Pharmacology Biochemistry & Behavior, Vol. 16, pp. 631–635, 1982. Printed in the U.S.A.

Effect of Amphetamine and Cocaine on Seizure in Lead Treated Mice[1]

RICHARD G. BURRIGHT, PETER J. DONOVICK, KATHLEEN MICHELS,[2]
RICHARD J. FANELLI[3] AND ZELIG DOLINSKY[4]

Department of Psychology and Center for Neurobehavioral Sciences
State University of New York at Binghamton, Binghamton, NY 13901

Received 2 August 1980

BURRIGHT, R. G., P. J. DONOVICK, K. MICHELS, R. J. FANELLI AND Z. DOLINSKY. *Effect of amphetamine and cocaine on seizure in lead treated mice.* PHARMAC. BIOCHEM. BEHAV. **16**(4) 631–635, 1982.—Mice, genetically selected for differences in brain weight were employed. Lead administration (0.5% lead acetate) from conception increased the proportion of 21 day old mice exhibiting seizures; total duration of observed seizures was also increased. Mice from the low brain weight line more frequently exhibited seizures than either mice from the high brain weight line or the Binghamton heterogeneous stock. Although genome and lead administration alter bodyweight, the inability of bodyweight to predict seizure occurrence and/or total duration of seizure within conditions also was noted. Lead administration from conception through testing increased the probability and duration of transcorneally induced electroconvulsive seizures of 21 day old mice within all three genotypes, and both cocaine and amphetamine injections 15 min prior to ECS reduced the number of animals exhibiting seizures as well as the duration of seizures in both lead treated and control mice.

Lead acetate Seizures Amphetamine Cocaine Brain weight Mouse genotype

THE toxic effects of lead have been known since the time of the ancient Greeks. Early reports were indicative of its adverse influences on the nervous system and lead is now known to alter: brain growth [9], EEG activity [1], patterns of sleep [10], neurotransmitter levels and turnover rates [8,9], and seizures induced by chemical convulsant agents [14] or electroconvulsive shock (ECS) [5]. Hawkins (cited in [14]) also noted that lead exposure increased the priming effect in rats for audiogenic seizures. Silbergeld *et al.* [14] suggested that lead-induced changes in the GABA system may be the critical link producing changes in chemically-induced seizures. However, mechanisms underlying seizures induced in various ways may well be different (e.g., [3, 6, 13]). For instance, among 21 day old mice of the Fuller Brain Weight Lines, we noted [3] that low brain weight mice were more susceptible to ECS induced seizures and showed more severe seizures than medium and high brain weight mice. In contrast, Fuller reported [6] that the high- and mid-brain weight lines were more prone to display audiogenic seizures than low brain weight mice following priming.

Silbergeld and Goldberg [13] suggest that differential responsivity to lead may be related to early and relatively rapid developmental changes in the organism (cf. [8]). An examination of the role which lead may play in influencing the development and regulation of mechanisms may provide a better understanding of individual differences in response to the toxic effects of lead. Thus, we used the Fuller Brain Weight Lines (BW) of mice which display both differential rates of brain development and terminal brain weights (e.g., [6]), and the Binghamton Heterogeneous Stock (HET) from which those lines were derived, to examine the effects of early administration of lead on transcorneal, ECS-induced seizures in 21 day old pups. In light of the interactive effects of cocaine on transcorneally induced seizures [3,15] and the potential for changes in both peripheral and central catecholamine systems with lead administration [8,9], we also examined the effects of a single administration of cocaine or amphetamine on the number of animals which exhibited seizures and the duration of ECS-induced seizures.

METHOD

Subjects

Male and female offspring of the Fuller high (HBW) and low (LBW) Brain Weight Lines and the Binghamton Heterogenous stock (HET) were used [7]. The brain weight lines were developed from the systematically outbred HET stock. All mice in this study were derived from matings in our laboratory. Mating pairs were established and subsequently maintained in transparent plastic cages with ad lib access to Charles River Mouse Chow and either 5 g of lead acetate/liter of deionized water (0.5%) or deionized water only. The lead solutions were mixed in deionized water and prepared twice weekly to minimize lead precipitation. All

[1]This research was supported in part by funds from NSF (DAR 7911233). Requests for reprints should be sent to Peter J. Donovick, Department of Psychology, SUNY-Binghamton, NY 13901.

[2]Currently at Department of Neurobiology, University of North Carolina, Chapel Hill, NC 27514.
[3]Currently at the Veterans Administration Medical Center, Duke University, Durham, NC 27705.
[4]Currently at Department of Psychiatry, University of Connecticut Health Center, Farmington, CT 06032.

offspring tested at 21 days of age were produced by 10–15 mating pairs in each of the six genotype × fluid conditions. Both parents were continuously maintained in the same cage with the same fluid source throughout the course of the experiment. Lead was thus administered from the time of mating through the time of testing the 21 day old offspring. This regimen has resulted in blood-lead levels in offspring of about 100 μg% in this laboratory (e.g., [2,17].

At birth, litters were culled to a maximum of eight pups with four males and four females wherever possible. The vivarium in which the mice were housed was maintained at 22°±1°C with white lights on between 8:00 a.m. and 8:00 p.m.; red lights were on between 8:00 p.m. and 8:00 a.m. When the pups were 21 (occasionally 22) days of age they were taken from their home cages, weighed and placed individually in opaque, wire-grid covered, mouse cages with wood shavings on the floor. Mice from each litter were distributed among the three drug groups (20 mg/kg cocaine, 5 mg/kg dextroamphetamine, or equal volume 0.9% saline) with approximately equal numbers of males and females in each. We employed only a single "intermediate" dose of each of these drugs, thus direct interpretative dose-response comparisons between cocaine and amphetamine groups is not warranted.

Two partial replications were conducted (November 1979 and April 1980), resulting in a total of 611 mice (ca. 100 mice in each genotype by fluid history condition) used in this experiment. Following procedures previously developed [3], each mouse received a single dose of the appropriate drug between 10:00 a.m. and 12:00 noon (EST). The experimenter was "blind" regarding the drug being injected. Fifteen minutes after intraperitoneal drug administration, pups were subjected to transcorneal ECS administered by a Lafayette apparatus (Model 86175B). The electrodes were lightly coated with Hewlett Packard Redux Creme and 750 volts were passed through the mouse and a 180,000 ohm resistor for 0.5 sec, resulting in a shock current of approximately 4 mA [3,15]. The mouse was then placed in a transparent cage and duration and characteristics of any subsequent seizure were recorded. Seizures were recorded as clonic, tonic, clonic + tonic, or leading to death; we also noted occasional wild-running especially in amphetamine treated groups. Under the conditions employed, the vast majority of seizures observed were clonic only, and they occurred almost immediately so that no latency measures were obtained (see [3], and Results below). All animals were sacrificed after being tested.

RESULTS

Neither replication nor sex effects were apparent and the data were pooled across these factors for purposes of statistical analysis (but see [3]). Overall, 79% (246/313) of the males and 81% (242/298) of the females seized. Thus, of the 611, 21-day-old mice tested, 80% (488) showed signs of seizure, and only 9% of the observed seizures (46/488) displayed tonus. As previously reported [3], HBW mice were least apt to display seizure (122/197, 62% seized), LBW mice were most prone to these ECS-induced seizures (235/242, 97% seized), and HET mice were intermediate in this regard (131/172, 76% seized). Lead increased the proportion of mice that seized, and injections of CNS excitant drugs 15 min. prior to ECS exposure attenuated the seizures.

A total of 30/611 (5%) of the weanling-age mice died following ECS—all 30 displayed seizures with a tonic component, and for purposes of our analysis they were assigned duration of 125 sec. When clonic (or tonic + clonic) seizures occurred without resultant death, total seizure durations ranged from 2 sec to 122 sec. No tonic-only seizures were seen in these mice, and although the tonic portion of the relatively few tonic + clonic seizures may have been quite short, the total duration of seizures which included a tonic component was quite long (see below).

For purposes of quantitative data analysis we chose total seizure duration on the following grounds: a sizeable number of animals did not seize (0 duration); by far the most common type of seizure observed in these weanling mice under our conditions were clonic-only seizures which were distributed quite continuously in time; the relatively few clonic + tonic seizures which did not result in death had long total seizure durations, despite the fact that the tonic component itself may have been rather short; and finally, the few mice that died, all of which displayed a tonic component in their seizures, were arbitrarily assigned a "maximal" total seizure duration of >125 sec. As a result of these considerations, five, equal-size seizure category groups ([0] – [4]) containing ca. 20% (about 122 mice) of all 611 mice observed were established on the basis of total seizure duration: 0 sec [0]; 1–12 sec [1]; 13–19 sec [2]; 20–29 sec [3]; ≥30 sec [4]. All 46 of the seizures that displayed tonus were categorized as category [4] seizures using this criterion. The overall (N=611) median total seizure duration (including no seizure animals) was 16 sec; of the 488 animals that did seize, median total duration was 19 sec.

We then proceeded with a "non-parametric," partioned χ^2 analysis (cf. [16]) of these categorized data. Obviously, the obtained distribution of total seizure durations could be categorized in other ways, but would not result in a substantially different description of our observed data. The 3 genomes (G: HBW, HWT, LBW) by 3 injections (D): saline, 5 mg/kg d-amphetamine, 20 mg/kg cocaine) by 2 fluid exposure histories (H: water, 0.5% lead acetate) by 5 seizure categories (Sz) resulted in an 18 × 5 (90 cell) contingency table into which observed frequencies of occurrence (number of animals) were cast. Based on the marginal totals in this 18 × 5 table, expected cell frequencies ranged from approximately 3.5–10.9, with 78% of the cells having expected frequencies > 5. The resulting χ^2 (68 *df*) was subsequently partitioned [16] into 7 components reflecting all possible "interactions" of the 3 experimental variables with the seizure category variable.

The percent (relative frequency) of mice in each of the 90 cells are shown in the 9 panels of Fig. 1. If neither genome, history, nor drug had any influence on seizure category, all bars in each of the 9 panels of Fig. 1 would be around a value of 20%. The open bars denote the mice from litters exposed only to water and the shaded bars indicate mice from litters exposed to the 0.5% lead acetate solution. In general, distributions skewed to the left indicate relatively many animals in the no or low seizure categories, whereas those skewed to the right represent relatively many animals in the higher seizure categories.

Exposure to lead (primarily via the mother) from conception to age at testing (21 days) resulted in an increased proportion of mice which exhibited seizures and increased the total duration of such seizures induced by transcorneal ECS (H × Sz: $\chi^2(4)=43.07$, $p<0.01$). (Of the 46 seizures which displayed tonus, 34 (78%) occurred in lead-treated mice.) Furthermore, both proportion and duration of seizures were reduced by injections of either amphetamine or cocaine 15

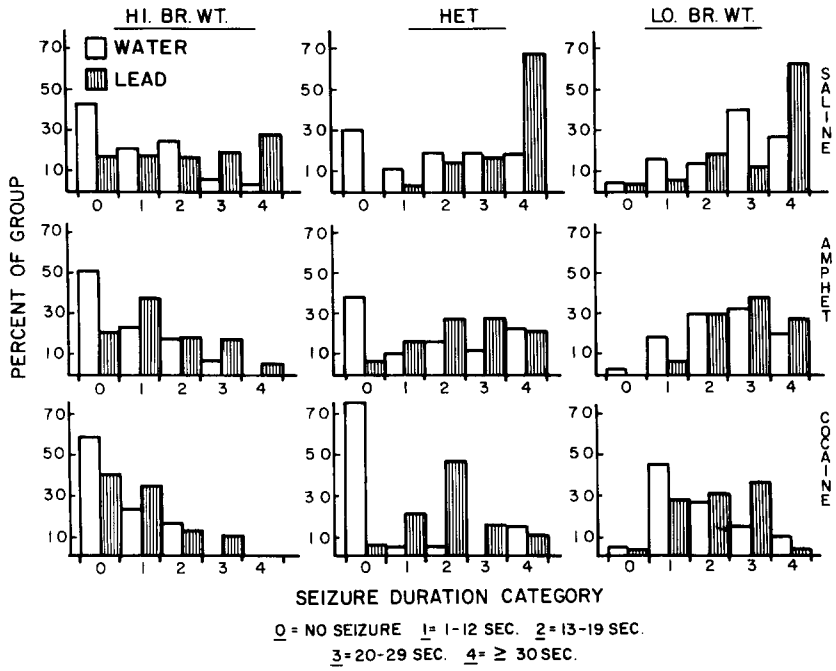

FIG. 1. The percent of mice from the 18 groups (3 Genomes by 3 Drug injections by 2 fluid exposure Histories) that fell into each of the five seizure duration categories. (Note that all 46 mice that died and/or displayed a tonic component in their seizures were included in seizure category [4].)

min prior to ECS (D × Sz: $\chi^2(8)=82.61, p<0.01$); 80% of the 46 seizures involving tonicity occurred in saline injected mice. The differential response to ECS of the 3 genomes employed also is apparent in Fig. 1; LBW mice are highly susceptible to seizures, HET stock less so, and HBWs least (G × Sz: $\chi^2(8)=133.55, p<0.01$).

The general statements above broadly represent the data, and the three χ^2 components reported above account for approximately 69% of the overall χ^2 ($\chi^2(68)=377.7, p<0.01$); however, the G × H × Sz and H × D × Sz components were both significant ($p<0.01$) and the G × H × D × Sz component approached significance ($p<0.06$). Together, these three complex components accounted for approximately 28% of the total χ^2. Only the G × D × Sz component did not approach significance. Indeed, Fig. 1 reveals that the characteristics of the water history and saline injection distributions related to each of the genomes (white bars, top three panels, Fig. 1) are differentially altered by lead history (dark bars) and drug injection (other panels) within each genotype; however, the changes are more in "degree" than direction, thus suggesting that while lead has a dramatic impact on the mouse, the degree of this change is effected by genotype.

Issues related to the influence of genome and history variables on bodyweight also need to be addressed (e.g., [11]). Indeed, an unweighted-means analysis of variance in the context of a Genome × History factorial design indicates the statistically significant impact of these variables on between group variability in body weight of these weanling age mice. Overall, body weight of these 21-day-old mice ranged from 3.9–17.8 g. As reported in various sources [3], 21-day-old HBW and HET animals typically weigh more (ca. 11.9–12.5 g) on average than LBW mice (ca. 9.6 g), (G: $F(2,605)=74.6$, $p<0.001$). Furthermore, not unexpectedly, the 0.5% lead acetate regimen clearly altered mean body weight (H: $F(1,605)=353.1, p<0.001$). The HBW and HET lead-history animals were, on the average, about 3 g lighter than their control (water history) counterparts, whereas the smaller, LBW lead-treated weanlings were less than 2 g lighter than their water-reared counterparts (G × H: $F(2,605)=10.2$, $p<0.001$).

These statistically significant genome and lead-history influences on group-mean body weights suggest that seizure probability and total duration are a simple function of bodyweight; i.e., they increase as body weight decreases. However, the range of individual body weights within groups is quite substantial and results in marked overlap among groups. Indeed, body weight does not predict seizure duration/category within genotype by history groups (see Fig. 2). Thus, the transcorneal ECS-induced seizure patterns observed in Fig. 1 cannot be simply explained on the basis of body weight alone. To help clarify this point, Fig. 2 provides scatter-plots of seizure duration vs bodyweight for the 246 saline-injected animals in each of the six, genome × fluid history conditions. It is also of interest to note that 37 of the 46 seizures which displayed tonus are represented in Fig. 2 (●), and that 29 of those 37 seizures occurred in animals with a prior history of lead acetate as their sole fluid source from conception to 21 days of age. The complexity of questions

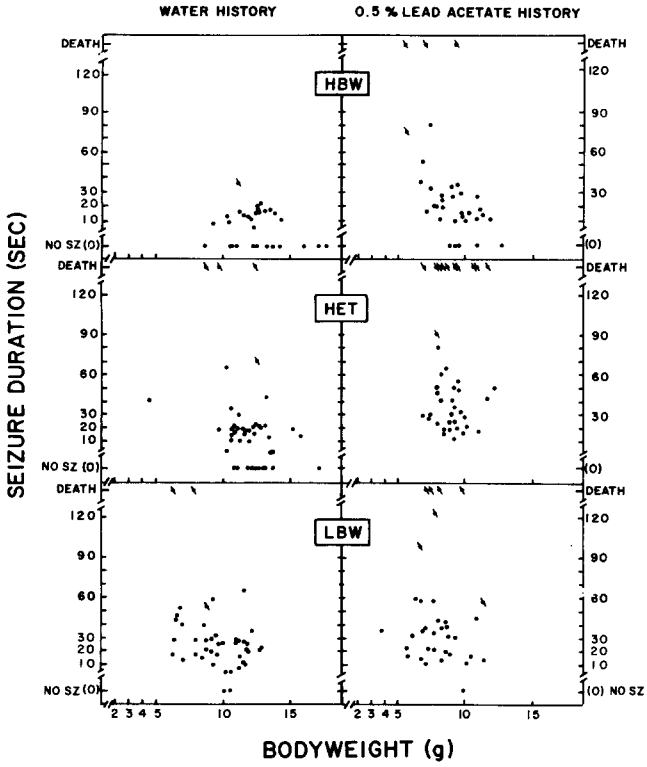

FIG. 2. Scatter-plots of seizure duration (including no (0) seizures and seizures which resulted in death (4)) vs bodyweight for all 246 mice which received saline injections 15 min prior to transcorneal, ECS exposure. The six separate graphs represent the 3 Genomes (HBW, HET, LBW) by 2 fluid Histories (water or lead). Those data points with diagonal lines through them (▀) indicate seizures which displayed a tonic component (see text).

regarding lead toxicity or malnutrition also have been illustrated in activity scores and responses to amphetamine in 35-day-old, CD-1 mice [11].

DISCUSSION

Susceptibility to environmental insult necessarily has multifaceted determinants which include the rate of development and age of the organism during the time of such insult. Our choice of the Fuller Brain Weight Lines of mice for this research reflects the potential for genotypic-related developmental differences in susceptibility to the toxic effects of low-level lead exposure as suggested by changes in seizure susceptibility. In support of our earlier findings [3], 21 day old mice from the LBW line were more sensitive to transcorneally induced seizures from HBW or HET mice. In the current study we found that lead enhanced sensitivity to ECS, but this was influenced somewhat by genetic background. Thus, the interactions found between exposure to low-lead toxicity and genotype were expected, but are not easily explained. Nonetheless, lead did result in enhanced seizure activity in all lines, and the excitant drugs attenuated reactions to ECS.

Further, the translation of lead toxicity to seizure susceptibility also cannot be simply understood in terms of clearly illustrated changes in neurochemical systems. Hrdina, Hanin and Dubas [9] reviewed the effects of lead administration on several neurotransmitter systems including catecholamines, indolamines and the cholinergic system. In general, studies have yielded conflicting results; at the present time we do not have a coherent picture of how lead may alter functioning in those neurotransmitter systems. Hrdina *et al.* [9] suggest that factors such as lead exposure levels, duration of exposure, time of performing biochemical or behavioral tests, lack of information on actual brain content of lead achieved by various routes and levels of exposure, as well as nutritional variables may contribute to the varied results.

In the context of the present study, 0.5% lead acetate drinking solution, administered throughout gestation to 21 days of age has been shown to enhance seizure susceptibility in select strains of mice. Further, this effect of lead was attenuated by IP injections of amphetamine or cocaine 15′ prior to transcorneally applied ECS. A simple explanation of these effects based on chemical factors is difficult to offer for several reasons. First, the apparently most relevant transmitters (NE and DA) have been reported either to increase, or to decrease, or not to change following lead administration [9]. One relevant dimension is the apparent differential sensitivity of the developing vs adult organism to lead [8]. Further, it is reasonable to assume that lead may have interactive influences on several neurotransmitters operating within a dynamic system. As might be expected, we have even less insight into how lead may alter the interrelationships among neurotransmitter systems, and there is little information of the effects of other purported neurotransmitter/neuromodulatory systems such as the enkephalins, prostaglandins, histamine. Finally, while the possibility is often acknowledged, few researchers emphasize the fact that pharmacological agents such as amphetamine and cocaine may alter functioning in systems besides those which have been accepted traditionally.

Thus, it is possible that lead may be acting to increase seizure susceptibility by altering levels of acethycholine or the catecholamines [9]; or by decreasing levels of GABA [13]. Alternatively, the interrelationship between these, as well as other neurotransmitter/neuromodulatory systems, may be modified by lead exposure. Amphetamine may attenuate lead's effects by acting directly on the catecholamines, or by increasing levels of GABA [12]. Likewise cocaine may well be acting on other systems besides the catecholamines.

In summary, lead was seen to interact with genotype in altering sensitivity to transcorneal, ECS-induced seizures. The importance of better understanding the role of genotype in sensitivity to environmental toxins is obvious. Too frequently we ignore the genetic and environmental history of the organism in describing/explaining our data. Better understanding of issues of environmental toxicity demands our attention to these factors even though often more questions are raised than answered.

REFERENCES

1. Cooper, G. S. and C. D. Sigwart. Neurophysiological effects of lead. In: *Lead Toxicity*, edited by E. L. Singhal and J. A. Thomas. Baltimore, MD: Urban & Schwarzenberg, 1980.

2. Dolinsky, Z., E. Fink, R. Burright and P. Donovick. The effects of lead, d-amphetamine, and time of day or activity levels in the mouse. *Pharmac. Biochem. Behav.* **14**: 877–880, 1981.

3. Donovick, P. J., R. G. Burright, R. J. Fanelli, B. Symchowicz and A. N. Ritz. Cocaine and seizure protection in mice of varying brain weights. *Pharmac. Biochem. Behav.* **14:** 409–414, 1981.
4. Dudek, B. C. and P. J. Berman. Biochemical correlates of selective breeding for brain size. In: *Development and Evolution of Brain Size,* edited by M. E. Hahn, C. Jensen and B. C. Dudek. New York: Academic Press, 1979.
5. Fox, D. A., S. R. Overmann and D. E. Woolley. Neurobehavioral ontogeny of neonatally lead-exposed rats II. Maximal electroshock seizures in developing and adult rats. *Neurotoxicology* **1:** 149–170, 1979.
6. Fuller, J. L. Fuller BWS lines: History and results. In: *Development and Evolution of Brain Size,* edited by M. E. Hahn, C. Jensen and B. C. Dudek. New York: Academic Press, 1979.
7. Fuller, J. L. and B. Herman. Effect of genotype and practice upon behavioral development in mice. *Devl Psychobiol.* **7:** 21–30, 1974.
8. Goldman, D., J. R. Hejtmancik, Jr., B. J. Williams and M. G. Ziegler. Altered noradrenergic systems in lead-exposed neonatal rat. *Neurobehav. Toxicol.* **2:** 337–343, 1980.
9. Hrdina, P. D., I. Hanin and T. C. Dubas. Neurochemical correlates of lead toxicity. In: *Lead Toxicity,* edited by E. L. Singhal and J. A. Thomas. Baltimore, MD: Urban & Schwarzenberg, 1980.
10. Jason, K. M. and C. K. Kellogg. Behavioral neurotoxicity of lead. In: *Lead Toxicity,* edited by E. L. Singhal and J. A. Thomas. Baltimore, MD: Urban & Schwarzenberg, 1980.
11. Loch, R. K., L. S. Rafales, I. A. Michaelson and R. L. Bornschin. Role of undernutrition in animal models of hyperactivity. *Life Sci.* **22:** 1963–1970, 1978.
12. Lynch, M. A. and B. E. Leonard. Changes in brain-aminobutyric acid concentrations following acute and chronic amphetamine administration and during post amphetamine depression. *Biochem. Pharmac.* **27:** 1853–1855, 1978.
13. Silbergeld, S. S. and A. M. Goldberg. Problems in experimental studies of lead poisoning. In: *Lead Toxicity,* edited by E. L. Singhal and J. A. Thomas. Baltimore, MD: Urban & Schwarzenberg, 1980.
14. Silbergeld, E. K., L. P. Miller, S. Kennedy and N. Eng. Lead, GABA, and seizures: Effects of subencephalopathic lead exposure on seizure sensitivity and GABAergic function. *Envir. Res.* **19:** 371–382, 1979.
15. Swinyard, E. A. Electrically induced convulsions. In: *Experimental Models of Epilepsy,* edited by D. P. Purpura, J. K. Penry, D. B. Tower, D. M. Woodbury and R. D. Walter. New York: Raven Press, 1972.
16. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1962.
17. Zimering, R. T., R. G. Burright and P. J. Donovick. Effects of pre-natal and continued lead exposure on activity levels in the mouse. *Neurobehav. Toxicol. Teratol.* **4:** 9–14, 1982.