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Discriminative Stimulus Properties of Amphetamine and Other Stimulants in Lead-Exposed and Normal Rats[1]

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ROSEN, J B , A M YOUNG, F C BEUTHIN AND R T LOUIS-FERDINAND *Discriminative stimulus properties of amphetamine and other stimulants in lead-exposed and normal rats* PHARMACOL BIOCHEM BEHAV **24**(2) 211–215, 1986 —The present study examined the discriminative stimulus properties of amphetamine (AMP) at progressively lower doses in lead-exposed and normal rats In addition, generalization gradients of AMP, apomorphine, methylphenidate, and caffeine to both high and low training doses of AMP were determined in these rats Under the high AMP training dose condition (1 0 mg/kg, IP) generalization gradients of AMP were similar for lead-exposed and control rats When the training doses were progressively lowered, the lead-exposed rats tended to require a higher range of AMP doses (0 24–0 49 mg/kg) than did control rats (0 18–0 32 mg/kg) to maintain discriminative control In parallel with this, the minimal discriminable doses tended to be higher for lead-exposed rats than for control rats Methylphenidate generalization gradients were different for lead-exposed and control rats under the high AMP training condition but became similar under the low AMP training condition No differences attributable to training dose or lead exposure were evident for apomorphine or caffeine

Lead Lead exposure Drug discrimination Amphetamine Methylphenidate Apomorphine Caffeine

LOW level lead exposure has previously been shown to impair a learner's ability to acquire stimulus control by a wide range of environmental stimuli (see [4] for review) Temporal patterns of responding under fixed-interval [1, 5, 17] and fixed-ratio [15] schedules of reinforcement and acquisition of auditory and visual discriminations [6,9] may be altered by lead exposure In addition, lead exposure has been shown to alter the behavioral actions of certain psychoactive drugs (e g , [16,20]) Particular interest in drug effects in lead-exposed rats has focused on *d*-amphetamine (AMP) because of a possible link between lead exposure in neonates and subsequent behavioral hyperactivity [7] Results of several animal studies have demonstrated an attenuated response to AMP following postnatal or chronic lead exposure [13, 20, 22]

Drug-stimulus discrimination paradigms may be effective in demonstrating altered effects of drugs induced by lead exposure Zenick and Goldsmith [24] have reported that amphetamine's ability to function as a discriminative stimulus is altered by lead exposure Lead-exposed rats were found to be less sensitive to the discriminative stimulus properties of AMP than were nontreated controls By systematically decreasing the training dose of AMP, these investigators showed that the threshold of discriminability was higher for lead-treated rats than for controls The present study extended this work by exploring generalization gradients for AMP and other psychomotor stimulants in lead-exposed rats Since both the amphetamine dose-response curve and the generalization of other drugs to the AMP stimulus can vary with the dose used as a training stimulus [23], dose-response curves for AMP and other stimulants were examined both before and after exposure to decreasing AMP training doses

METHOD

Subjects and Apparatus

Eight male Long-Evans rats served as subjects Upon birth, two litters were culled to eight pups each Four pups from each litter were assigned to the treatment and control groups, respectively From the first through the twentieth days after birth, rats received either 10 mg/kg lead acetate or

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equimolar sodium acetate (IP) Four lead-exposed and four control rats were randomly selected from these litters for the experiments Prior to discrimination experiments all rats had similar experiences in a number of behavioral tasks [18] None had received any drug treatment, except lead, prior to the present experiment

At 90 days of age, each rat was housed in an individual cage, with water freely available At 180 days, each was reduced to 85% of its ad lib feeding weight by restricting access to food for 2 days Thereafter, each rat received approximately 15 g of laboratory rat chow daily following the experimental session or at midday on weekends The light cycle in the animal colony was from 7 a m to 7 p m Experiments were carried out between 3 p m and 6 p m five days a week

Experiments were conducted in operant chambers containing two levers, stimulus lamps, and a pellet feeder The levers were mounted on each side of the food receptacle and required a minimal force of 0 21 N to record a response The experimental chamber was placed in a sound attenuating cubicle with an exhaust fan Programming, recording, and data collection were accomplished by Rockwell AIM 65 computers

Procedure

Training AMP (1 0 mg/kg) and saline were established as discriminative stimuli for food-maintained responses (cf [10]) Each session began with a 15 min pretreatment interval during which the chamber was dark and responses had no scheduled consequences Immediately following the pretreatment interval the chamber lights were illuminated, and responses on either the right or left lever, contingent upon whether saline or 1 mg/kg AMP had been administered, were reinforced by delivery of one 45 mg food pellet during 15 min sessions Following AMP administration, responses on only the left lever produced food, following saline administration, responses on only the right lever produced food Across sessions, the response requirement for food delivery was increased from an initial value of 1 to a final value of 20 consecutive responses on the appropriate lever Each response on the incorrect lever reset this response requirement AMP and saline administration alternated daily Training continued until two criteria were met for nine consecutive sessions The criteria were (1) emission of fewer than 40 responses prior to the first food delivery and (2) distribution of at least 90% of the total responses in a session on the appropriate lever Injections of AMP and saline alternated during the first five sessions in which criteria were met During the final four days, two consecutive saline sessions were followed by two consecutive AMP sessions

Dose-Response Determinations

Once the above criteria were met, dose-response curves were determined for AMP Generalization test sessions were identical to training sessions, with the exception that 20 consecutive responses on either lever were reinforced AMP or saline training sessions alternated with test sessions If a rat failed to meet the performance criteria during a training session, further test sessions were postponed until two consecutive successful AMP and saline training sessions occurred

After completion of AMP dose-response gradients, dose-response curves were determined for apomorphine, methylphenidate, and caffeine, in that order The order of doses of each drug tested in each rat was unsystematic The largest doses used were those that reduced responding so markedly that fewer than 40 responses were emitted or no reinforcers were delivered

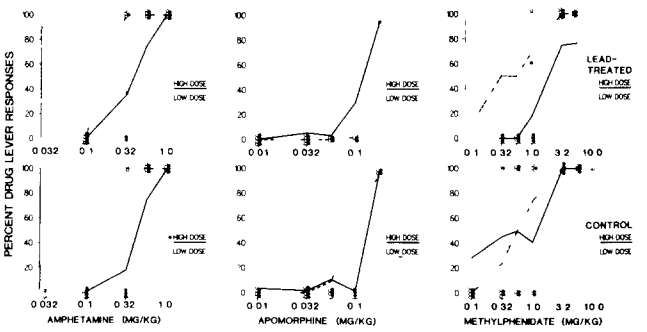

FIG 1 Generalization gradients for AMP, apomorphine, and methylphenidate in rats trained to discriminate amphetamine and saline The upper graphs are gradients for lead-treated rats (n=4), while the lower gradients are for control rats (n=4) Ordinate mean drug lever responses, expressed as a percentage of total responses emitted during the session Abscissa doses of drugs in one-quarter log cycle intervals (e g , for AMP, 0 032, 0 056, 0 1, 0 18, 0 32, 0 56, and 1 0 mg/kg) Solid lines are gradients under the high AMP training dose condition, and the broken lines are gradients under the low AMP training dose condition Solid and open circles represent percentage of drug lever responses for individual rats under the high and low AMP training doses, respectively Left panel Amphetamine generalization gradients The doses tested ranged from 0 032 to 1 0 mg/kg Middle panel Apomorphine generalization gradients The doses tested ranged from 0 01 to 0 18 mg/kg Right panel Methylphenidate generalization gradients The doses tested ranged from 0 1 to 10 0 mg/kg

Systematic Lowering of Training Dose

Once all dose-response gradients were determined, the AMP training dose for an individual rat was successively reduced in decrements of approximately 30% (cf [14]) If the rat met performance criteria in 8 out of 10 sessions at each new training dose, the dose was again decreased If not, training continued for an additional 10 sessions If after 20 sessions the criteria were not met, the training dose was raised by 30% and the rat retested for successful performance in 8 out of 10 sessions

Redetermination of Dose-Response Curves at the Lowered Training Doses

After an individual rat reached criteria at its lowest possible dose of AMP, generalization tests again were conducted for each drug, as described above

Data Analysis

The number of responses emitted on the AMP-appropriate lever, expressed as a percentage of the total number of responses emitted during a 15 min session, was examined as a function of dose for each drug tested The distribution of responses was not evaluated if fewer than 40 responses were emitted during a test session For each subject, the minimal discriminable dose was defined as the lowest dose of a test drug that occasioned greater or equal to 90% AMP-appropriate responding The small subject sample

precluded statistical analysis Therefore, results from individual subjects have been presented in graphic and tabular form

Drugs

d-Amphetamine sulfate, methylphenidate hydrochloride, apomorphine hydrochloride, and caffeine (anhydrous) were dissolved in saline Stock solutions of *d*-amphetamine and methylphenidate were used for several weeks Apomorphine and caffeine solutions were prepared daily Injection volumes were generally 1 mg/kg body weight All drugs were given IP except apomorphine, which was given SC Appropriate saline control injections were given SC during apomorphine testing

Blood Lead Level Determination

Blood lead levels of rats, taken from the tail vein, were analyzed using a model 170-70 Hitachi Atomic Absorption Spectrophotometer at 383 6 nM Samples (100 μl) of control and experimental blood were diluted with 1% Triton X-100 Lead concentrations were determined from a standard curve

RESULTS

Blood Lead Levels and Body Weight

The lead exposure regimen used in the present study produces a 40 to 50 fold elevation in blood lead levels (mean±SD=157 8±26 0 μg/100 ml) at postnatal day 21 compared to sodium acetate treated control rats (2 8±0 3 μg/100 ml) (Roginski, Louis-Ferdinand and Beuthin, *Toxicol Appl Pharmacol*, submitted) At 180 days of age, adult lead-treated rats still showed significantly higher levels of blood lead than did control rats (lead-treated mean±SD=8 0±2 5 μg/100 ml, control mean±SD=5 4±1 4 μg/100 ml, $t(13)=5\ 34$, $p<0\ 001$), although these levels can be considered within the nontoxic range of control blood levels [8]

Body weight differences between the groups were not apparent At 90 days of age the mean±SD weight of the lead-exposed group was 383 2±45 8, while that of the control group was 382 5±17 6 We have previously reported that other rats undergoing this lead dosing protocol showed transient weight reductions at 25 days of age, but did not differ in weight from control subjects by 50 days of age [18]

Amphetamine Training Doses and Generalization Gradients

The groups did not differ in the number of sessions needed for acquisition of the initial discrimination (range of the number of sessions to reach criterion performance 32–40 for both groups) However, 1 0 mg/kg AMP severely suppressed responding in two rats, one from each group The training dose for these two rats was therefore lowered to 0 56 mg/kg during initial discrimination training Initial AMP generalization gradients were also similar for control and lead-treated rats (Fig 1, left panel, solid lines and circles) AMP doses of 0 56 or 1 0 mg/kg occasioned primarily drug-appropriate responses in both groups, while lower doses occasioned primarily saline-appropriate responses Doses higher than 1.0 mg/kg abolished lever press responses in all subjects As shown in Table 1 (left columns), the minimal discriminable doses of AMP for individual rats were similar in both groups under the high training dose condition

When the AMP training dose was systematically lowered, stimulus control tended to be lost at higher AMP doses in lead-exposed rats than in controls Lead-treated subjects required AMP doses of 0 49 to 0 24 mg/kg for continued discriminative control, while control subjects required 0 32 to 0 18 mg/kg (Table 1) Lowering the AMP training dose also lowered the minimal discriminable doses, with lower AMP test doses generating drug-appropriate responses in subjects who attained lower training doses than in subjects who required higher training doses (Table 1, right columns) Therefore, the AMP generalization gradients generated after establishment of discriminative control by the lower AMP dose also tended to differ between the groups (Fig 1, left panel, dotted lines and open circles) Two subjects in the control group responded on the drug-appropriate lever at 0 1 mg/kg AMP or lower, while no subject in the lead-treated group did so

TABLE 1

INDIVIDUAL TRAINING AND MINIMAL DISCRIMINABLE DOSES (MDD) OF *d*-AMPHETAMINE FOR LEAD-EXPOSED AND CONTROL SUBJECTS

	High amphetamine training dose condition		Low amphetamine training dose condition	
	Training dose	MDD (mg/kg)	Training dose	MDD (mg/kg)
Lead-exposed subjects				
LT-1	1 0	0 32	0 49	0 32
LT-9	1 0	0 56	0 32	0 18
LT-7	1 0	1 0	0 24	0 18
LT-4	0 56	0 56	0 32	0 18
Control subjects				
LT-14	1 0	0 56	0 32	0 32
LT-15	1 0	1 0	0 24	0 18
LT-10	1 0	0 56	0 18	0 056
LT-8	0 56	0 56	0 24	0 10

Apomorphine, Methylphenidate, and Caffeine Generalization Gradients

Under the high AMP training dose condition, a dose of 0 18 mg/kg apomorphine occasioned AMP-appropriate responses in two control subjects and one lead-treated subject (Fig 1, middle panel) However, apomorphine doses of 0 056 mg/kg and above severely decreased or abolished responding in one or more subjects in both groups Under the low AMP training dose condition, 0 32 mg/kg apomorphine occasioned AMP-appropriate responses in only two control subjects, and doses of 0 056 mg/kg and above again abolished responding in one or more subjects in both groups

Under the high AMP training dose condition, methylphenidate (3 2 or 5 6 mg/kg) occasioned complete AMP-appropriate responses in all control subjects, but in only 3 of 4 lead-treated subjects One control subject generalized to methylphenidate doses as low as 0 32 mg/kg For control subjects, lowering the AMP training dose did not alter the methylphenidate generalization gradient appreciably However, the gradient for lead-treated subjects shifted left under the low AMP training dose condition, so that the final methylphenidate gradients were similar for both groups

Overall, there were considerable differences among individual subjects in the lowest doses of methylphenidate that maintained drug-appropriate responding, resulting in shallow dose-response curves

Caffeine (3 2 to 100 mg/kg) did not occasion AMP-appropriate responses under the high AMP training dose condition Under the low AMP training dose condition, doses of 3 2 to 100 mg/kg occasioned AMP-appropriate responses in one or more subjects in both groups, but the pattern of such generalization bore no consistent relation to dose (data not shown)

DISCUSSION

In agreement with Zenick and Goldsmith's [24] report of lead-induced changes in AMP discrimination, lead-exposed rats in the present experiment required 0 24 to 0 49 mg/kg AMP to maintain discriminative behavior under a stimulus fading procedure However, these doses were only slightly higher than those required by control rats (0 18 to 0 32 mg/kg) This contrasts with the approximately 2-fold difference in lowest discriminable doses reported by Zenick and Goldsmith

The lack of a large difference between groups in the present study may have resulted from the subjects' more lengthy exposure to the initial high AMP dose training condition, as compared to the earlier report Indeed, the differences observed by Zenick and Goldsmith [24] were not accompanied by differences in acquisition of the initial high dose discrimination Instead, the initial differences appeared only in response to a training challenge (cf [3]) By extending the initial training period to allow for generalization tests, we may have minimized the extent to which this challenge could reveal differences between the groups

For amphetamine, lowest training doses and minimal discriminable doses for individual rats in both groups were not predicted by differences in acquisition of the initial AMP discrimination or in initial generalization gradients Any differences between groups appeared only in response to a training challenge For methylphenidate, lowering the AMP training dose did not systematically shift the generalization gradient in control rats, but shifted that in lead-exposed rats to the left As a result, final methylphenidate gradients were similar in lead-exposed and control rats Neither apomorphine nor caffeine produced unequivocal stimulus generalization to caffeine under any condition

Any apparent differences in lead-exposed subjects may have resulted from growth retardation rather than lead exposure itself However, rats receiving an identical lead exposure regimen showed only transient decreases in body weight at 25 days of age [18] Additionally, in the present study, body weights of the lead-exposed and control groups did not differ at the time of testing Although interference with somatic growth may confound the effects of lead with those of nutritional factors, Silbergeld and Goldberg [21] suggest that the effect of lead on growth rate may be viewed as a lead-related effect and not strictly as a confounding variable

Considering the control rats alone, lowering the AMP training dose shifted the generalization gradient for amphetamine itself left, but did not uniformly alter that for methylphenidate These results are in general agreement with Stolerman and D'Mello's [23] systematic examination of the role of training dose in modulating AMP's quantitative and qualitative generalization profile, and replicate previous reports of generalization between AMP and methylphenidate (e g , [11]) In contrast to Stolerman and D'Mello's report, however, apomorphine shared stimulus properties with both the higher and the lower AMP dose in those rats able to respond These results thus add to the number of reports showing widely varying degrees of generalization between AMP and apomorphine (e g , [2, 12, 19, 23]), controlling variables remain to be determined

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