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Effects of Chronic Lead Exposure on Cocaine-induced Disturbance of Fixed-interval Behavior

ROBERT T. BURKEY,* JACK R. NATION,*
CATHY A. GROVER,† AND GERALD R. BRATTON‡

Department of Psychology, Texas A&M University, College Station, TX 77843 , †Department of Medical Pharmacology and Toxicology, Texas A&M University, College Station, TX 77843 and ‡Department of Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX 77843

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BURKEY, R. T., J. R. NATION, C. A. GROVER AND G. R. BRATTON. *Effects of chronic lead exposure on cocaine-induced disturbance of fixed-interval behavior.* PHARMACOL BIOCHEM BEHAV **56**(1) 117–121, 1997.—Chronic lead exposure has been shown to attenuate cocaine-induced increases in extracellular dopamine levels in the region of the nucleus accumbens, and antagonize the locomotor stimulating effects of the drug. The purpose of this study was to determine if similar lead-induced disturbances in the effects of cocaine include the impact of the drug on schedule-controlled responding. Adult male rats exposed ad libitum to water containing 500 ppm lead acetate (Group Lead), or a comparable concentration of sodium acetate (Group Control), were placed on a restricted diet (12–15 g food/day) prior to commencing fixed-interval (FI-5 min) schedule training on Day 33 of exposure. After 27 days of operant training, animals received a sequence of no injection, saline injection, and cocaine injection tests, repeating the sequence for 3, 10, 20 and 40 mg/kg cocaine HCl (ip). Local rates were determined for successive 30 s segments of the interval and the pattern of responding was compared under conditions of saline and cocaine injection. For both groups, cocaine increased responding, especially early in the interval. However, the rate enhancing effects of cocaine were less pronounced in lead-exposed animals than controls, at least at the 20 mg/kg dose. These data extend earlier findings and accent the need to examine further the interactive relations between the external chemical environment and drug sensitivity.

Behavior Cocaine Lead Fixed-interval schedules

LEAD is an environmentally ubiquitous contaminant that places many children and adults at risk. The manifold nature of exposure routes, coupled with compelling evidence of neurotoxicity from low-level lead exposure, has increased concerns over the number of people who may suffer lead-related pathology. Employing the Centers for Disease Control (CDC) revised definition of elevated blood lead as $\geqslant 10$ μg/dl (down from 25 μg/dl), it is estimated that 1 in 6 children in the United States have unsafe blood lead levels (3). Although less vulnerable than children (19), adults also are deleteriously affected by low-level lead exposure (7).

With the awareness that lead toxicity threatens a greater segment of society than previously believed, investigators in this area have begun to re-examine the range of influence of environmental lead. In recent years, the possibility that lead poisoning may alter the behavioral and neurochemical profile of drugs of abuse has been a topic of interest. In this regard, the effects of toxicant exposure on cocaine-related changes in behavior has been examined. Preliminary behavioral investigations have shown that recurrent lead (8) or cadmium (17) exposure attenuates the psychostimulatory effects of an ip administration of 10 mg/kg cocaine HCl. Coupled with the finding of reduced cocaine-induced elevation of extracellular dopamine in the nucleus accumbens of lead-exposed rats (16), these data suggests a pattern of diminished responsiveness to cocaine among metal-exposed animals.

Because previous investigations have focused on simple behavioral systems (locomotor activity), or isolated neurochemical changes, not much is known about the interactive relation between metals and cocaine in operant preparations that involve more complex, integrated response systems. Accordingly, this study examined the effects of chronic lead exposure on cocaine-related disturbance of schedule-controlled responding. Specifically, the effects of lead contamination on

fixed-interval (FI) behavior following ip injections of 0, 3, 10, 20, or 40 mg/kg cocaine HCl were determined. Because FI schedules commonly produce low rates of responding early in the interval and high rates of responding late in the interval, such schedules are ideally suited for investigating the effects of cocaine on operant phenomena expressing a relatively high level of behavioral complexity (9,13,22).

METHOD

Subjects

The animals used in this study were 17 viral free adult male Sprague–Dawley rats (Holtzman Company, Madison, WI) approximately 50 days old at the beginning of the experiment. Initial animal weights ranged between 180 and 200 g. Eight of the animals (Group Control) were exposed ad libitum to a distilled water solution containing 500 ppm sodium acetate trihydrate [FW 136.1] (Sigma Chemical Co., St. Louis, MO). The remaining 9 animals (Group Lead) were presented ad libitum with distilled water containing 500 ppm lead acetate trihydrate [FW 379.3] (Sigma Chemical Co., St. Louis, MO). For both conditions, animals were placed on a restricted diet of 12 to 15 g/day of Teklad 4% Mouse/Rat Diet 7001 (Harlan Sprague–Dawley, Inc., Madison, WI). This feeding schedule has been shown to provide adequate nutrition, while maintaining stable body weights (cf. 1).

Cocaine

The cocaine HCl used in this study was provided gratis by the Research Technology Branch of the National Institute on Drug Abuse (NIDA).

Apparatus

Eight identical small animal test chambers (Coulbourn E10-10) served as the apparatus for operant lever press training (details of the apparatus may be found in [18]).

Procedure

Throughout the experiment, animals were maintained on a 12 h/12 h dark/light cycle. Weekly body weight as well as the amount of fluid (sodium acetate or lead acetate) consumed was determined for each animal over the course of exposure and training.

Animals were exposed to their respective control or lead solutions for 33 days prior to commencing training. With the exposure regimen continuing throughout the study, and the restricted diet in place, lever responding for a 45 mg food pellet was shaped on a continuous reinforcement schedule. Once an animal reached a criterion of 50 responses within a 30 min session, they were placed on a fixed-interval 30 s (FI-30) schedule of reinforcement for one day, an FI-2 min schedule the next day, and then were trained under an FI-5 min schedule for the remainder of the experiment. Relatively uniform response rates were evident for control and lead-exposed animals across 27 sessions (1 h/day) of training, prior to beginning the cocaine test sequence. At the end of the 27th session, clear and reliable temporal discrimination patterns had emerged wherein animals were making relatively few responses early in the interval, while exhibiting increased responding late in the interval.

The cocaine test procedures employed in this study were based on a test methodology used previously (13). Starting on Day 28 of training (Day 65 of exposure), a 3 day injection sequence began. Prior to being tested under the FI-5 min schedule for 1 hr, animals received either no injection, a saline injection, or an injection of cocaine. Maintaining the integrity of this sequence, cocaine doses of 3, 10, 20, or 40 mg/kg were dissolved in saline and administered ip in a volume of .1 ml/100 g body weight. An equivalent volume of the same saline solution without cocaine added served as the vehicle-only condition. All injections were administered 10 min prior to placement in the operant test chamber. So, in this procedure each cocaine challenge was preceded the previous day by an FI-5 min test session where saline was administered before the session began. This permitted local rates of responding under cocaine conditions to be compared with rates emitted the previous day after saline injections.

Cocaine doses used here were selected on the basis of levels previously used in this laboratory (8,16,17), as well as in previous investigations of cocaine-induced changes in FI behavior (9,13). Lever responding was recorded for control and lead-exposed animals in successive tenths (30 s segments) of the 5 min (300 sec) interval. Local rates were calculated for each segment under saline and cocaine treatment conditions. For each animal, responding under the saline test conducted the day prior to a given cocaine test was used in determining the effects of cocaine on local rates of responding within the FI (see [13]).

Chemical Analysis

Twenty-four hours after the final day of testing, animals were rendered unconscious in a bell jar with CO_2 and then were decapitated. After each animal had been sacrificed, trunk blood was collected. The concentration of lead in blood was accomplished via dry ashing and atomic absorption spectrophotometry as described elsewhere (15).

RESULTS

Fluid Intakes and Animal Body Weights

Fluid intake decreased for both groups over the period of exposure. A 2 Groups X 11 Weeks repeated measures analysis of variance (ANOVA) test performed on these data revealed a significant interaction effect ($F(10, 160) = 2.71$, $p < .01$). Individual comparisons of means (Tukey's HSD procedure) indicated that although control animals consumed more fluid early in the exposure period ($ps < .05$), over the last 6 weeks group separation was not evident ($ps > .05$); mean weekly intake range was 173–212 ml/wk/group. The mean lead exposure for Group Lead animals over the course of the experiment was 205.27 ($\pm$5.69) mg/kg/wk.

An identical analysis performed on the body weight measure also failed to find any reliable evidence of group differences ($Fs < 1$). The restricted food intake schedule was equally effective for both Group Control and Group Lead animals with respect to maintaining body weight at around 290 g throughout the study.

Behavioral Data

Local rates. The mean response rates (responses/sec) for each group at each saline test session (left) and each cocaine dose (right) are shown in Fig. 1. Separate Groups (Control, Lead) X Type of Injection (Saline, Cocaine) X Segments [bins] (1-10) ANOVAs were performed on the data at each dose.

At the 3 mg/kg dose, neither the groups main effect ($F(1, 15) = .04$, $p > .05$) nor the group interaction with type of

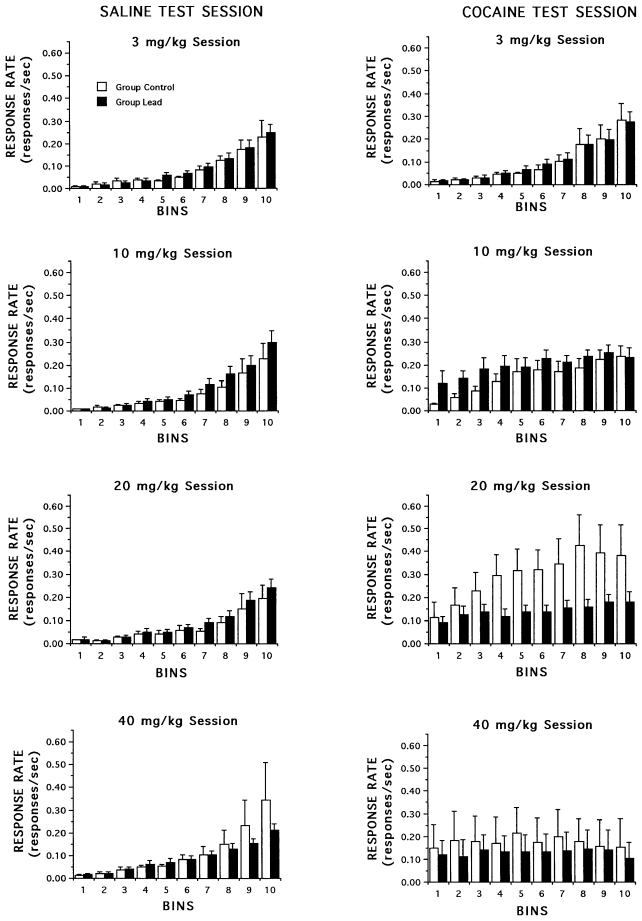

FIG. 1. Effects of 3, 10, 20, and 40 mg/kg cocaine on fixed-interval (FI-5 min) responding for each group (Group Control, $N = 8$; Group Lead, $N = 9$), expressed as group means (bars) and SEM (error lines). Saline responding prior to the appropriate cocaine dose is shown on the left and cocaine responding is shown on the right across successive 30 s segments of the interval (bins).

injection ($F(1, 15) = .08, p > .05$) reached an acceptable level for statistical significance. Consistent with the visual profile of the data depicted in Fig. 1, at this lowest dose it appears that cocaine had minimal effects on lever responding for food at any segment of the FI ($F(9, 135) = .30, p > .05$).

The analysis of the lever press results at 10 mg/kg again failed to reveal any evidence of group separation in terms of the main effect test ($F(1, 15) = 1.63, p > .05$), or any of the interaction tests (Fs < 1.3). However, at this dose of cocaine, it was shown that under conditions of the cocaine challenge response rates were elevated relative to saline rates ($F(1, 15) = 13.85, p < .05$), and these effects were most prominent in early segments of the interval ($F(9, 135) = 4.22, p < .001$). Thus, cocaine-induced changes in schedule-controlled responding emerged at a dose of 10 mg/kg, but there was no indication of lead-related disturbances at this dose.

The most dramatic differences in group responding in this study were evident at the 20 mg/kg dose. Although the groups did not differ with respect to overall response rate ($F(1, 15) = 2.65, p > .05$), the analysis performed on the data presented in Fig. 1 did yield a main effect for type of injection ($F(1, 15) = 10.90, p < .01$), and more importantly a marginal groups X type of injection interaction effect ($F(1, 15) = 4.34, p = .054$). Individual comparison of means indicated that cocaine-induced enhancement of lever press responding was evident for both control and lead-treated animals, and that this effect was more apparent among controls. Further statistical documentation of this effect was provided by the report of a significant groups X type of injection X segments interaction ($F(9, 135) = 3.35, p < .001$). Employing Tukey's HSD post hoc procedure and controlling comparisons family-wise, it was shown that cocaine-induced increases in response rate were greater for controls than lead-exposed animals for all segments other than the two initial bin periods. With respect to group comparisons of saline responding, only the final bin (segment) showed any evidence of group differences, with lead-exposed animals responding at a higher rate than controls.

Finally, regarding the analysis of the data at a dose of 40 mg/kg, the only test to reach an acceptable level for statistical significance was type of injection X segments interaction test ($F(9, 135) = 7.59, p < 001$). This result was due to increased responding by both groups in the early segments of the interval. The group main effect and interactions with group were found to be nonsignificant (all Fs < 1). Although there was some indication that controls responded at higher rates than lead-exposed animals late in the interval (see Fig. 1), the high within group variability yielded nonsignificant findings.

The local response data were analyzed further for general reliability of responding across saline test sessions. The results of the Groups X Doses X Segments ANOVA performed on the saline data failed to reveal any evidence of significant changes in responding across saline test sessions, at least this was the case collapsed across groups ($F(3, 45) = 2.14, p > .05$). Nor, for the combined group data, was there any indication that overall performance patterns throughout the interval shifted over saline test sessions ($F(27, 405) = .79, p > .05$). However, the 3-way interaction involving groups did reach an acceptable level for statistical significance ($F(27, 405) = 2.50, p < .05$). Subsequent comparisons controlled family-wise (Tukey's HSD) indicated that this difference was due to higher rates of responding in control animals during bins (segments) 9 and 10 at the 40 mg/kg test session relative to all other saline test sessions. The saline response patterns for Group Lead animals did not change significantly across test sessions. Perhaps a conditional (contextual) component associated with the motor stimulating properties of cocaine played a role in altering saline responding during the session prior to the 40 mg/kg test (cf. [23]), and such associative processes were compromised by lead exposure. In any event, in terms of the omnibus statistical analysis, it appears that repeated experience with cocaine produced only modest changes in local response rates under conditions of saline.

Regression plots. To further determine the differential effects of cocaine dose on local rates of responding within the FI, cocaine-induced rates (responses/sec) for each segment of the interval were divided by the comparable saline rates. Figure 2 plots these values for each animal as a function of their saline rates of responding, using a log scale. Least squares linear regression was performed on the log of these data, and the slope and coefficient of determination (r-square) were determined for Group Control and Group Lead animals at each cocaine dose.

There are two points to be made regarding the presentation of the data in Fig. 2. First, inasmuch as more negative regression slopes reflect relatively greater cocaine-induced enhancement of lower as contrasted with higher response rates, it is evident that the impact of cocaine increased for both groups

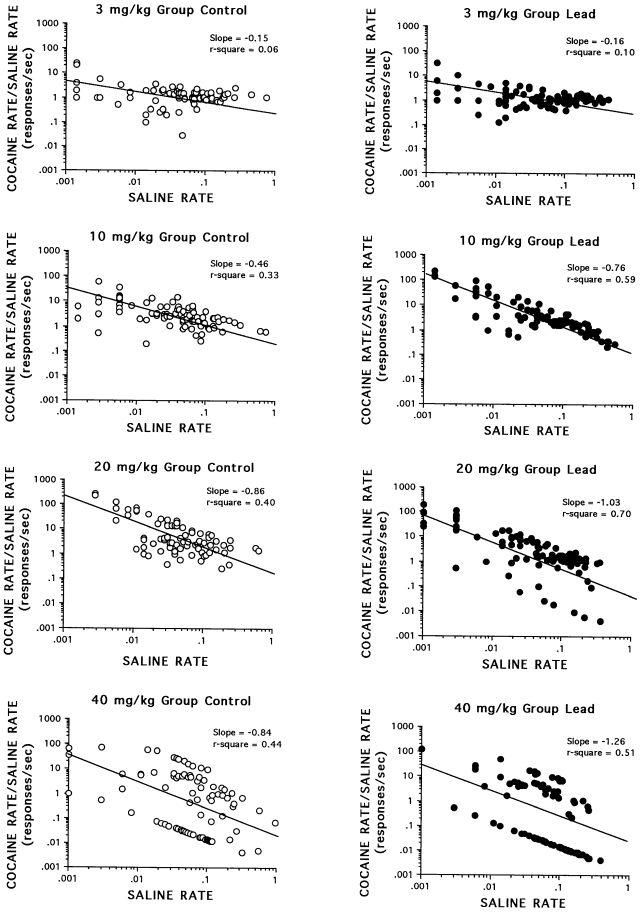

FIG. 2. Effects of 10 mg/kg cocaine HCL on local rates of fixed-interval (FI-5) for each group (Group Control, $N = 8$; Group Lead, $N = 9$). Saline response rates for individual animals, derived from successive 30 s segments of the interval, are plotted on the abscissa (log scale), and the ratios of cocaine rate/saline rate are plotted on the ordinate (log scale). Least squares linear regression was performed on the pooled logarithmic data.

Lead/Cocaine

as dose increased. Second, it should be noted that for the bulk of data points at each dose, the cocaine rate/saline rate ratios were above 1.0. Ostensibly, the rate enhancing effects of cocaine on slower response rates characteristic of early segments of FI responding was comparatively more of an issue in this study than the rate decreasing effects of cocaine later in the interval (9,13,22), and it was the prior effects that were blunted by lead exposure. Certainly, this is consistent with the data reported in Fig. 1.

Blood Lead Concentrations

Blood lead concentrations were determined for the 8 control and 9 lead-exposed animals used in this experiment. Assuming unequal variances, an analysis of lead residues in blood confirmed that metal burdens were substantially greater in Group Lead animals (mean = 25.11 μg/dl; SEM = 1.36) than controls (mean = 1.27 μg/dl; SEM = .46), $t(9.8) = 16.57$, $p < .01$. These values agree with those obtained in earlier lead/cocaine studies completed in this laboratory (7,15), and are consistent with "moderate levels" reported in studies of low level contaminant exposure in adult male humans (2).

DISCUSSION

The results of this experiment indicated that acute ip administration of cocaine altered the pattern of responding under an FI-5 min schedule of reinforcement for control and lead-exposed animals. Specifically, following an injection of 10, 20, or 40 mg/kg cocaine, normally low level response rates at the beginning of the interval increased in adult male rats exposed ad libitum to water containing 500 ppm sodium acetate (Group Control) or 500 ppm lead acetate (Group Lead). Additionally, for control but not lead-exposed animals, at the 20 mg/kg dose it was observed that this pattern of elevated responding persisted throughout the interval. And, it was apparent that the increases in response rates at the 20 mg/kg dose of cocaine were greater for control than lead-exposed animals. This pattern of lead-induced attenuation of the rate-increasing effects of cocaine was observed in the absence of reliable lead-related disturbances in fluid intake or body weight.

The finding that cocaine increases low level response rates within the FI agrees partly with earlier reports on the effects of cocaine on temporal response patterns. For both rats (9,13) and pigeons (22), acute cocaine administration has been shown to increase local rates of responding early in the interval. But conspicuous rate-decreasing effects were observed in these studies that reported cocaine-induced declines in high rates of responding late in the interval. Why such "rate-dependent" effects were not expressed here is unclear. Certainly, differing animal ages, duration of isolation, test chambers, and other methodological variables may have contributed to the discrepant results.

The results obtained in this investigation of the differential effects of 20 mg/kg cocaine on schedule-controlled responding in control and lead-exposed animals are congruent with earlier studies of lead/cocaine interactions. As indicated, the stimulatory properties of cocaine (8), and cocaine-induced elevation of nucleus accumbens dopamine (16), are attenuated by chronic low-level lead exposure. It is worth noting that in each of these studies disturbances in the sensitivity to cocaine were observed in animals that had blood lead concentrations, which only a few years ago, would have fallen at or below the threshold for defining human health risks, i.e., 25 ug/dl. Moreover, the relatively modest exposure regimen employed in this study likely would have produced even higher body burdens of lead had we not employed an enriched diet (Teklad Diet 7001). Rodent diets containing lowers amounts of protein and minerals are known to increase lead absorption dramatically (cf. [4, 21]). Coupled with our data and the finding that lead absorption from the gut is ten times greater in humans than rats (14), such evidence would seem to affirm the recent decision of CDC to take a more severe stand on what constitutes an acceptable level of lead exposure.

The finding that the rate enhancing effect of cocaine increased from 10 to 20 mg/kg , and then diminished at 40 mg/kg (see Fig. 1), is not surprising. Commonly, the motor stimulating effects of increasing doses of cocaine follow such a biphasic pattern (20). Of greater interest is the fact that evidence of lead/cocaine antagonism in this study was restricted largely to the 20 mg/kg comparison. Perhaps nominal drug-related changes at lower dose levels and insurmountable effects at high doses renders a window of influence for the toxicant. Whatever, attenuation by lead of the rate changing effects of cocaine at a moderate dose was clearly evident here.

With respect to possible mechanisms associated with lead-related attenuation of cocaine effects, there is an expanding and relevant literature which centers on lead-induced disturbances of presynaptic dopamine kinetics (see [5] for a recent review). There is evidence that lead impairs the biosynthesis (12) and release (11) of mesolimbic dopamine, and that these results are due to alterations in autoreceptor-mediated regulatory activity. Supporting findings are available from the aforementioned microdialysis report of decreased extracellular dopamine levels in the nucleus accumbens for lead-exposed rats following cocaine administration (16), as well as the D1 and D2 supersensitivty that is believed to be an upregulatory consequence of decreased dopamine availability in the region of the ventral striatum (6). Because blockade of dopamine reuptake in the nucleus accumbens by cocaine is believed to be an integral component of the drug's psychoactive properties (10), it follows that events (such as lead exposure) that reduce the amount of dopamine available for reuptake should minimize cocaine's effects (a more detailed discussion of this issue is presented by Cory-Slechta [5]). In this context, the attenuation of cocaine-induced disturbances in fixed-interval responding observed here for lead-exposed animals would be expected.

Whatever the mechanism(s) responsible for the effects reported here, it is increasingly clear that inorganic lead alters cocaine sensitivity. At this juncture, it is unclear what impact the contaminant may have on the selection for or degree of use of this commonly abused psychoactive agent. Self-administration of drugs in the human population is a serious problem and surely multiply determined. In any event, insofar as environmental elements contribute to these activities and play a mediational role in abuse patterns, a more deliberate exploration of the mechanisms underlying these effects is mandated.

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