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Neonatal Lead Exposure: Effects on Development of Behavior and Striatal Dopamine Neurons[1]

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JASON, K. M. AND C. K. KELLOGG. *Neonatal lead exposure: Effects on development of behavior and striatal dopamine neurons.* PHARMAC. BIOCHEM. BEHAV. 15(4)641–649, 1981.—Rats treated directly with water or lead acetate (25 or 75 mg/kg) on postnatal days 2 through 14 were tested for the development of spontaneous activity and for locomotor responses to *d*-amphetamine as weanlings. The development of striatal dopamine (DA) levels, uptake, release, and the disappearance of DA after synthesis inhibition were also examined. Blood and brain lead levels were markedly increased at 15 days in exposed animals; by 35 days blood lead levels had decreased 90% while lead level in neostriatum decreased only 55–60%. The effects of the early lead exposure fell into two classes. Shortly after cessation of lead treatment, changes were observed in exposed animals in the development of motor activity, as well as in the disappearance of DA after synthesis inhibition, accumulation of labelled DA, and endogenous DA levels. As brain lead decreased from 15 to 35 days many of the behavioral and neurochemical abnormalities also dissipated. However, at 35 days, striatal DA levels remained decreased in animals given the higher lead dose with a resultant decrease in turnover rate and alterations were noted in activity responses to *d*-amphetamine in lead-treated animals.

Neonatal lead exposure Rats Striatal dopamine Development Behavior Activity
d-Amphetamine Blood lead Brain lead

LEAD is one of several heavy metals known to produce deleterious effects on the human central nervous system (CNS), as manifested by neurologic and behavioral disorders. It is also well-documented that the young, in whom the CNS is undergoing rapid development, are especially susceptible to the neurotoxic effects of lead exposure [24].

Hyperactivity, one of the commonly reported symptoms of minimal brain dysfunction (MBD) in children [10,43], has been correlated with exposure to lead in some cases [13,14]. Experimental investigations of developmental lead exposure in animals have reported increases [31], decreases [34] or transient alterations in motor activity [16,33].

Stimulant drugs such as amphetamine, effective in alleviating symptoms in some hyperactive children [5,6], decrease activity levels of hyperactive lead-treated mice [40]. Others have found that amphetamine-induced increases in activity are attenuated in lead-exposed animals [28, 32, 34, 42].

Amphetamines exert their pharmacologic actions primarily through central catecholamine (CA) systems [1], and locomotor activity is believed to de dependent on the functional integrity of brain dopamine (DA) neurons [12]. Several studies have attempted to correlate behavioral effects of lead with CA levels in the brain, but results have not been consistent for either whole brain levels [19,36] or regional brain CA levels [16,17]. Species differences and methods of exposure may account for some of the discrepancies.

Measurement of amine levels to study the effects of lead on neurotransmitters is a very general method for evaluating CNS changes, and of little use in determining function in neurons. Developmental aspects of physiologic processes in neurons (e.g., synthesis, reuptake) may be more susceptible to environmental manipulations than steady-state transmitter levels. After postnatal lead exposure, no changes were found in whole brain DA synthetic rate in mice [37] or rats [29]. One study measuring DA metabolites reported evidence of decreased DA synthesis in striatum of postnatally lead-treated rats [20]. In lead-treated mice synaptosomal uptake of DA and the DA precursor tyrosine into forebrain were decreased [41] and lead added *in vitro* inhibited DA uptake in caudate synaptosomes without affecting DA release [38].

Most investigations have examined either behavior or neurochemistry in subjects whose lead exposure began at birth and continued throughout lactation and beyond weaning. Such prolonged exposure periods confound the issue of whether exposure to lead during a restricted early period of brain development alone is a cause of the behavioral or neurochemical changes reported. The present set of experiments was designed to determine the effects of low-level, short-

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term exposure to lead, emphasizing changing effects of lead treatment on behavioral development and the development of several processes within striatal DA neurons. Determinations were made immediately after cessation of the exposure and up to 3 weeks later. Blood and brain lead levels were also measured immediately and 3 weeks after exposure ended so that behavioral and neurochemical changes could be correlated with the presence of lead in the body.

GENERAL METHOD

Animals

Young male and female Long-Evans hooded rats were obtained from Blue Spruce Farms (Altamont, NY) and allowed to acclimate to laboratory conditions. Animals were bred when females were 14–16 weeks of age. During the third week of gestation, females were transferred to individual plastic cages and allowed to litter normally. Births were noted within 12 hours, with day of birth designated as day 0. Litters were adjusted after 2 days to 8–10 pups per dam. Pups received oral doses of the lead solution or vehicle once daily on days 2 through 14. Pups not used earlier were weaned at 22 days and housed by litter in metal hanging cages after that time. All animals were exposed to standard laboratory chow and water ad lib throughout the experiment, and were kept under constant temperature conditions and a 12 hr–12 hr light-dark cycle.

Lead Exposure

Three exposure groups were used in all experiments. Lead acetate (Fisher, Certified, A.C.S.) was dissolved in boiled distilled water. Experimental animals were intubated with either 25 or 75 mg/kg of lead acetate per day in a volume of 0.02 ml/g of body weight. Controls received the volume of water only, also by oral intubation. A curved, 30 gauge needle with a tip of polyethylene tubing was used to intubate animals 2 to 6 days old, and a similarly-modified 26 gauge needle was used on days 7 through 14. Due to the problem of possible contamination, all animals in a litter received the same dose. Each behavioral and neurochemical experiment included animals from 2 to 4 litters per group, and approximately equal numbers of males and females.

EXPERIMENT 1

METHOD

Physical Development

Animals were weighed daily on days 2 through 14 and at 17, 21, 28 and 35 days of age. Offspring were monitored for the appearance of eye-opening and the appearance of fur.

Histology

Five 35-day-old animals from each group were sacrificed and complete necropsy examinations were performed. Tissues were fixed in 10% neutral buffer and Formalin and processed by routine methods of paraffin embedment. Hematoxylin-eosin stained samples of brain, thymus, heart, lung, stomach, intestine, kidney, liver, pancreas, adrenals and gonads were examined for histopathologic change by light microscopy. The pathologist's samples were not identified by lead group.

Lead Analyses

Blood. At 15 and 35 days, 4 to 6 animals per group were anesthetized with ether, blood was collected by cardiac puncture, and animals were sacrificed. Lead in whole blood was measured by atomic absorption spectrophotometry [15].

Brain. At 15 and 35 days, 3 control animals and 5 animals from each lead group were killed by cervical dislocation. The brain was removed and dissected into five parts: midbrain-brainstem, (caudal to a pre-collicular cut), cerebellum, diencephalon, striatum (caudate-putamen), and cortex (rest of telencephalon). Lead was measured using an anodic stripping voltammeter (Model 3010, Environmental Science Associates, Bedford, MA). Tissue samples, in glass test tubes, were dissolved in 0.3 ml concentrated (67%) nitric acid, placed on a sand bath and taken to dryness at 100°C after which 0.2 ml of a solution of 67% nitric acid, 70% perchloric acid and 96% sulfuric acid (24:24:1) was added and the temperature raised to 250°C until almost all moisture had evaporated (1 to 2 drops remained). Tubes were removed from the sand bath, allowed to cool, and 2.0 ml of Metexchange M (Environmental Science Associates) was mixed into samples which then sat for 10 min before being poured into analysis cells (Environmental Sciences Associates). Each tube was rinsed twice with 0.5 ml of 1.0 M sodium acetate in 0.2 M sodium chloride and rinses were added to the analysis cells. Samples were then analyzed and lead content calculated using a standard curve with values ranging from 10–100 ppb lead. Tissue weights ranged from 57 to 400 mg. Calculation of control values required extrapolation because most of these readings fell below the lower ranges of the standard curve.

Behavioral Studies

Apparatus. Locomotor activity was measured in a 10 in. × 18 in. activity chamber as previously described [26]. The floor is divided into squares, and horizontal activity is measured by passing a small (approximately 50 μA) current through the animal each time a square is crossed. Counts are recorded by an Anadex printout counter connected to the activity meter. The activity chamber was located in a sound-proof room so that animals could be observed with minimal disturbance, and was cleaned after use for each animal.

Development of locomotor activity. Motor activity of young rats was measured by repeated placing of each rat individually in the activity chamber for 30 min on days 11, 13, 15, 18, 21, and 28. Twelve rats from each group were monitored in this manner. Each session took place at the same time of day, approximately 4 to 6 hr after the onset of light.

Effects of amphetamine on locomotor activity. At 35 days of age, naive animals were injected IP with saline in a volume of 0.1 ml/100 g body weight, returned to the home cage for 15 min and then placed individually in the activity chamber for a 30 min baseline period. After this time, animals were removed from the apparatus and injected with the appropriate drug dose (1 or 5 mg/kg *d*-amphetamine phosphate, or saline). After 15 min animals were returned to the activity chamber for a second 30 min period. Testing for all animals took place approximately 7–9 hr after light onset.

Analyses

Data were analyzed using one- or two-way analysis of

TABLE 1
LEAD LEVELS

Group	Blood μg/100 ml		Neo-Striatum ng/g	
	15 Days	35 Days	15 Days	35 Days
Control	1.13 ± 0.32	0.83 ± 0.36	7.0 ± 3.5	not detectable
25 mg/kg	50.07 ± 5.33	4.00 ± 0.28	434.6 ± 17.3	175.4 ± 54.2
75 mg/kg	98.64 ± 9.89	10.17 ± 0.28	626.2 ± 31.5	278.0 ± 51.9

Animals were directly exposed to lead daily on postnatal days 2 through 14. Lead analyses were made at 15 and 35 days postnatal age. Each value represents the mean ± S.E.M. of 3–5 determinations.

variance (ANOVA) with appropriate post-hoc comparisons when overall F ratio was significant and development of activity levels was also analyzed using *t*-tests for repeated measures [22].

RESULTS

No differences were found between the growth patterns of lead-treated and control rats up to 35 days of age. There were no effects of lead on mortality, weight, age at eye opening, or the appearance of fur. Light microscopic examinations of 35-day-old animals revealed no histopathologic changes in sections from any organ of any of the groups. In particular, there was no evidence of neuron degeneration, demyelination or vascular change in brain tissue sections.

Lead Concentrations

Blood. Lead levels in blood are presented in Table 1. Across ages, blood lead levels in lead groups differed significantly from controls, $F(2,30)=36.59$, $p<0.001$. Blood lead levels in both experimental groups decreased by 90% from day 15 to day 35.

Brain. Analyses of regional brain lead concentration within each lead-treated group demonstrated no significant differences among brain regions in lead accumulation at either age. In this preparation, only striatal lead levels are reported. As can be observed in Table 1, striatal lead levels decreased only 55–60% from day 15 to day 35. Statistical analysis indicated significant differences among all treatment groups across ages, $F(2,20)=54.71$, $p<0.001$.

Behavior

The development of locomotor activity is presented in Fig. 1. All animals demonstrated increases in activity reaching maximum levels by day 15. Whereas the overall pattern was similar in lead-exposed and control animals, there were some observable differences. Activity decreased in control animals following peak activity at day 15, and this decline between 15 and 18 days was significant ($p<0.05$). However, the peak activity was sustained in lead-exposed animals through day 18. A significant decrease in activity occurred in the experimental animals between days 18 and 21 ($p<0.05$ for the 25 mg/kg group and $p<0.01$ for the 75 mg/kg group). Hence this difference in the age at which the characteristic decrease in activity occurs [9] resulted in a significant effect of lead on activity levels at 18 days of age, $F(2,35)=3.92$, $p<0.05$.

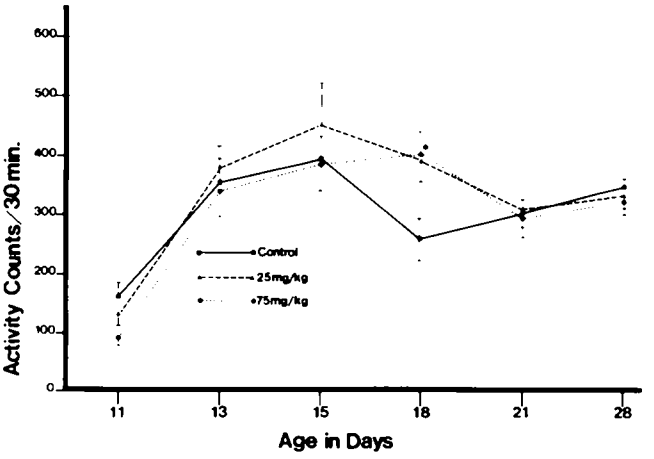

FIG. 1. Development of activity levels in control and lead-treated rats. Each point is the mean+S.E.M. of 12 animals. *Both lead groups differ significantly from controls, $p<0.05$.

Effects of d-Amphetamine

At 35 days of age there were no significant differences between lead and control groups in activity during the 30 min pre-drug period. Baseline activity counts (mean±S.E.M.) were: controls, 298±17; 25 mg/kg, 293±20; 75 mg/kg, 289±16. There were no sex differences in activity levels under saline or amphetamine.

Figure 2 depicts activity scores during the 30 min drug test, expressed as percent of baseline counts. All groups showed a reduction in activity under saline relative to baseline activity, probably representing habituation. Two-way ANOVA (amphetamine × lead) revealed significant main effects of amphetamine, $F(2,44)=21.80$, $p<0.001$, and of lead, $F(2,44)=7.95$, $p<0.001$, and a significant amphetamine × lead interaction, $F(4,44)=2.77$, $p<0.05$. Post-hoc *t*-tests revealed that the 25 mg/kg lead group had significantly lower scores than controls under both saline ($p<0.05$)

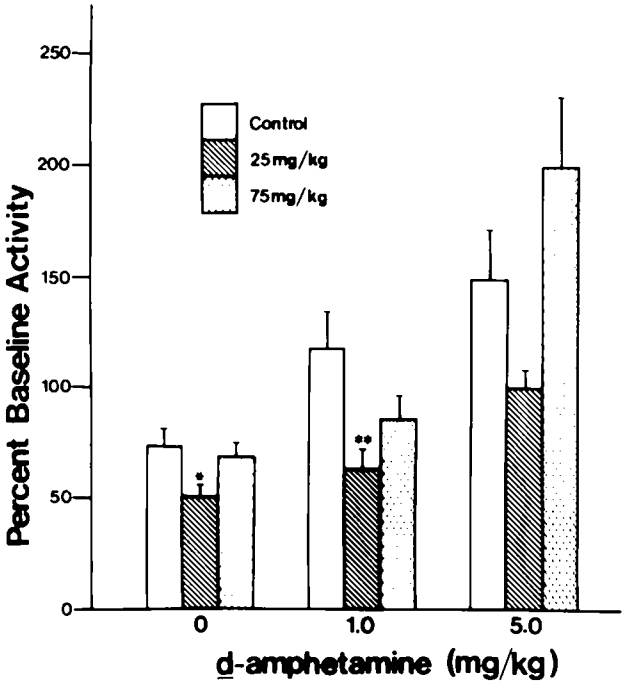

FIG. 2. Effects of *d*-amphetamine on activity in 35-day-old control and lead-treated rats. Each bar is the mean±S.E.M. of 5 animals. *Significantly different from controls, $p<0.05$. **Significantly different from controls, $p<0.02$.

and 1 mg/kg *d*-amphetamine ($p<0.02$). The 75 mg/kg lead group did not differ significantly from controls at any dose. At 5 mg/kg of *d*-amphetamine, the activity chamber, limited to detecting horizontal movement, did not accurately record the effects of the drug. Although all animals exhibited strong responses to 5 mg/kg *d*-amphetamine the responses were not entirely quantifiable as horizontal activity. Observation revealed qualitative differences in the response to 5 mg/kg *d*-amphetamine between animals receiving 25 mg/kg of lead acetate and the other two groups; these anmals exhibited more rearing responses and stereotypic behavior and less running than the other two groups.

EXPERIMENT 2

Experiment 1 demonstrated that early postnatal lead exposure produced (1) discrete changes in the development to mature activity levels in rat without affecting baseline activity levels at a later age, and (2) a change in the behavioral responses to *d*-amphetamine at the later age. It also reaffirmed that lead levels in blood decrease faster than in brain and that the amphetamine response in lead-treated animals is altered at a point when blood levels have dropped 90%. In Experiment 2, three ages were chosen for the study of striatal DA neurons in lead-exposed animals. Levels of DA were measured in striatum and DA utilization was estimated by following the decline in amine levels after synthesis inhibition [2].

METHOD

Procedure

Animals were exposed to lead as described above. Groups of animals aged 15, 18 and 35 days were injected with saline or 250 mg/kg αMT (*d*-, *l*-α-methyl-p-tyrosine methyl ester HCl) in a volume of 0.1 ml/10 g and sacrificed 2 or 4 hr later. Ages were selected based upon the previous experiment in which activity differences between groups were apparent at 18 days but not at 15 and 35 days. The times selected for analysis of DA after synthesis inhibition were based on previous observations [25]. Saline-injected animals were used to determine *in vivo* DA levels and served as the 0 time point. After sacrifice, brains were removed and the striatum quickly dissected over ice and stored at 80°C until assay.

Dopamine Assay

Samples were homogenized in 0.4 N perchloric acid, centrifuged at 12,000 × G for 10 min at 4°C, and the CA's separated on strong cation exchange columns (Dowex 50 × 4 resin, 200–400 mesh, 85 mm × 4 mm), according to methods described by Atack and Magnusson [4]. Striata from two animals were pooled per sample in 15-day and 18-day studies. DA was assayed spectrophotofluorimetrically, according to Atack [3].

Analyses

Two-way (lead × age) ANOVA was used to analyze the data for DA levels. One-way ANOVA with post-hoc *t*-tests for lead effects were carried out when a significant main effect of lead or lead × age interaction occurred. In the study evaluating DA utilization, regression coefficients were determined and compared by ANOVA among treated and control groups [22].

RESULTS

DA levels in control and lead-treated animals are presented in Fig. 3. There was a significant lead × age interaction for striatal DA levels, $F(4,31)=2.88$, $p<0.05$. Levels of DA in the 75 mg/kg group were significantly lower than controls at 15 and 35 days, as determined by post-hoc *t*-tests ($p<0.05$ at 15 days and at 35 days). There were no significant differences between groups at 18 days, although DA levels appeared to be higher in the high lead group than in the other two groups.

Figure 4 depicts the disappearance of DA after synthesis inhibition. At 15 days, analysis of regression coefficients revealed no significant differences. However, both lead-treated groups exhibited biphasic decay curves over the 4 hr following synthesis inhibition. Significant non-linear components [22] were found in decay curves of both the 25 mg/kg group, $F(1,10)=29.07$, $p<0.01$, and the 75 mg/kg group, $F(1,8)=9.14$, $p<0.05$, but there was no significant non-linear contribution to the decay curve for controls.

At 18 days, regression analysis revealed a significant effect of lead, $F(4,28)=6.06$, $p<0.01$, and further comparisons of regression coefficients revealed that there was no significant difference between the 25 mg/kg group and controls, but that the 75 mg/kg group differed significantly from both controls, $F(2,21)=12.15$, $p<0.01$, and from the 25 mg/kg group, $F(2,17)=4.67$, $p<0.02$. This difference may reflect the higher baseline values for DA in this group. Again at this age there were significant non-linear contributions to DA decay curves in the 25 mg/kg group, $F(1,6)=10.92$, $p<0.05$ and the 75 mg/kg group, $F(1,9)=10.66$, $p<0.01$, which did not occur in the control group.

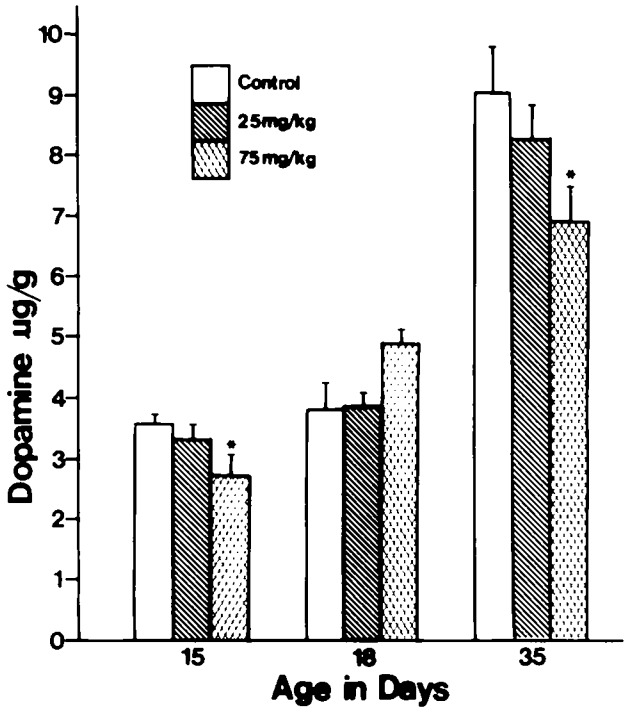

FIG. 3. Development of striatal DA levels in control and lead-treated rats. Each bar is the mean+S.E.M. of 3–6 samples. *Significantly different from controls, $p<0.05$.

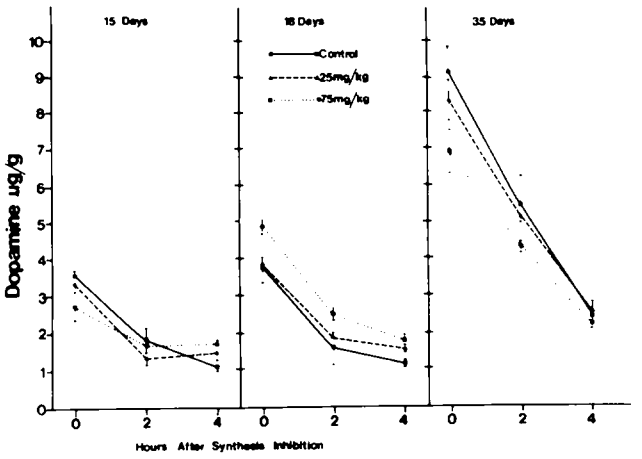

FIG. 4. Disappearance of DA following synthesis inhibition. DA was determined after saline injection (0 point) and at 2 and 4 hrs following administration of α-MT (250 mg/kg). Each point is the mean of 3–6 samples.

Thirty-five-day-old control and lead-treated animals did not differ with respect to regression coefficients nor did significant non-linear components occur in the DA decay curves of any of the three groups. Since at this age, the decay curves for all groups were linear over the time period analyzed, values for turnover rate (μg/g/hr) and turnover rate constant were evaluated [8] and are presented in Table 2. These data demonstrate that although the fractional rate constant does not differ among groups (as previously indicated by the regression analysis), the turnover rate, which equals the product of the rate constant and endogenous levels, is decreased by 35% in the high lead group.

TABLE 2

KINETICS OF DA UTILIZATION (35 DAY-OLD RATS)

Group	DA Level (μg/g±S.E.M.)*	Turnover Rate μg/g/hr	Rate Constant (hr^{-1})±S.E.M.
Control	9.07 ± 0.85	3.03	0.33 ± 0.05
25 mg/kg	8.32 ± 0.66	2.54	0.30 ± 0.03
75 mg/kg	6.94 ± 0.64	1.96	0.28 ± 0.03

The decrease in DA levels was followed over a 4 hr period following the inhibition of tyrosine hydroxylase by α-methyl-p-tyrosine (250 mg/kg). The fractional rate constant was calculated from the slope of the line and the turnover rate equals the product of the rate constant and basal levels of DA.

*DA level represents the concentration of DA measured at time 0, prior to administration of the drug.

EXPERIMENT 3

Experiment 2 demonstrated that in addition to behavioral changes early exposure to lead produced some changes in striatal DA neurons. Further experiments were performed to investigate the effects of lead on the development of mechanisms of DA uptake, storage and release because alterations in any of these could contribute to the observed changes in DA neurons. The pharmacologic effects of *d*-amphetamine are exerted by direct release of DA and by inhibition of reuptake [18,23] and these membrane-related processes may be particularly vulnerable to the effects of lead. Experiment 1 demonstrated that lead does not readily leave the brain, and there is evidence that at the cellular level, lead binds preferentially to membranes [7]. Animals at 15 and 35 days of age were selected for study in this experiment because in the previous experiment, DA levels and/or the disappearance of DA following synthesis inhibition were altered in lead-treated animals at one age but not the other.

Uptake was examined after 5 or 20 min incubation with exogenous transmitter. Previous work has demonstrated that shorter incubation time is more reflective of uptake at the level of the cell membrane and the longer incubation time is more indicative of uptake at the level of the synaptic vesicle [27]. Additionally, the development of potassium-stimulated release was investigated.

METHOD

Procedure

Animals were killed, the brain immediately removed , and striatum dissected over ice as previously described. Uptake and release procedures were carried out accordingly to Ziance and Rutledge [44].

In Vitro *Uptake*

Each sample consisted of both striata from one animal

Fresh tissue was chopped into approximately 0.3 mm square sections using a McIlwain tissue chopper, placed in a flask containing a small volume of cold physiological medium [21], and kept at 4°C until incubation commenced. Immediately before incubation, fresh medium, which had been equilibrated with 95% O_2-5% CO_2 was added to each sample to make a volume of 1.9 ml. The medium for uptake studies contained 3.1×10^{-4} M pargyline and 3.7×10^{-4} M tropolone to inhibit the CA metabolizing enzymes monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT), respectively. Incubation at 37°C was carried out under an atmosphere of 95% O_2-5% CO_2. After 10 min pre-incubation, ^{3}H-DA (1.29 μCi: specific activity of 6.45 Ci/mmol; New England Nuclear) in a volume of 0.1 ml, was added to samples for a final concentration of 10^{-7} M, and incubation was continued for 5 or 20 min. Samples kept at 4°C throughout the experiment provided blank values for each group.

After incubation, flasks were emptied into chilled centrifuge tubes and centrifuged at 4°C for 10 min at 12,100×G. A 0.1 ml aliquot of the supernatant (medium) was placed in a scintillation vial containing 3 ml of absolute ethanol and 10 ml of 0.5% PPO (2,5-diphenyloxazole) in toluene and the remainder of the supernatant discarded. The pellet was washed with 2 ml fresh medium, recentrifuged, and the supernatant discarded. Centrifuge tubes were dried by wiping away excess fluid, the tissue was transferred to preweighed glass scintillation vials, and tissue weight determined. Protein solubilizer (Protosol, New England Nuclear) was added to tissue samples (1 ml/sample) which were kept at room temperature until all tissue was dissolved (approximately 48 hr) after which 10 ml of 0.5% PPO in toluene was added. All samples were dark-adapted for at least 3 hr before radioactivity was counted in a liquid scintillation spectrometer. Separate blanks and standards were used for tissue and medium samples, and for each experiment. Tissue to medium ratios of ^{3}H-DA were calculated as the ratio of μCi/g weight of tissue to μCi/ml in medium for each sample.

In Vitro *Release*

To provide enough tissue for release experiments it was necessary to pool striatal regions from two 15-day-old animals per sample. Uptake of ^{3}H-DA in physiological medium without MAO and COMT inhibitors present was terminated by mild vacuum filtration after 20 min incubation. Each sample was thoroughly rinsed with physiological medium and then transferred to a flask containing 2 ml of either physiological medium or high-potassium medium (50 mM potassium as compared to 5.6 mM potassium in physiological medium, with an isomolar quantity of sodium removed to retain the isotonicity). Flasks were then returned for an additional 20 min incubation, after which release was stopped by pouring contents of each flask into a cold centrifuge tube. Samples were treated as described for the uptake procedure, except that tissue was not rinsed and recentrifuged. Radioactivity was expressed as the percent of total tritium (tissue plus medium) present in the medium.

RESULTS

Uptake

Results are expressed as the tissue:medium ratios obtained without subtraction of values for blanks kept at 4°C throughout incubation because no group differences were found upon comparison of those samples. Tissue:medium values for 4°C uptake at 5 min were 4.26±0.82 at 15 days and 3.39±0.43 at 35 days. These values for 20 min uptake were 6.51±1.42 at 15 days and 4.87±0.37 at 35 days. Data from the two incubation periods were analyzed separately using two-way ANOVA (lead × age).

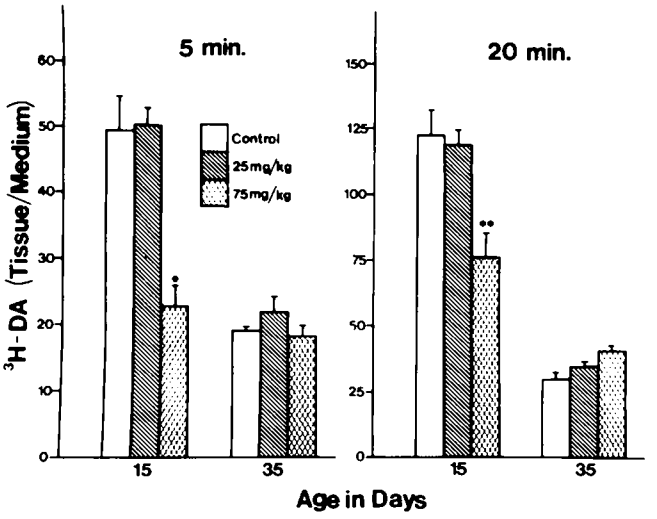

FIG. 5. Development of ^{3}H-DA uptake into striatum of control and lead-treated rats. Tissue minces were incubated in the presence of inhibitors of MAO and COMT. Substrate concentration was 10^{-7} M. Each point represents the mean±S.E.M. of 4–6 samples. *Significantly different from controls ($p<0.01$) and 25 mg/kg ($p<0.01$). **Significantly different from controls ($p<0.01$) and 25 mg/kg ($p<0.05$).

Uptake of ^{3}H-DA into striatum is presented in Fig. 5. Lead significantly altered uptake after both 5 min incubation, $F(2,14)=9.48$, $p<0.01$, and after 20 min incubation, $F(2,13)=4.96$, $p<0.05$ and the lead × age interactions were also significant at 5 min, $F(2,14)=6.61$, $p<0.01$ and at 20 min $F(2,13)=9.96$, $p<0.01$. When ages were separated and one-way ANOVA performed, the lead-related changes occurred at both incubation periods at 15 days, $F(2,8)=12.06$, $p<0.01$ at 5 min and $F(2,7)=8.47$, $p<0.05$ at 20 min, but not at 35 days. Post-hoc t-tests indicated that after both 5 and 20 min incubation, ^{3}H-DA taken up in the 75 mg/kg group at 15 days was significantly less than in controls or in the 25 mg/kg group ($p<0.01$).

Release

Data for release(Table 3) were analyzed using three-way ANOVA for lead × age × medium (physiological vs high potassium). No significant main or interactive effects of lead occurred. Significant main effects for medium, $F(1,47)=229.46$, $p<.001$, indicated greater potassium-stimulated than spontaneous release; the significant effect for age, $F(1,47)=43.55$, $p<0.001$, reflected greater release in both media in the 35-day-old animals in all groups.

GENERAL DISCUSSION

The data collected demonstrated that doses of lead administered in the present study produced no obvious neurotoxicity or gross histologic changes. This observation

TABLE 3

IN VITRO RELEASE

Group	15 Days		35 Days	
	5.6 mM Potassium	50 mM Potassium	5.6 mM Potassium	50 mM Potassium
Control	31.7 ± 1.9*	58.5 ± 3.2	41.6 ± 1.4	65.6 ± 2.1
25 mg/kg	27.3 ± 1.9	56.7 ± 2.8	40.7 ± 2.4	67.8 ± 2.5
75 mg/kg	29.4 ± 2.6	58.9 ± 2.6	41.3 ± 2.3	67.8 ± 0.09

*Values represent the percent of tritium released into either physiologic (5.6 mM potassium) or high potassium (50 mM) medium after 20 min pre-incubation with 10^{-7} M ^{3}H-DA. Each value is the mean ± S.E.M. of 3–4 determinations.

is consistent with earlier reports on exposure to similar levels of lead [19, 31, 42]. However, the present exposure levels and the limited time of exposure still produced observable changes in the offspring. Two classes of lead-related changes in behavioral and neurochemical development were observed. Some of the effects which occurred only in the period immediately following lead exposure (when both brain and blood lead levels were high) appeared to be reversible with time and cessation of exposure. Other effects persisted after blood and brain lead levels had decreased substantially, although brain lead levels were still appreciable.

During the period immediately following cessation of lead treatment differences in locomotor activity were expressed as a prolongation of peak activity levels with a resultant delayed decrease to mature activity levels in lead-treated animals. The characteristic developmental pattern of locomotor activity measured in isolated rats (increase to a peak value followed by an abrupt decrease to adult levels) has been attributed to asynchronous rates of maturation of arousal and arousal-inhibitory systems [9]. Hence, while lead-related differences in activity were not pronounced, the altered development of activity observed may reflect interference by lead with mechanisms responsible for the normal developmental pattern. Altered maturation of activity patterns has also been reported in rats nursed by dams receiving 5% lead carbonate in food and then exposed to 50 ppm lead in drinking water after day 16 [33]. More extreme changes in activity have been observed only in animals exposed to higher doses of lead than those used in this study and/or continued on lead well past weaning [30, 35, 39, 40].

There were no baseline differences in locomotor activity in animals tested at 35 days, when blood and brain levels had decreased by 90% and 55–60%, respectively. Behavioral differences at this age were expressed as attenuated activity responses to *d*-amphetamine. Animals given the lower dose of lead showed the most pronounced differences. This group's lower scores under saline (see Fig. 2) may also reflect differences in habituation. The limitations of the testing apparatus are such that not all types of movement are detected and differences in type of activity generated by *d*-amphetamine in the two exposure groups could account for an apparent effect only in the low-exposure group. Certainly, the attenuated response to *d*-amphetamine noted in the low exposure group is similar to observations made by others [28,42]. However, in previous studies, the duration of time between testing and the cessation of lead exposure was shorter than in the present study; consequently, brain lead levels were probably higher.

Based upon literature reports linking activity changes with nigrostriatal DA neurons (vida supra), the development of these neurons was investigated. Pronounced differences in DA nuerons were observed in lead-exposed animals immediately following cessation of lead treatment, but many of these differences dissipated with the loss of lead from the body.

Differences in DA levels in the striatum were apparent only in animals exposed to the high lead dose. At both 15 and 35 days, DA levels were significantly decreased in this group compared to the other groups. However, at day 18, the DA levels were slightly higher in this group than in the other groups. This increase may reflect compensatory mechanisms within DA neurons (perhaps activated by the withdrawal of lead) attempting to restore normalcy within these neurons. If so, such mechanisms apparently could not sustain the necessary increases which occur with continued development of the striatum and hence, DA levels in this structure are again decreased as animals approach maturity.

In both lead-exposed groups, the disappearance of DA following synthesis inhibition was altered at 15 and 18 days. These groups differed from controls in that the decay curves had a biphasic pattern and statistically were determined to have non-linear components. Such decay curves have previously been reported in animals younger than 15 days of age [25]. A precise explanation for these biphasic curves cannot be made at this time, but whatever the underlying mechanisms they were no longer operative at 35 days of age in the lead-treated animals.

To further explore whether mechanisms underlying DA function may exist within DA neurons in lead-treated animals, the accumulation and release of exogenous DA was evaluated. These studies demonstrated decreased accumulation of ^{3}H-DA in the high-lead group at 15 days but not at 35 days of age. Therefore at one age decreased DA levels correlate with decreased accumulation of exogenous transmitter while at another age they do not. Decreased uptake of DA has previously been reported by Silbergeld in caudate synaptosomes from untreated adult rats when lead was added to the incubation medium [39]. Lead has also been demonstrated to bind preferentially to cell membranes [7]. The reduction in accumulation of ^{3}H-DA by striatal slices from 15-day-old animals may indicate that high brain lead levels at this age interfere with membrane-dependent processes. A lead-related interference in membrane processes

may also have contributed to the biphasic decay curves discussed above.

The results of all the neurochemical studies suggest that at 15 days, decreased DA levels in the high lead group may reflect lead-related interferences with the mechanisms that sustain DA levels. Similar interference may be operative in the low lead group, as DA utilization also differed in this group, although ^{3}H-DA uptake and DA levels appeared normal. However, at 35 days the decreased DA levels noted in the high lead group do not appear to be related to alterations in the mechanisms which maintain levels. At this age the decreased levels could reflect decreased terminal density. This suggestion is supported by the lower turnover rates measured in this group which reflect lower DA levels rather than a difference in the fractional rate constant.

Many of the effects noted immediately following a limited period of exposure to lead during development appear to be due to the presence of high levels of lead in the brain, rather than to any permanent alteration in cellular mechanisms induced by the lead. This observation is important in light of questions concerning the permanency of effects observed in children with a limited exposure to lead. The blood lead levels reached in this study fall within the range of values considered indicative of increased lead burden in children [24].

However, in the present study, lead levels in the brain were still pronounced at 35 days of age. The alterations in responsiveness to *d*-amphetamine (noted in the low lead group) and the decreased DA levels (noted in the high lead group) observed at this age may reflect lasting alterations brought on either by the early lead exposure or by the persisting lead in the brain. The decreased DA levels (but yet normal ability to accumulate exogenous DA) may reflect alterations in the content: capacity ratio of DA neurons [11] and such differences could account for altered responses to drugs. Such altered responses may be a critical long-term effect of early developmental exposure to many chemical substances.

To obtain a more precise correlation between changes in behavior and changes in neurochemistry, a refinement of both behavioral testing and neurochemical measurement is needed. These present studies, however, by limiting the exposure period to lead and measuring brain lead as well as blood lead have demonstrated how slowly lead leaves the brain and have allowed correlation of behavioral and neurochemical effects with brain lead. Such comparisons have not previously been demonstrated.

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