 50 μg of DA made it possible to obtain recovery values for each sample. Recovery values for DA, as determined fluorometrically (activation of 225 nm and emission at 325 nm), were always greater than 80%. Each [^{3}H]DA value was corrected for recovery. The newly-synthesized [^{3}H]DA found in the medium was expressed as a percentage of [^{3}H]DA in both tissue and medium (see above).

Accumulation and catabolism of [^{3}H]DA by rat forebrain slices. The accumulation of [^{3}H]DA by DA-containing neurons within rat forebrain slices was studied by using the procedure of Snyder *et al.* (1968) with only minor modifications. Forebrain slices were prepared with a mechanical tissue chopper at 0.3-mm intervals (see above). The tissue (4 to 6 mg of protein) was preincubated for 5 min at 37°C (95% O_2–5% CO_2) in centrifuge tubes containing 2 ml of a physiological salt solution (Besson *et al.*, 1969). [^{3}H]DA (New England Nuclear; 8.8 Ci/mmol) was added to give a final concentration of 0.1 μM and the incubation was continued for an additional 5 min. The active accumulation of [^{3}H]DA was terminated by rapidly chilling the tubes on ice, immediately followed by centrifugation at 10,000 × *g* for 5 min (4°C). The accumulated [^{3}H]DA was extracted by disrupting the pellets in 2 N HCl by using a tissue grinder. Acidified medium and tissue extracts were adjusted to pH = 6.5 and passed over a 30 × 5 mm Dowex 50W-X4 (200–400 mesh; Na^+ form) cation-exchange column. [^{3}H]DA was eluted in 2 N HCl and assayed by liquid scintillation spectrometry using a toluene based scintillation fluor containing Triton X-100. The column recovery of previously added DA (50 μg) was determined by spectrophotofluorometry (activation at 285 nm and emission at 325 nm). Recoveries for DA ranged from 75 to 85%. The [^{3}H]DA in each sample was corrected for recovery.

Total tissue protein was determined by the biuret method (Layne, 1957). Tissue wet weights in each sample were obtained according to the method of Ziance and Rutledge (1972). Diffusion blanks were obtained by incubating the tissue in the presence of 0.1 μM [^{3}H]DA for 5 min on ice (4°C).

In some experiments, the accumulation and subsequent intraneuronal catabolism of [^{3}H]DA was examined in control and Pb-treated rat pup forebrain slices. These experiments were performed as above except that in addition to [^{3}H]DA an aliquot of the column effluent was also assayed for [^{3}H]deaminated catabolites of [^{3}H]DA. An estimate of the nonenzymatic catabolism of [^{3}H]DA was determined at 4°C.

The activity of DA-sensitive adenylate cyclase. Striata from 30-day-old rat pups were rapidly dissected (Glowinski and Iversen, 1966) and homogenized in 25 volumes (w/v) of 2 mM tris(hydroxymethyl)aminomethane-maleate buffer (ph=7.4) containing 2 mM ethylene glycol bis(β-aminoethyl)N,N′-tetraacetic acid. Basal and DA-sensitive adenylate cyclase activity was obtained by using the procedure described by Kebabian *et al.* (1972). A 50-μl aliquote of the striatal homogenate was assayed in a total volume of 300 μl. 3-Isobutyl-1-methylxanthine (1 mM; Sigma Chemical Company) was used in the incubation to inhibit the activity of phosphodiesterase (Beavo *et al.*, 1970). The incubation was performed at 30°C for 2.5 min in a shaking water bath. The amount of cyclic adenosine 3′:5′-monophosphate (cAMP) formed per assay tube was determined by the competitive protein binding assay of Gilman (1970) as modified by Tovey *et al* (1974) by using commercially available binding protein (Diagnostic Products Corp., Los Angeles, CA). The activity of DA-sensitive adenylate cyclase was obtained by subtracting the basal activity from that obtained in the presence of various concentrations (1 μM–1 mM) of DA (Kebabian *et al.*, 1972).

Statistical analysis. All behavior data (locomotor activity) was statistically evaluated by using the nonparametric Mann-Whitney *U* test for significance (Siegel, 1956). Biochemical data was statistically analyzed using Students *t* test (Fryer, 1966).

Drugs and chemicals. *d*-Amphetamie SO_4 (a donation from Smith Klein & French Laboratories, Philadelphia, PA); apomorphine HCl (a donation from Merck & Co., Rahway, NJ). All chemicals were obtained commercially in the highest purity. Radioisotopes used were 1-[1-^{14}C] tyrosine, 1-[*ring*-2,6-^{3}H]tyrosine and 3,4-[*ethyl*-2-^{3}H]N-dihydroxyphenylethylamine (DA).

Results

Locomotor activity after chronic postnatal $PbCO_3$ exposure. Rat pups were chronically exposed, from birth to 40 ppm of Pb. As expressed in table 1, this level of Pb exposure produced a 5-fold increase in the whole-brain content of Pb. In spite of this large increase in brain Pb content, the rat pups appeared otherwise normal; no signs of high-dose Pb intoxication (Pentschew and Garro, 1966).

The results of the locomotor studies are summarized in table 2. A 1.5- to 2.0-fold increase ($P < .05$) in the spontaneous (saline) locomotor activity of 25- to 35-day-old rat pups was observed after chronic $PbCO_3$ exposure. When *d*-amphetamine or apomorphine was administered to these animals, a progressive, dose-related increase, above basal (saline) locomotor activity, was observed. However, the magnitude of the drug-related increase (above basal) in locomotor activity in Pb-exposed pups was not proportional to that seen in pair-fed control animals. This observation is best demonstrated by re-expressing the data as a percentage of the appropriate saline injected group (table 2, percentage of saline). At each dose of *d*-amphetamine or apomorphine, the increase in locomotion, above spontaneous (saline) activity, was significantly less ($P < .05$) than that seen in pair-fed control pups.

Prejunctional alterations at DA synapses after chronic postnatal $PbCO_3$ exposure. *DA synthesis in rat forebrain synaptosomes.* The synthesis of DA in synaptosomal-rich fractions of rat pup forebrain was examined in pair-fed untreated and $PbCO_3$-exposed animals (table 3). Synaptosomes from 25-, 30- and 35-day old Pb-exposed rat pups showed the same capacity to synthesize DA as did those obtained from pair-fed control animals.

Accumulation of [^{3}H]DA by rat forebrain slices. [^{3}H]DA accumulation after chronic $PbCO_3$ exposure is summarized in table 4. As can be seen from the tissue/medium (T/M) ratio for [^{3}H]DA, rat forebrain slices were effective in concentrating this amine 14-fold over that found in medium. However, no change in the T/M ratio for [^{3}H]DA was noted in slices from $PbCO_3$-

TABLE 1

Whole-brain Pb content after postnatal exposure to $PbCO_3$

Pups were exposed to Pb through the maternal milk. Assays for whole-brain levels of Pb were performed at 27 days of age. Numbers in parentheses, number of pups.

Pair-Fed Control Pups[a]	$PbCO_3$-Exposed Pups[a]
μg/g wet wt.	μg/g wet wt.
0.27 ± 0.02	1.36 ± 0.13[b]
(8)	(8)

[a] The values represent the mean ± S.E.M. of eight rat pups.
[b] $P < .005$ as compared to pair-fed control.

TABLE 2

Locomotor activity after *d*-amphetamine or apomorphine in postnatal $PbCO_3$-exposed rat pups

All animals were permitted a 30-min habituation period in the testing environment before injection (i.p.); injection volume = 0.2 ml/100 g b.wt.

Agent	Pair-Fed Control			$PbCO_3$-Exposed		
	N	Total locomotor activity[a]	% of saline	N	Total locomotor activity[a]	% of saline
d-Amphetamine SO_4						
0 (saline)	10	1412	100	10	2161[b]	100
0.5 mg/kg	10	4264[c]	301	10	2285[c]	105[b]
1.0 mg/kg	10	4361[c]	308	10	4621[c]	213[b]
1.5 mg/kg	10	6321[c]	447	10	7071[c]	327[b]
2.0 mg/kg	10	7743[c]	548	10	8660[c]	400[b]
Apomorphine HCl						
0 (saline)	18	450	100	18	916[b]	100
0.5 mg/kg	14	1721[c]	382	15	1674[c]	182[b]
1.0 mg/kg	13	3600[c]	800	12	2944[c]	321[b]
2.5 mg/kg	12	4955[c]	1101	12	5127[c]	559[b]
5.0 mg/kg	12	5829[c]	1295	12	5393[c]	588[b]

[a] Median responses of number (*N*) of animals tested for a period of 120 min (*d*-amphetamine) or 70 min (apomorphine).
[b] $P < .05$ as compared to pair-fed control.
[c] $P < .05$ as compared to saline.

TABLE 3

The synthesis of DA by synaptosomal-rich fractions of rat pup forebrain after chronic lead carbonate exposure

Mean ± S.E.M. of four to seven expeiments.

Age[a]	Treatment	
	Pair-fed control	$PbCO_3$
	p-mol $^{14}CO_2$/mg protein/30 min	
25	22.72 ± 1.58	23.74 ± 0.66
30	21.62 ± 0.77	21.88 ± 0.45
35	23.23 ± 1.01	21.66 ± 0.71

[a] Days after birth.

exposed rat pups. Moreover, careful examination of the data shows that tissue and medium [^{3}H]DA content as well as the formation of total deaminated catabolites of [^{3}H]DA remained unchanged after $PbCO_3$ exposure.

KCl and d-amphetamine-evoked release of [^{3}H]DA from rat forebrain slices. The effects of $PbCO_3$ exposure on the K^+-evoked release of [^{3}H]DA are found in figure 1. The percentage of [^{3}H]DA found in the medium containing normal K^+ concentrations (2.4 mM) was similar with tissue from either pair-fed control or $PbCO_3$-exposed animals. When the K^+ concentration in the medium was raised to 15, 30 or 45 mM, a concentration-dependent efflux of accumulated [^{3}H]DA was observed. However, at each elevated K^+ concentration, the percentage of [^{3}H]DA found in the medium of slices from Pb-exposed animals was significantly ($P < .05$) less than that seen from control brain slices. This data suggests that $PbCO_3$ was effective in reducing the release of exogenous [^{3}H]DA by KCl.

d-Amphetamine elicits the release of DA from neuronal stores (Besson *et al.*, 1969; Azzaro and Rutledge, 1973), although by a mechanism which differs from that of K^+ (Ziance *et al.*, 1972). We therefore examined the amphetamine-evoked release of [^{3}H]DA from brain slices of $PbCO_3$-exposed rat pups. The results of these studies are found in figure 2. In control forebrain slices, *d*-amphetamine elicited the release of exogenous [^{3}H]DA in a concentration-related manner (fig. 2A). At 0.1 mM *d*-amphetamine, nearly 60% of the total [^{3}H]DA was found in the medium, demonstrating the effective releasing properties of this agent. The release of exogenous [^{3}H]DA (fig. 2A) by *d*-amphetamine was unchanged by chronic $PbCO_3$ exposure, although there was a small consistent trend toward a reduction in the percentage of [^{3}H]DA found in the medium (nonsignificant). Since the behavioral effects of *d*-amphetamine are associated with the release of a newly synthesized pool of DA (Dominic and Moore, 1969; Svennson, 1970; Thornburg and Moore, 1973), the experiments were repeated in tissue allowed to form [^{3}H]DA from the natural precursor substrate [^{3}H]tyrosine (fig. 2B). the *d*-amphetamine-induced release of newly synthesized [^{3}H]DA (fig. 2B) was similar to the release of exogenous [^{3}H]DA (fig. 2A). As before, *d*-amphetamine released [^{3}H]DA in a manner that was directly proportional to concentration and nearly 60% of the newly synthesized [^{3}H]DA was found in the medium at the highest concentration of *d*-amphetamine tested. $PbCO_3$ exposure was also ineffective in altering the release of newly synthesized [^{3}H]DA from rat pup forebrain slices.

Postjunctional alterations at DA synapses after chronic postnatal $PbCO_3$ exposure. Several investigators have demonstrated a DA-sensitive adenylate cyclase in brain tissue which appears closely associated with the postjunctional DA receptor (Kebabian *et al.*, 1972; Horn *et al.*, 1974; Miller *et al.*, 1974). The DA and apomorphine-evoked stimulation of adenylate cyclase activity was therefore used as an index of the postjunctional response to DA receptor activation.

In these studies, rat pup neostriatal homogenates were used because of the high density of postsynaptic DA receptors present in this brain region (Creese *et al.*, 1975). The activity of adenylate cyclase in the absence of DA or apomorphine (basal activity) was unchanged by chronic $PbCO_3$ exposure (table 5). DA concentrations of 0.01, 0.1 and 1 mM produced a significant ($P < .05$) elevation (above basal) in the activity of adenylate cyclase in control and Pb-exposed tissues (table 5). This effect of DA was directly related to concentration. However, the magnitude of the DA-evoked activation in $PbCO_3$-treated tissue was significantly ($P < .05$) less than that seen in control tissue at 0.1 and 1 mM concentrations of DA. This apparent effect of chronic $PbCO_3$ exposure was even more dramatic when apomorphine-evoked activation of adenylate cyclase was studied (fig. 3). In the latter experiments, a significant ($P < .05$) reduction in the magnitude of the response was observed at 10 and 100 μM concentrations of this agent.

TABLE 4

Effect of chronic $PbCO_3$ exposure on [^{3}H]DA accumulation and deamination in 25- to 35-day-old rat pup forebrain slices

Each value represents mean ± S.E.M. of five experiments.

Treatment	Tissue [^{3}H]DA[a]	Medium [^{3}H]DA	Total [^{3}H]Deaminated Catabolites[b]	T/M Ratio [^{3}H]DA
	p-mol/g	*p-mol/ml*	*p-mol/g*	
Pair-fed control	571.12 ± 31.81	39.19 ± 2.14	1171.03 ± 96.08	14.58 ± 0.44
$PbCO_3$	584.32 ± 33.82	44.82 ± 3.14	1059.99 ± 71.59	13.10 ± 0.39

[a] Diffusion blanks/control = 32.10 ± 4.29 pmol/g; $PbCO_3$ = 38.16 ± 4.35 pmol/g.
[b] Represents the sum of [^{3}H]deaminated catabolites found in tissue and medium.

TABLE 5

DA stimulation of adenylate cyclase activity in homogenates of 25- to 35-day-old rat pup neostriatum after chronic lead carbonate exposure

Control			$PbCO_3$		
DA	Adenylate cyclase activity[a]	% of basal	DA	Adenylate cyclase activity[a]	% of basal
0 (basal)	72.60 ± 1.69	100	0 (basal)	70.32 ± 1.95	100
10^{-6} M	74.28 ± 4.33	102	10^{-6} M	71.40 ± 4.67	101
10^{-5} M	87.00 ± 3.27[b]	119	10^{-5} M	83.16 ± 6.02[b]	118
10^{-4} M	106.88 ± 3.00[b]	147	10^{-4} M	94.20 ± 3.47[b,c]	133
10^{-3} M	109.02 ± 5.22[b]	150	10^{-3} M	92.08 ± 3.36[b,c]	130

[a] Pico-moles of cAMP per 50 µl/2.5 min; mean ± S.E.M. of five experiments in duplicate.
[b] $P < .05$ with respect to basal activity.
[c] $P < .05$ with respect to pair-fed control animals.

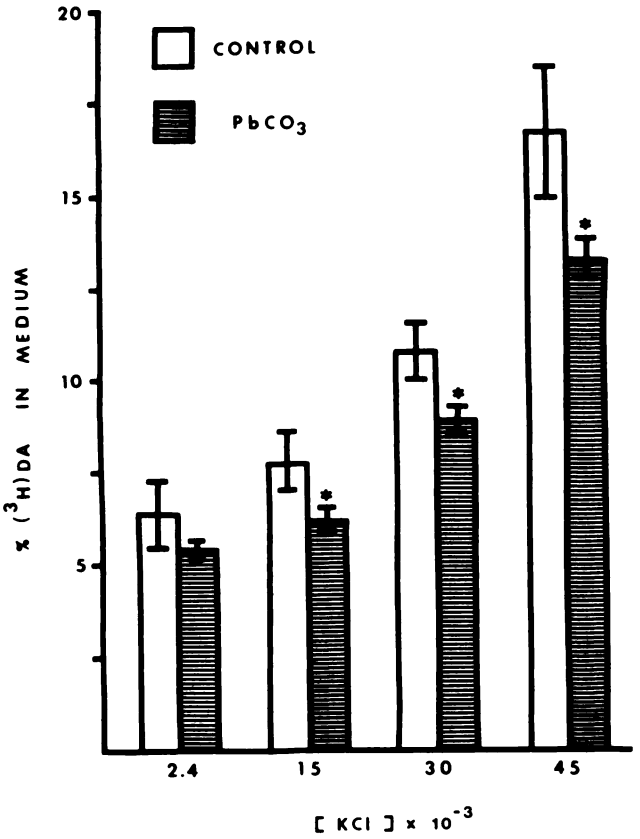

Fig. 1. The KCl-evoked release of exogenous [^{3}H]DA from rat forebrain slices. Forebrain slices were prepared from pair-fed control and $PbCO_3$-exposed rat pups (25–35 days of age) and allowed to accumulate exogenous [^{3}H]DA. The release of [^{3}H]DA from the tissue to the incubation medium after 2.4, 15, 30 and 45 mM KCl was examined. Release is expressed as [^{3}H]DA in the medium as a percentage of the total [^{3}H]DA in the tissue and medium. Each value represents the mean ± S.E.M. of six experiments. * a significant difference from pair-fed controls; $P < .05$.

Discussion

The actions of drugs on the CNS are, for the most part, associated with specific alterations in the neurochemical properties of synaptic transmission. It is therefore reasonable to assume that industrial wastes and other environmental pollutants may interact in similar ways with these same neuronal mechanisms to produce CNS toxicity. The behavioral studies presented here confirm that chronic postnatal exposure to low levels of Pb will produce a locomotor hyperactivity in developing rodents (Silbergeld and Goldberg, 1973; Sauerhoff and Michaelson, 1973). Moreover, the previously reported attenuated locomotor response to *d*-amphetamine and apomorphine was also observed (Silbergeld and Goldberg, 1974; Sobotka and Cook, 1974; Wince and Azzaro, 1977). This latter effect was of particular interest since both of these agents are thought to enhance locomotion through actions at dopaminergic synapses within the brain (see "Introduction"). Biochemical studies were therefore designed to investigate the possibility of a Pb-evoked alteration in the neurochemical properties of central dopaminergic neurotransmission.

Initially, our attention was directed toward the effects of Pb on prejunctional biochemical properties at central DA synapses. It was demonstrated that chronic $PbCO_3$ exposure was unable to elicit a change in either the capacity of brain tissue to form DA from [^{14}C]tyrosine (table 3) or to accumulate exogenously administered [^{3}H]DA (table 4). Since the accumulation of [^{3}H] catecholamines by brain slices reflects the ability of nerve terminals to transport, store and catabolize this amine neurotransmitter substrate (Azzaro and Smith, 1975), it would suggest that each of these metabolic events associated with the DA-containing neuron is unaffected by chronic postnatal low-level Pb exposure.

The accumulation data presented here was somewhat unexpected since Silbergeld and Goldberg (1975) reported a small (20%) but significant reduction in the forebrain accumulation of exogenous [^{3}H]DA after chronic postnatal Pb acetate expo-

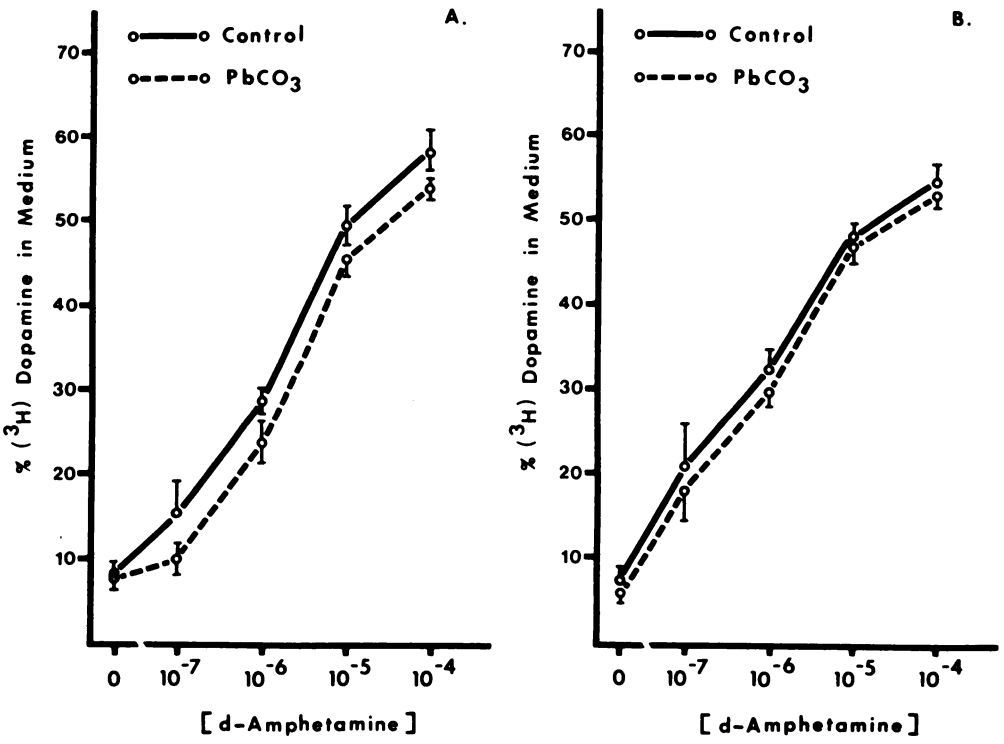

Fig. 2. The effects of chronic $PbCO_3$ exposure in the *d*-amphetamine-induced release of exogenous [^{3}H]DA (A) or newly synthesized [^{3}H]DA (B) from forebrain slices. Forebrain slices from pair-fed control or $PbCO_3$-exposed rat pups (25–30 days of age) were prepared, allowed to accumulate exogenous [^{3}H]DA (A) or synthesize [^{3}H]DA from [^{3}H]tyrosine (B) and then were exposed to *d*-amphetamine SO_4 (10^{-7}–10^{-4} M). The release of [^{3}H]DA is expressed as [^{3}H]DA in the medium as a percentage of the total [^{3}H]-DA in the tissue and medium. Each value represents the mean ± S.E.M. of five to six experiments.

sure. Although several differences exist between our studies and those of Silbergeld and Goldberg (1975), which may account for the discrepancy, one important difference resides in the method employed for the exposure of animals to Pb salts. Silbergeld and Goldberg exposed mice pups to Pb by administering Pb acetate (5 mg/ml) in the drinking water of nursing dams. Although there is no indication as to the brain content of Pb, this alternate method of exposure may have resulted in a Pb concentration that was higher than that presented here. Silbergeld (1977) demonstrated that 0.1 mM concentrations of Pb (*in vitro*) are required to significantly reduce the accumulation (by 20%) of [^{3}H]DA. The data presented in table 1 would suggest that brain Pb concentration in our animals are only in the micromolar range. Therefore, if the level of Pb exposure relates directly to the effects of this cation on [^{3}H]DA accumulation, it would suggest that the concentration o Pb obtained in our studies was too low to produce an inhibitory effect. effect.

Although no change was observed in the capacity of DA-containing neurons to synthesize, transport, store and catabolize radiolabeled DA, chronic Pb exposure did alter the release of exogenously administered [^{3}H]DA from these same neural elements. In this regard, Pb was effective in significantly reducing the K^+-induced release of [^{3}H]DA from brain slices. However, this action of Pb was only observed at elevated K^+ concentrations (fig. 1) and was not seen when *d*-amphetamine was used as the releasing agent (fig. 2). The inability of chronic Pb exposure to alter the *d*-amphetamine-evoked release of [^{3}H]DA from brain slices is significant. KCl is well established as a releasing agent whose depolarizing action of neuronal membranes is very similar to physiological stimulation (Hillman and McIlwain, 1961; Nowycky *et al.*, 1975). Accordingly, the strict Ca^{++} requirement for the K^+-induced release of neurotransmitters has been demonstrated (Rubin, 1970). In contrast, the *d*-amphetamine-induced release of monoamines is essentially independent of Ca^{++} (Ziance *et al.*, 1972). The reduction in the K^+ but not the *d*-amphetamine-induced release of [^{3}H]DA from Pb-exposed brain slices suggests the possibility of a competition between P^{++} and Ca^{++} for sites associated with the Ca^{++}-dependent release of [^{3}H]DA.

Previous work by other investigators support the concept of a competition between Pb^{++} and Ca^{++} with regard to neurotransmitter release. Kostial and Vouk (1957) demonstrated a reduction in the stimulus-evoked release of acetylcholine (ACH) from perfused superior cervical ganglion after the addition of $PbNO_3$ to the perfusion medium. These investigators were able to reverse the inhibitory effects of $PbNO_3$ by elevating the Ca^{++} concentration of the perfusion medium. Similar inhibitory effects on ACH release have been observed at the neuromuscular junction after *in vitro* and *in vivo* Pb exposure (Silbergeld *et al.*, 1974a, 1974b; Manalis and Cooper, 1973) and in cortical minces after chronic low-level Pb acetate exposure (Carroll *et al.*, 1977). In each of these studies, the suggestion was made of a competition between Pb^{++} and Ca^{++} as the mechanism for the inhibitory effects of Pb on ACH release. Thus, the inhibitory action of Pb on [^{3}H]DA release from brain tissue may prove to be a nonspecific effect associated with the Ca^{++}-dependent release of all neurotransmitter substances.

Postjunctional properties at synaptic sites within the CNS are more difficult to assess. However, we examined the effect of chronic postnatal Pb exposure on the activity of DA-sensitive adenylate cyclase as an index of the postjunctional response to DA receptor activation. The rationale for this approach was based upon previous studies which show the close association of postsynaptic norepinephrine (NE) and DA receptors with the membrane-bound adenylate cyclase enzyme (Kebabian *et al.*, 1972; Horn *et al.*, 1974; Miller *et al.*, 1974). Activation of this enzyme by NE or DA results in the intracellular formation of cAMP; a substance which is postulated as the 2nd messenger in mediating the postjunctional effects of these catecholamine neurotransmitter substances (Klainer *et al.*, 1962; Greengard, 1976; Nathanson, 1976).

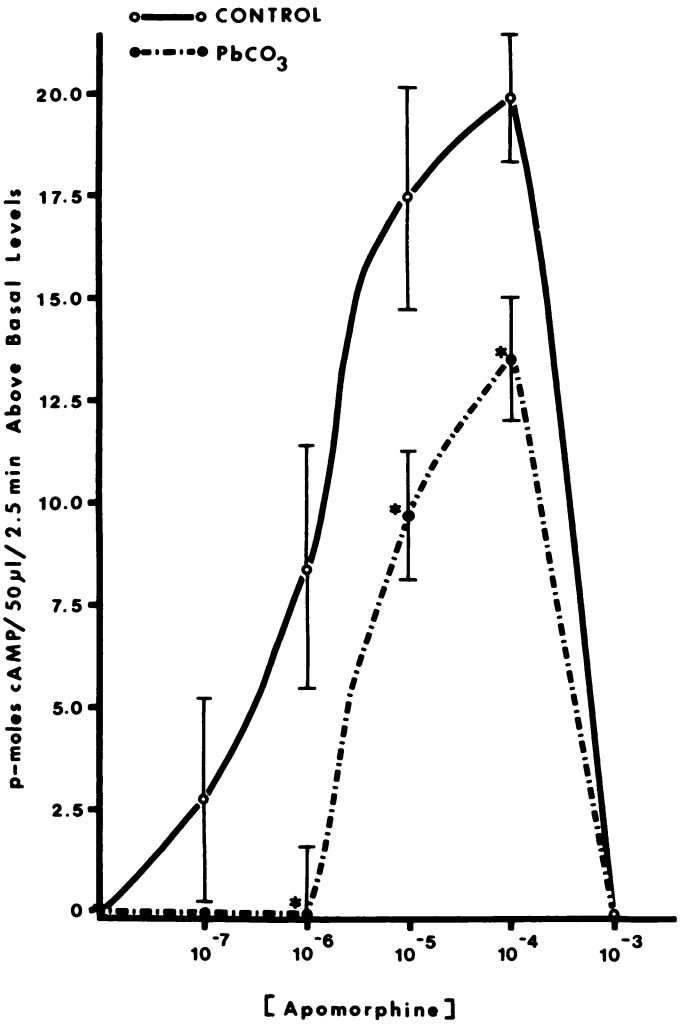

Fig. 3. The effect of chronic $PbCO_3$ exposure on the apomorphine-evoked stimulation of neostriatal adenylate cyclase activity. Homogenates of the neostriatum were prepared from pair-fed control and $PbCO_3$-exposed rat pups (25–35 days of age) and the activity of adenylate cyclase was determined in the absence (basal) and presence of apomorphine HCl. The results are expressed as an increase in cAMP formed above basal levels. Each value represents the mean ± S.E.M. of five experiments. * significant difference from pair-fed controls; $P < .05$.

The ability of DA or apomorphine to stimulate the activity of adenylate cyclase was depressed by chronic $PbCO_3$ exposure (table 5; fig. 3). This observation was made in the absence of an alteration in the basal activity of adenylate cyclase. These results suggest the possibility of a postjunctional subsensitivity to DA and other DA receptor agonists.

Taylor *et al.*, (1978) have recently provided electrophysiological evidence for a Pb-evoked postjunctional subsensitivity to NE in the cerebellum. In their studies it was shown that the iontophoretic application of Pb^{++} to Purkinje cells antagonized the receptor-mediated inhibition of Purkinje cell firing by NE. A NE-sensitive adenylate cyclase has been shown to be closely associated with the Purkinje cell NE receptor (Nathanson *et al.*, 1976; Bloom *et al.*, 1972; Siggins *et al.*, 1973). Of interest is that acute addition of $PbCl_2$ (1–30 µM) to cerebellar homogenates inhibits the activation of this adenylate cyclase enzyme by NE (Nathanson and Bloom, 1976).

It remains to be established whether the acute and chronic effects of Pb on adenylate cyclase activity are mediated by similar mechanisms. Acute *in vitro* $PbCl_2$ (1–30 µM) inhibits proportionally both the basal activity and the NE-evoked activation of adenylate cyclase (Nathanson and Bloom, 1976). This observation has been duplicated in our own laboratory with DA-sensitive adenylate cyclase after *in vitro* addition of $PbNO_3$ to striatal homogenates (unpublished observations). However, chronic postnatal low-level $PbCO_3$ exposure is without effect on the basal activity of striatal adenylate cyclase and only inhibits the DA receptor-mediated activation of this enzyme (table 5). Additional studies will be required to resolve this dilemma.

Although speculation on the role of observed synaptic changes with regard to various behavioral responses must be made with extreme caution, our neurochemical findings at the DA synapse would seem to at least partially explain the behavioral alterations in the drug-induced locomotor activity after chronic low-level $PbCO_3$ exposure. Much evidence has accumulated to suggest that both *d*-amphetamine and apomorphine enhance locomotion through actions at central DA synapses (see "Introduction"). Amphetamine is believed to act indirectly on postjunctional DA receptors by increasing the synaptic concentration of DA through the prejunctional release of this neurotransmitter substance (Besson *et al.*, 1969; Azzaro and Rutledge, 1973). Apomorphine, on the other hand, is independent of prejunctional DA and acts directly as a postjunctional DA receptor agonist (Anden *et al.*, 1967). If the inhibition in the receptor-mediated stimulation of DA-sensitive adenylate cyclase, observed in our animals, truly reflects a postjunctional subsensitivity to DA and other DA receptor agonists, then a reduction in the locomotor response to *d*-amphetamine and apomorphine might be expected.

The spontaneous locomotor hyperactivity observed in chronic $PbCO_3$-exposed rodents is more difficult to explain. Locomotor hyperactivity is complex and is more likely associated with alterations at a number of neurotransmitter synaptic sites (see Wender, 1974). However, it has been demonstrated that the selective destruction of DA-containing neurons, in neonatal rats, is associated with a locomotor hyperactivity appearing between 12 and 22 days of age (Shaywitz *et al.*, 1976). In their study (Shaywitz *et al.*, 1976) it was demonstrated that only a partial reduction (about 50%) in the number of DA-containing neurons was sufficient for the expression of hyperactivity. Therefore, it is tempting to speculate that the pre- and postjunctional changes observed in our studies may represent a sufficient depression of DA synaptic activity to at least partially account for the observed changes in spontaneous locomotion. However, the possibility of an effect of Pb on the metabolism of other neurotransmitters (e.g. ACh) cannot be excluded and therefore additional neurochemical studies will be required to fully understand the effect of this cation on locomotion.

Acknowledgments

The authors wish to express their gratitude to Mrs. Renee Schoepp for her assistance in preparing this manuscript.

References

ANDEN, N.-E., RUBENSON, A., FUXE, K. AND HOKFELT, T.: Evidence for dopamine receptor stimulation by apomorphine. J. Pharm. Pharmacol. **19:** 627–629, 1967.

AZZARO, A. J. AND RUTLEDGE, C. O.: Selectivity of release of norepinephrine, dopamine and 5-hydroxytryptamine by amphetamine in various regions of rat brain. Biochem. Pharmacol. **22:** 2801–2813, 1973.

AZZARO, A. J. AND SMITH, D. J.: The role of storage and catabolism in the accumulation of norepinephrine after short and long incubation times. J. Neurochem. **24:** 811–813, 1975.

BEAVO, J. A., ROGERS, N. L., CROFFORD, O. B., HARDMAN, J. G., SUTHERLAND, E. W. AND NEWMAN, E. V.: Effects of xanthine derivatives on lipolysis and on cAMP phosphodiesterase activity. Mol. Pharmacol. **6:** 597–603, 1970.

BEATTIE, A. D., MOORE, M. R., GOLDBERG, A., FINLAYSON, M. J., MACKIE, E. M., GRAHAM, J. F., MAIN, J. C., MCLAREN, D. A., MURDOCK, R. M. AND STEWARD, G. T.: Role of chronic low-level lead exposure in the etiology of mental retardation. Lancet **1:** 589–591, 1975.

BESSON, M. J., CHERAMY, A., FELTZ, P. AND GLOWINSKI, J.: Release of newly-synthesized dopamine from dopamine-containing terminals in the striatum of rat. Proc. Natl. Acad. Sci. U.S.A. **62:** 741–748, 1969.

BLOOM, F. E., HOFFER, B. J., BUTTENBERG, E. F., SIGGINS, G. T., STEINER, A. L., PARKER, C. W. AND WEDNER, J. F.: Adenosine 3′,5′-monophosphate is localized in cerebellar neurons: Immunofluorescence evidence. Science (Wash. DC) **177:** 436–438, 1972.

BROWN, D. R.: Neonatal lead exposure in the rat: Decreased learning as a function of age and blood lead concentrations. Toxicol. Appl. Pharmacol. **32:** 628–637, 1975.

CANNON, H. AND BOWLES, J. M.: Contamination of vegetation by tetraethyl lead. Science (Wash. DC) **137:** 765–766, 1962.

CARROLL, P. T., SILBERGELD, E. K. AND GOLDBERG, A. M.: Alteration of central cholinergic function of chronic lead acetate exposure. Biochem. Pharmacol. **26:** 397–402, 1977.

CARSON, T. L., VAN GELDER, G. A., KARAS, G. C. AND BUCK, W. V.: Slowed learning in lambs prenatally exposed to lead. Arch. Environ. Health **29:** 154–156, 1974.

CHOW, T. J.: Lead accumulation in roadside soil and grass. Nature (Lond.) **225:** 295–296, 1970.

CREESE, I., BURT, D. R. AND SNYDER, S. H.: Dopamine receptor binding: Differentiation of agonist and antagonist states with (^{3}H)dopamine and (^{3}H)haloperidol. Life Sci. **17:** 993–1002, 1975.

DAVID, O., CLARK, J. AND VOELLER, K.: Lead and hyperactivity. Lancet **2:** 900–903, 1972.

DAVID, O., MCGANN, B., HOFFMAN, S., SVERD, J. AND CLARK, J.: Low lead levels and mental retardation. Lancet **2:** 1376–1379, 1976.

DE LA BURDE, B. AND CHOATE, M. S.: Early asymptomatic lead exposure and development at school age. J. Pediatr. **87:** 638–642, 1975.

DOMINIC, J. A. AND MOORE, K. E.: Acute effects of α-methyl-tyrosine on brain catecholamine levels and on spontaneous and amphetamine stimulated motor activity in mice. Arch. Int. Pharmacodyn. Ther. **178:** 166–176, 1969.

DRISCOLL, J. AND STEGNER, S.: Behavioral effects of chronic lead ingestion in laboratory rats. Pharmacol. Biochem. Behav. **4:** 411–417, 1976.

ERNST, A. M.: Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. Psychopharmacologia **10:** 316–323, 1967.

FRYER, H. C.: Concepts and Methods of Experimental Statistics, pp. 164–182, Allyn and Bacon, Boston, 1966.

GILMAN, A.: A protein binding assay for 3′,5′-cyclic monophosphate. Proc. Natl. Acad. Sci. U.S.A. **67:** 305–312, 1970.

GLOWINSKI, J. AND IVERSEN, L. L.; Regional studies of catecholamines in the rat brain. I. The disposition of (^{3}H)norepinephrine, (^{3}H)dopamine and (^{3}H)dopa in various regions of the brain. J. Neurochem. **13:** 655–669, 1966.

GOLTER, M. AND MICHAELSON, I. A.: Growth, behavior and brain catecholamines in lead-exposed rats: A reappraisal. Science (Wash. DC) **187:** 359–361, 1975.

GOYER, R. A. AND RHYNE, B. C.: Pathological effects on lead. *In* International Reviews of Experimental Pathology, ed. by G. W. Richter and M. A. Epstein, vol. 12, pp. 2–77, Academic Press, New York, 1973.

GRAY, E. G. AND WHITTAKER, V. P.: The isolation of nerve endings from brain: An electron-microscopic study of cell fragments derived by homogenization and centrifugation. J. Anat. **96:** 79–87, 1962.

GREENGARD, P.: Possible role for cyclic nucleotide and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters. Nature (Lond.) **260:** 101–108, 1976.

HILLMAN, H. H. AND MCILWAIN, H.: Membrane potential in mammalian cerebral tissue in vitro: Dependence on ionic environment. J. Physiol. (Lond.) **157:** 263–278, 1961.

HOLLISTER, A. S., BREESE, G. R. AND COOPER, B. R.: Comparison of tyrosine hydroxylase and dopmaine-B-hydroxylase inhibition with the effects of various 6-hydroxydopamine treatments on d-amphetamine-induced motor activity. Psychopharmacologia **36:** 1–16, 1974.

HORN, A. S., CUELLO, A. C. AND MILLER, R. J.: Dopamine in the mesolimbic system of the rat brain: Endogenous levels and the effects of drugs on the uptake mechanism and stimulation of adenylate cyclase activity. J. Neurochem. **22:** 265–270, 1974.

JACOBS, B. L., WISE, W. D. AND TAYLOR, K. M.: Is there a catecholamine-serotonin interaction in the control of locomotor activity? Neuropharmacology **14:** 501–506, 1975.

KEBABIAN, J. W., PETZOLD, G. L. AND GREENGARD, P.: Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain and its similarity to the "dopamine receptor." Proc. Natl. Acad. Sci. U.S.A. **69:** 2145–2149, 1972.

KELLY, P. H., SEVIOUR, P. W. AND IVERSEN, S. D.: Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. **94:** 507–522, 1975.

KLAINER, L. M., CHI, Y. M., FREIDBERG, S. L., RALL, T. W. AND SUTHERLAND, E. W.: Adenylate cyclase. IV. The effects of neurohormones on the formation of adenosine-3′,5′ phosphate by preparations from brain and other tissues. J. Biol. Chem. **237:** 1239–1243, 1962.

KOSTIAL, K. AND VOUK, V. B.: Lead ions and synaptic transmission in the superior cervical ganglion of the cat. Brit. J. Pharmacol. **12:** 219–222, 1957.

KUCZENSKI, R. AND SEGAL, D. S.: Intrasynaptosomal conversion of tyrosine to dopamine as an index of brain catecholamine biosynthetic capacity. J. Neurochem. **22:** 1039–1044, 1974.

LANDRIGAN, P. J., BALOH, R. W., BARTHEL, W. V., WITHWORTH, R. H., STAEHLING, N. W. AND ROSENBLUM, G. F.: Neuropsychological dysfunction in children with chronic low-level lead absorption. Lancet **1:** 708–712, 1975.

LAYNE, E.: Spectrophotometric and turbidimetric methods for measuring protein. Methods Enzymol. **3:** 447–454, 1957.

LIN-FU, J. S.: Undue absorption of lead among children—A new look at an old problem. N. Engl. J. Med. **286:** 702–710, 1972.

MABRY, P. D. AND CAMPBELL, B. A.: Serotonergic inhibition of catecholamine-induced behavioral arousal. Brain Res. **49:** 381–391, 1973.

MANALIS, R. S. AND COOPER, G. P.: Presynaptic and postsynaptic effects of lead at the frog neuromuscular junction. Nature (Lond.) **243:** 354–355, 1973.

MICHAELSON, I. A.: Effects of inorganic lead on levels of RNA, DNA, and protein of developing neonatal rat brain. Toxicol. Appl. Pharmacol. **26:** 539–548, 1974.

MICHAELSON, I. A., GREENLAND, R. D. AND ROTH, W.: Increased brain norepinephrine and turnover in lead-exposed hyperactive rats. Pharmacologist **16:** 250, 1974.

MICHAELSON, I. A. AND SAUERHOFF, M. W.: An improved model of lead-induced brain dysfunction in the suckling rat. Toxicol. Appl. Pharmcol. **28:** 88–96, 1974.

MILLER, R. J., HORN, A. S. AND IVERSEN, L. L.: The action of neuroleptic drugs on dopamine-stimulated cAMP production in rat neostriatum and limbic forebrain. Mol. Pharmacol. **10:** 759–766, 1974.

MURPHY, L., MINDEN, E. E., ELLER, P. M. AND PETERING, H. G.: Atomic absorption determination of zinc, copper, cadmium and lead in tissue solubilized by aqueous tetramethylammonium hydroxide. Anal. Biochem. **53:** 365–372, 1973.

NATHANSON, J. A.: Cyclic nucleotides and nervous system function. Physiol. Res. **57:** 157–256, 1976.

NATHANSON, J. A. AND BLOOM, F. E.: Heavy metals and adenosine cyclic 3′,5′-monophosphate metabolism: Possible relevance to heavy metal toxicity. Mol. Pharmacol. **12:** 390–398, 1976.

NATHANSON, J. A., FREEDMAN, R. AND HOFFER, B. J.: Lanthanum inhibits brain adenylate cyclase and blocks noradrenergic depression of Purkinje cell discharge independent of calcium. Nature (Lond.) **261:** 330–332, 1976.

NEILL, D. B., GRANT, L. D. AND GROSSMAN, S. P.: Selective potentiation of locomotor effects of amphetamine by midbrain raphe lesions. Physiol. Behav. **9:** 655–657, 1972.

NOWYCKY, M. C., SGARAGLI, G. P., COOPER, J. R. AND ROTH, R. H.: Effect of collagenase on the release of dopamine and acetylcholine from slices of rat corpus striatum. J. Neurochem. **24:** 1279–1281, 1975.

PARKER, M. M., HUMOLLER, F. L. AND MAHLER, D. J.: Determination of copper and zinc in biological material. Clin. Chem. **13:** 40–48, 1967.

PENTSCHEW, A. AND GARRO, F.: Lead encephalo-myelopathy of the suckling rat and its implications on the porphyrinopathic nervous disease. Acta Neuropathol. **6:** 266–278, 1966.

PIJNENBURG, A. J. J., HONG, U. M. M. AND VAN ROSSUM, J. M.: Inhibition of *d*-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens. Psychopharmacologia **41:** 87–95, 1975.

REITER, L. W., ANDERSON, G. E., LADKEY, J. W. AND CAHILL, D. F.: Developmental and behavioral changes in the rat during chronic exposure to lead. Environ. Health Perspect. **12:** 119–123, 1975.

ROBERTS, D. C. S., ZIS, A. P. AND FIBIGER, H. C.: Ascending catecholamine pathways and amphetamine-induced locomotor activity: Importance of dopamine and apparent non-involvement of norepinephrine. Brain Res. **93:** 441–454, 1975.

RUBIN, P. R.: The role of calcium in the release of neurotransmitter substrates and hormones. Pharmacol. Rev. **22:** 389–428, 1970.

SAUERHOFF, M. W. AND MICHAELSON, I. A.: Hyperactivity and brain catecholamines in lead-exposed developing rats. Science (Wash. DC) **182:** 1022–1024, 1973.

SHAYWITZ, B. A., YAGER, R. D. AND KLOPPER, J. H.: Selective brain dopamine depletion in developing rats: An experimental model of minimal brain dysfunction. Science (Wash. DC) **191:** 305–307, 1976.

SIEGEL, S.: Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill Book Co., New York, 1956.

SIGGINS, G. R., BATTENBURG, E. D., HOFFER, B. J., BLOOM, F. E. AND STEINER, A. L.: Noradrenergic stimulation of cyclic adenosine monophosphate in rat Purkinje neurons: An immunocytochemical study. Science (Wash. DC) **179:** 585–588, 1973.

SILBERGELD, E. K.: Interactions of lead and calcium on synaptosomal uptake of dopamine and choline. Life Sci. **20:** 309–318, 1977.

SILBERGELD, E. K., FALES, J. T. AND GOLDBERG, A. M.: Evidence for a junctional effect of lead on neuromuscular function. Nature (Lond.) **247:** 49–50, 1974a.

SILBERGELD, E. K., FALES, J. T. AND GOLDBERG, A. M.: The effects of inorganic lead on the neuromuscular junction. Neuropharmacology **13:** 795–801, 1974b.

SILBERGELD, E. AND GOLDBERG, A. M.: A lead-induced behavioral disorder. Life Sci. **13:** 1275–1283, 1973.

SILBERGELD, E. AND GOLDBERG, A. M.: Lead-induced behavioral dysfunction: An

animal model for hyperactivity. Exp. Neurol. **42**: 146–157, 1974.

SILBERGELD, E. AND GOLDBERG, A. M.: Pharmacological and neurochemical investigations of lead-induced hyperactivity. Neuropharmacology **14**: 431–444, 1975.

SNOWDON, C. T.: Learning deficits in lead-injected rats. Pharmacol. Biochem. Behav. **1**: 599–603, 1973.

SNYDER, S. H., GREEN, A. I. AND HENDLEY, E. D.: Kinetics of (^{3}H)norepinephrine accumulation into slices from different regions of the rat brain. J. Pharmacol. Exp. Ther. **164**: 90–102, 1968.

SOBOTKA, T. J., BRODIE, R. E. AND COOK, M. P.: Psychophysiologic effects of early lead exposure. Toxicology **5**: 175–191, 1975.

SOBOTKA, T. J. AND COOK, M. P.: Postnatal lead acetate exposure in rats: Possible relationship to minimal brain dysfunction. Am. J. Ment. Defic. **79**: 5–9, 1974.

SVENSSON, T. H.: The effect of inhibition of catecholamine synthesis on dextroamphetamine-induced central stimulation. Eur. J. Pharmacol. **12**: 161–166, 1970.

TAYLOR, D., NATHANSON, J., HOFFER, B., OLSON, L. AND SEIGER, A.: Lead blockade of norepinephrine-induced inhibition of cerebellar Purkinje neurons. J. Pharmacol. Exp. Ther. **206**: 371–381, 1978.

TAYLOR, W. A. AND SULSER, F.: Effects of amphetamine and its hydroxylated metabolites on central noradrenergic mechanisms. J. Pharmacol. Exp. Ther. **185**: 620–632, 1973.

THORNBURG, J. E. AND MOORE, K. E.: The relative importance of dopaminergic and noradrenergic neuronal systems for the stimulation of locomotor activity induced by amphetamine and other drugs. Neuropharmacology **12**: 853–866, 1973.

TOVEY, K. C., OLDHAM, K. G. AND WHELAN, J. A. M.: A simple direct assay for cAMP in plasma and other biological samples using an improved competitive protein binding technique. Clin. Chem. Acta **56**: 221–234, 1974.

UDENFRIEND, S., ZALTZMAN-NERENBERG, P. AND NAGATSU, T.: Inhibitors of purified beef adrenal tyrosine hydroxylase. Biochem. Pharmacol. **14**: 837–845, 1965.

WENDER, P. H.: Some speculations concerning a possible biochemical basis of minimal brain dysfunction. Life Sci. **14**: 605–621, 1974.

WINCE, L. C. AND AZZARO, A. J.: Dopamine synaptic function following chronic low-level lead carbonate exposure in neonatal rats. Pharmacologist **19**: 134, 1977.

WINCE, L. C. AND AZZARO, A. J.: Neurochemical changes of the central dopamine synapse following chronic lead exposure. Neurology **28**: 382, 1978.

WINNEKE, G., BROCKHAUS, A. AND BALTISSEN, R.: Neurobehavioral and systemic effects of long term blood lead-elevations in rats. I. Discrimination-learning and open field-behavior. Arch. Toxicol. **37**: 247–262, 1977.

ZIANCE, R. J., AZZARO, A. J. AND RUTLEDGE, C. O.: Characteristics of amphetamine-induced release of norepinephrine from rat cerebral cortex *in vitro*. J. Pharmacol. Exp. Ther. **182**: 284–294, 1972.

ZIANCE, R. J. AND RUTLEDGE, C. O.: A comparison of the effects of fenfluramine and amphetamine on uptake, release and catabolism of norepinephrine in rat brain. J. Pharmacol. Exp. Ther. **180**: 118–126, 1972.

Send reprint requests to: Dr. Albert J. Azzaro, Department of Neurology, West Virginia University Medical Center, Morgantown, WV 26506.