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Conditioned Place Preferences, Conditioned Locomotion, and Behavioral Sensitization Occur in Rats Treated With Diethylpropion

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REIMER, A. R., M. T. MARTIN-IVERSON, L. J. URICHUK, R. T. COUTTS AND A. BYRNE. *Conditioned place preferences, conditioned locomotion, and behavioral sensitization occur in rats treated with diethylpropion.* PHARMACOL BIOCHEM BEHAV 51(1) 89–96, 1995.—Diethylpropion is a centrally acting appetite-suppressing drug thought to act primarily through catecholamine pathways in the brain. In the present study, four doses of diethylpropion (0, 10, 20, and 40 mg/kg, intraperitoneally) were administered to rats to examine the hypothesis that the drug has psychomotor stimulant properties such as the ability to induce conditioned behaviours and behavioural sensitization. The rats were administered drug and then vehicle on alternating days, and confined to a "drug-" or vehicle-paired side of a two-compartment box for 16 pairings. Only the 10-mg/kg dose of diethylpropion increased spontaneous locomotor activity in comparison to vehicle; the 20- and 40-mg/kg doses significantly decreased spontaneous locomotion. All doses of diethylpropion decreased spontaneous rearing, and the 20-and 40-mg/kg doses produced significantly less rearing than the 10-mg/kg one. At the 10-mg/kg dose, conditioned place preferences, conditioned locomotion, and conditioned rearing were observed. The 40-mg/kg dose produced conditioned rearing and conditioned defecation. In response to a 5-mg/kg challenge injection of diethylpropion, behavioural sensitization in locomotion and rearing occurred in rats that had previously received any one of the three doses of diethylpropion. Over 36 days, decreased weight gain was observed only in the 20- and 40-mg/kg groups. The rats were killed 48 h after the last drug injection, and whole brain was analyzed for levels of the catecholamines, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT (not a catecholamine), and 5-hydroxyindoleacetic acid (5-HIAA) by HPLC with electrochemical detection. No significant differences from control values were found, indicating that diethylpropion has no long-term effects on levels of these brain chemicals. The results support the hypothesis that diethylpropion has amphetamine-like psychomotor stimulant properties.

Anorectics Behavioural sensitization Conditioned defecation Conditioned locomotion Conditioned place prefences Diethylpropion Psychomotor stimulants

THERE ARE two major categories of centrally acting appetite-suppressing drugs (anorectics): those such as amphetamine (AMP) or diethylpropion (DEP), primarily acting through catecholamine pathways, and those such as fenfluramine (FEN), proposed to be acting through serotonergic pathways (33). Intraventricular injection of 6-hydroxydopamine to rats pretreated with pargyline, a procedure that dramatically reduces brain catecholamine levels without significantly affecting the concentration of serotonin, blocks the anorectic effect of amphetamine and diethylpropion, but has no effect on food reduction induced by FEN and *p*-chloroamphetamine (11,29). Thus, DEP's anorectic effects appear to be catecholamine dependent. All members of the catecholamine category of appetite suppressants have some sympathomimetic and stimulant properties (33), although adverse effects with DEP occur less frequently than with other appetite suppressants in the catecholamine group (36). Some patients experience insomnia, and a risk of abuse in obese patients has been noted (6,8). DEP can result in stimulant-induced psychosis (7,8,16), and although the potential for DEP abuse is high, it is not as great as that for AMP, methamphetamine, or cocaine (8). However, since its reclassification as a controlled drug in

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Canada (in 1978), DEP's previous relatively widespread abuse in this country has been greatly reduced (8).

The neurochemical profile of DEP is similar to that of other psychomotor stimulants. DEP and D-AMP significantly increased norepinephrine (NE) and dopamine (DA) levels, but not serotonin [5-hydroxytryptamine (5-HT)] or 5-hydroxyindoleacetic acid (5-HIAA) levels (11). In comparison to D-AMP, L-AMP, mazindol, phentermine, DL-FEN, D-FEN, or L-FEN, DEP was the least potent DA or NE uptake inhibitor, and had a very weak effect on 5-HT uptake inhibition (11). In contrast to AMP, DEP protected noradrenergic, but not dopaminergic neurons from death, following intraventicular injections of 6-hydroxydopamine (6-OHDA) 30 min after the administration of pargyline (11). Similar to AMP, DEP had no effect on death of serotonergic neurons induced by a high dose of FEN (11). In another study, AMP was shown to be equal to or superior to DEP at inhibiting DA uptake in synaptosomes (24), and was more effective than DEP at inducing spontaneous release of synaptosomal DA (24). Mazindol is a more potent inhibitor of DA uptake than AMP or DEP (11,24), and is equipotent to DEP as a synaptosomal releaser of DA (24).

Past research has shown that DEP has stimulant-like behavioural effects. Reserpine and α-methyl-para-tyrosine (α-MPT) deplete the vesicular and newly synthesized pools of the catecholamines, respectively. Reserpine disrupts the uptake storage mechanism of vesicles of the catecholamines and irreversibly damages the vesicle, and α-MPT is an inhibitor of tyrosine hydroxylase. The locomotor activating effects of a 20-mg/kg dose of DEP was abolished by pretreatment with reserpine but not by α-MPT, whereas locomotor effects of a 3-mg/kg dose of *N*-methylamphetamine was unaffected by reserpine but abolished by α-MPT (23). In agreement with these results, DEP- and mazindol-induced locomotor activity was abolished by reserpine but unaffected by α-MPT (24). Amphetamine-induced locomotion is also blocked by α-MPT but not by reserpine (10,30,38). That the effects of α-MPT and reserpine on DEP- and mazindol-induced locomotor stimulation were identical suggests that, like mazindol and cocaine, DEP-induced locomotor effects rely more heavily on the vesicular pool of DA than on the newly synthesized pool of DA.

DEP is less potent than AMP as a psychomotor stimulant [see (14) for review]. For example, in one study, 7.5 mg/kg DEP produced the same amount of locomotor activity in rats as 2.5 mg/kg D-AMP, approximately 10 times the amount produced by vehicle injection (11). Rats intravenously (IV) self-administered DEP (2 mg/kg per infusion) to the same extent as a lower dose of AMP (0.25 mg/kg per infusion), whereas FEN and saline were not self-administered (13). Rats trained to lever press for IV infusions of AMP lever pressed for saline following a noncontingent injection of DEP or AMP, but not after FEN or saline, suggesting generalization between AMP and DEP. In rhesus monkeys trained to self-administer cocaine, DEP maintained self-administration, although the animals showed a significant preference for cocaine [(15) cited by (14)]. However, only low doses (≤ 1 mg/kg/infusion) of DEP were investigated in this study.

Some evidence suggests that the toxicities of DEP and AMP differ. Three investigations have shown that AMP was more toxic in grouped mice than single-caged mice, whereas housing did not affect the toxicity of DEP (5,18,35). Furthermore, AMP increased acoustic startle, but DEP (9.12 mg/kg) had no effect on this parameter (17). Thus, although it is similar to AMP for some parameters, DEP is different for a variety of others.

Although DEP has been quite widely used as an anorectic since 1957 (14), there is a lack of information available on the behavioural effects of the drug. The purpose of the present experiment was to examine the possibility that DEP can produce conditioned place preferences (CPP), conditioned locomotion, and behavioural sensitization, such as are observed with other psychomotor stimulants (1–3,25,27,31,32,34). Nothing was found in the literature on the testing of these effects with DEP. CPP has been suggested to predict abuse liabilities of drugs [20,26,34; for a review of CPP, see (37)]. Psychomotor stimulant effects can be conditioned to contextual stimuli (25,31,34), and some investigators believe that this plays a role in drug addiction (21,22). Because DEP can also produce a stimulant-like psychosis, and some researchers believe that behavioural sensitization is an animal model for stimulant-induced psychoses (1,27,32), we investigated the possibility that DEP can produce behavioural sensitization.

METHODS

Subjects

Forty-eight male Sprague–Dawley rats weighing between 250 and 350 g were obtained from Health Sciences Animal Services of the University of Alberta. The animals were on a 12 L : 12 D, 0700–1900 h. They were housed in pairs in shoebox cages with betachip and had free access to food and water. The environmental temperature was maintained at 22°C and the humidity at 50%. Testing occurred from approximately 1100–1700 h each day. All procedures were approved by the Health Sciences Animal Care Committee as following CCAC recommendations for animal use in research. One rat in the 40-mg/kg group died of unknown causes on day 13 of the experiment, and data from this animal were not used.

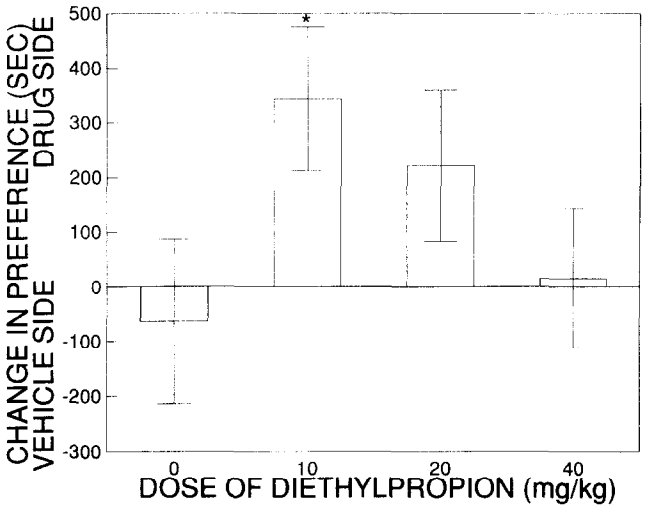

FIG. 1. Conditioned place preferences (CPP) to the drug-associated compartment comparing the change in time [seconds (SEC)] spent in the drug-associated compartment on a predrug day (habituation day 5) to the amount of time observed after eight pairings of a drug injection with confinement on the drug side. Animals receiving 10 mg/kg of diethylpropion showed CPP toward the drug compartment. Error bars represent ± SEM of each group. *Significantly different from the vehicle injected group, $p < 0.05$.

TABLE 1

MEDIAN PRETEST (DAY 5) VS. THE TEST (FOR CPP) TIMES SPENT IN DRUG COMPARTMENT OF CAGE

DOSE (mg/kg)	Pretest Times on Drug Side (s)	Semi-Interquartile Ranges (s)	Test Time on Drug Side (s)	Semi-Interquartile Ranges (s)
0	688	351-1370	740	377-1195
10	588	320-1118	1042	856-1391
20	800	326-1243	966	736-1345
40	917	721-1147	907	611-1345

Drugs

DEP (Merrell Dow Pharmaceuticals, Cincinnati, OH) was dissolved in double-distilled water and administered IP at dosages of 0, 10, 20, and 40 mg/kg per ml. Doses were selected on the basis of the literature (11,14,17,23,24,33) and on previous neurochemical work done in our laboratory. The time course of DEP was determined in a pilot study. Following an IP injection, DEP affected locomotor activity and rearing within 10 min, and the peak effect occurred within 20-30 min. All injections were made 10 min before placing rats in the experimental boxes.

Apparatus

Six CPP boxes (Acadia Instruments, Saskatoon, Saskatchewan, Canada) were used. These boxes had two compartments, with each compartment (30 L × 30 W × 25 cm H) consisting of clear Plexiglas sides and a distinctive floor (unique tactile cues). One floor was a grate with 1-cm squares, and the other consisted of 14 horizontal bars spaced 1.25 cm apart. The two compartments were separated by an opaque partition containing a 7.5-cm-long tunnel to allow animals access to both sides, and the tunnels had a removable door on either end. Each compartment was transected by two infrared photobeams 3 cm above the floor, which measured general locomotor behaviour on each side, and by eight infrared photobeams that transected the compartments 15 cm above the floor to assess rearing behaviour on each side. The compartments rested on a fulcrum such that the compartment tilted 2 mm when an animal crossed from one side to the other. A

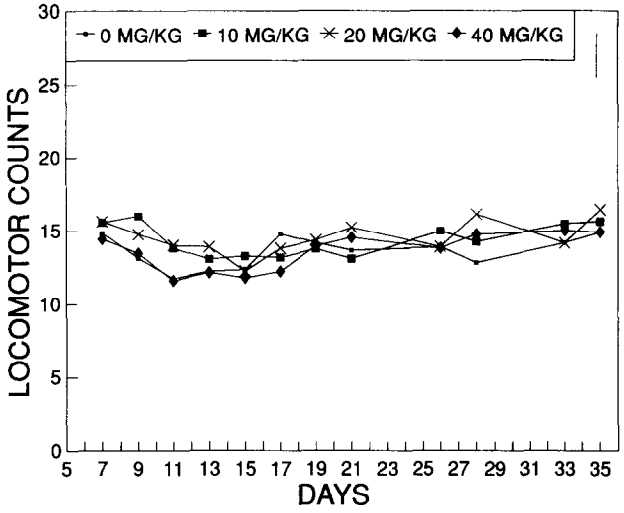

FIG. 3. Square root of locomotor activity (photobeam interruptions) in the vehicle-associated compartment on the 12 vehicle days. There were no significant differences between groups. The critical difference at $p < 0.05$ is 3.075 (bar in upper-right corner).

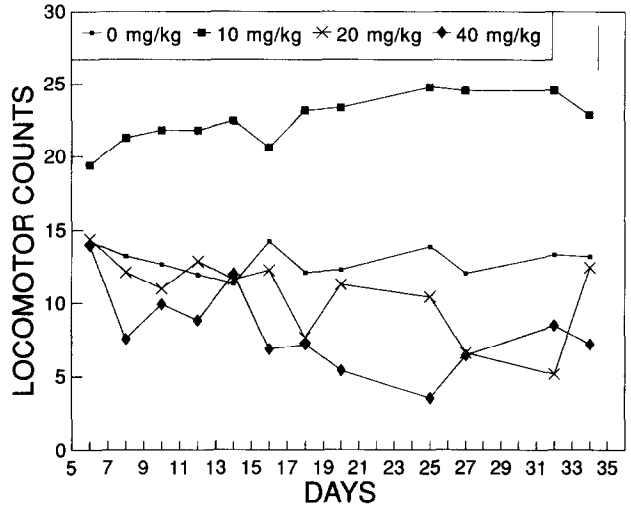

FIG. 2. Square root of locomotor activity (photobeam interruptions) in the drug-associated compartment on the 12 drug days. The 10-mg/kg dose of diethylpropion resulted in an increase in locomotor activty compared to all other groups, and a significant increase in locomotion over days (behavioural sensitization). The 40-mg/kg dose significantly decreased locomotion in comparison to vehicle except on days 6 and 14. The critical difference at $p < 0.05$ is 3.075 (bar in upper-right corner).

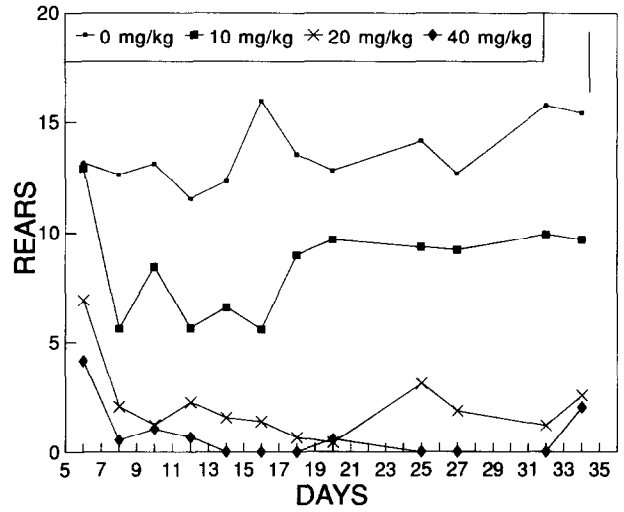

FIG. 4. Square root of the rears (photobeam interruptions) in the drug-associated compartment on the 12 drug days. The 10-mg/kg group had significantly fewer rears than controls except on day 1. The 20- and 40-mg/kg groups had significantly fewer rears than controls or the 10-mg/kg group on all drug days. The critical difference at $p < 0.05$ is 2.745 (bar in upper-right corner).

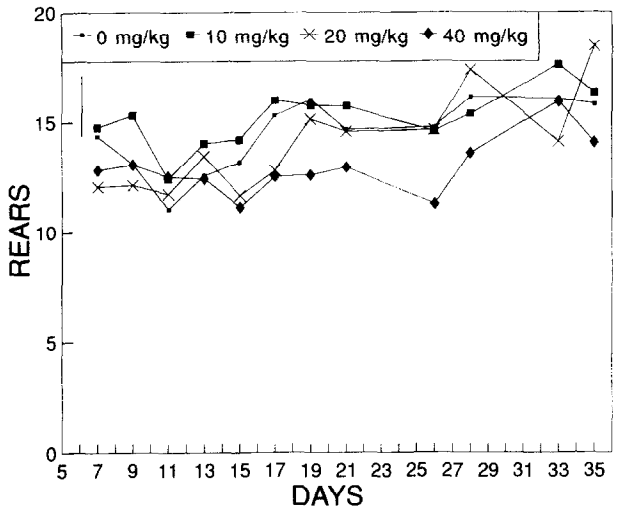

FIG. 5. Square root of the rears (photobeam interruptions) in the vehicle-associated compartment on the 12 vehicle days. The 40-mg/kg group had significantly fewer rears than the vehicle group on days 17, 19, and 26, and there were also other significant differences on several different days. The critical difference at $p < 0.05$ is 2.745 (bar in upper-left corner).

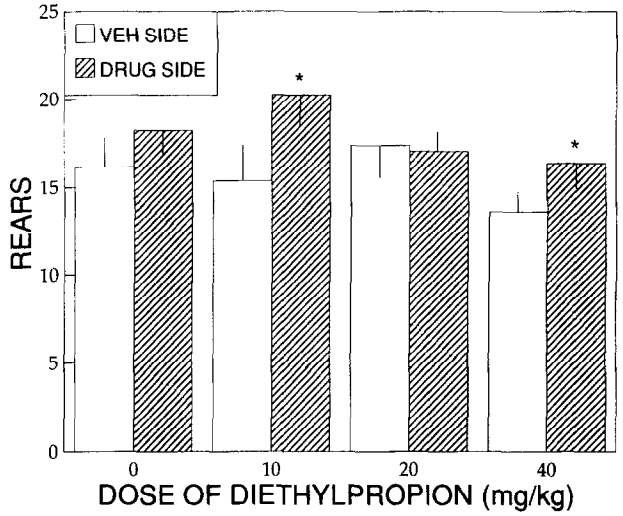

FIG. 7. Motor stimulant effects conditioned to the test context, as shown by square root of rears (photobeam interruptions). Error bars represent SEM of each group. The groups given previous injections of 10 or 40 mg/kg diethylpropion exhibited increases in rears relative to the most recent vehicle (VEH) day (day 28), although rearing during the conditioning (DRUG) days themselves was less than on vehicle days. *Significantly different from respective vehicle group, $p < 0.05$.

weight of 50 g near the entrance (20 g by the far end of the compartment) was sufficient to tilt the box. Tilting of a compartment broke an additional photobeam such that the time spent in each compartment could be determined. The photobeam arrays were connected to a computer, and interruptions of photobeams were counted by Turbo C software.

Procedure

The procedures for drug-induced CPP followed those already established (19,20). Prior to being placed in the CPP boxes, rats were kept in their home cages for 7 days. The animals were then randomly assigned to four groups of 12 each. The CPP procedure was unbiased. Each group was counterbalanced so that an equal number of rats in each group received drug on either of the two floor types. The test environment was illuminated with infrared light extending into the

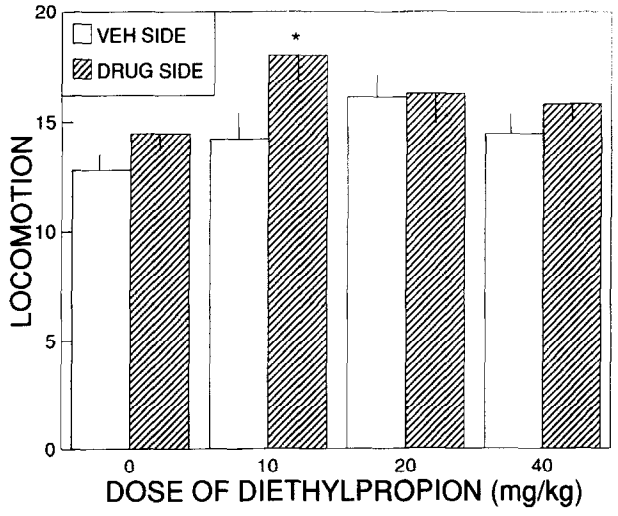

FIG. 6. Motor stimulant effects conditioned to the test context, as shown by square root of locomotor counts (photobeam interruptions). Error bars represent SEM of each group. The group given previous injections of 10 mg/kg diethylpropion exhibited increases in locomotion relative to the most recent vehicle (VEH) day (day 28). *Significantly different from respective vehicle group, $p < 0.05$.

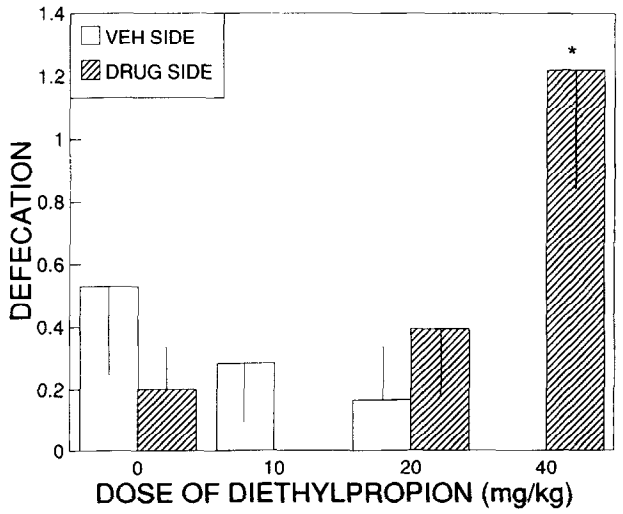

FIG. 8. Defecation conditioned to the test context, as shown by square root of the number of fecal boli. Error bars represent SEM of each group. Only the group given previous injections of 40 mg/kg diethylpropion exhibited increases in defecation relative to the most recent vehicle (VEH) day (day 28). There was no defecation in the 40-mg/kg group on the vehicle side. *Significantly different from respective vehicle group, $p < 0.05$.

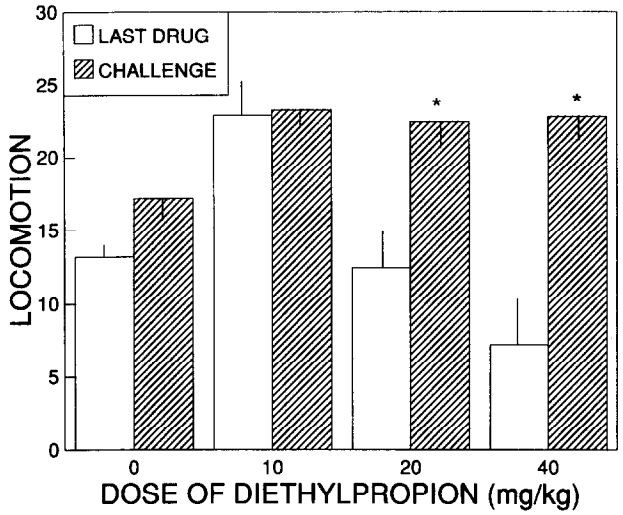

FIG. 9. Motor stimulant effects of a single treatment of diethylpropion (DEP) (5 mg/kg) to each of the indicated previous doses, comparing the last day of drug at the indicated dose (LAST DRUG) to the sensitization test challenge injection (CHALLENGE), as shown by square root of locomotor counts (photobeam interruptions). Error bars represent SEM of each group. All groups previously receiving injections of DEP exhibited behavioural sensitization, or at least an equivalent amount of locomotor activity in response to a lower dose of DEP, relative to the most recent drug day (day 34). *Significantly different from same dose on "last drug" day, $p < 0.05$.

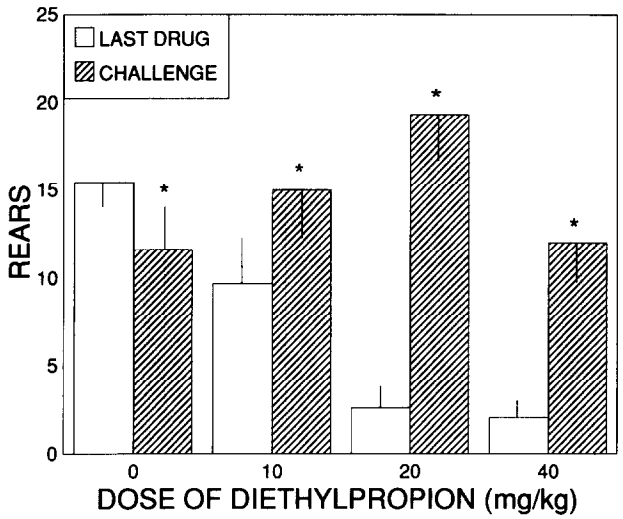

FIG. 10. Rearing effects of a single treatment of diethylpropion (DEP) (5 mg/kg) to each of the indicated previous doses, comparing the last day of drug at the indicated dose (LAST DRUG) to the sensitization test challenge injection (CHALLENGE), as shown by square root of rears (photobeam interruptions). Error bars represent SEM of each group. All groups previously receiving injections of DEP exhibited behavioural sensitization of rearing activity, relative to the most recent drug day (day 34), and the group previously receiving vehicle had significantly fewer rears than with a vehicle injection. *Significantly different from same dose on "last drug" day, $p < 0.05$.

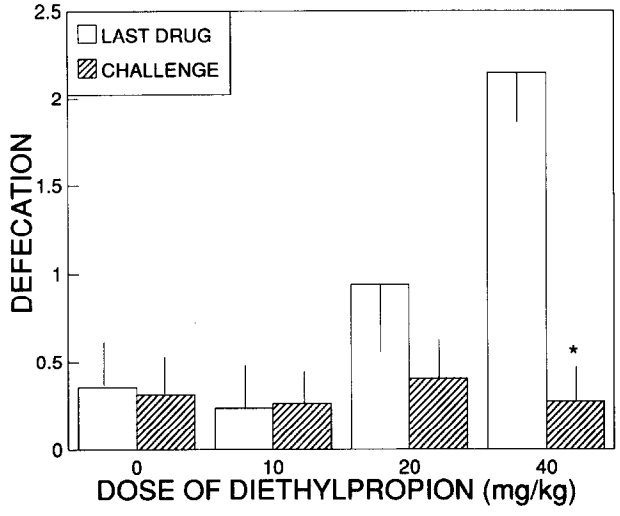

FIG. 11. Effects on defecation of a single treatment of diethylpropion (DEP) (5 mg/kg) to each of the indicated previous doses, comparing the last day of drug at the indicated dose (LAST DRUG) to the sensitization test challenge injection (CHALLENGE), as shown by the square root of the number of fecal boli (photobeam interruptions). Error bars represent SEM of each group. Only the group previously receiving injections of DEP at a dose of 40 mg/kg exhibited a significant decrease in defecation, relative to the most recent drug day (day 34). *Significantly different from same dose on "last drug" day, $p < 0.05$.

visible red frequency. Throughout the experiment, the cages were cleaned between runs with an ammonia-based cleaning fluid that was diluted with six parts of water to one part cleaning fluid. The first 5 days of the experiment (Part 1) were used to habituate the animals to the CPP boxes and to determine initial side preferences, by allowing them free access

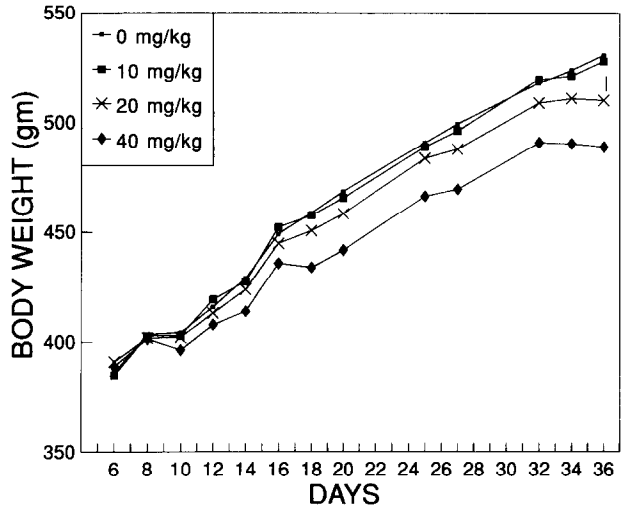

FIG. 12. The effect of diethylpropion on body weight, at the indicated doses, from days 6–36, in grams. The 40-mg/kg group weighed significantly less than the other groups from days 14–36, and the 20-mg/kg group weighed significantly less than the 0- and 10-mg/kg groups from days 32–36. The critical difference at $p < 0.05$ is 8.27 g (bar in upper-right corner).

to both sides for 30 min/day. In Part 2, each rat received drug injections while being restricted to one side of the cage for 30 min on odd days and vehicle injections while restricted to the other side of the cage for 30 min on even days. This procedure resulted in a total of eight drug injections and eight vehicle injections. The number of fecal boli per rat on each day was recorded beginning on the 8th day of Part 2. The animals were then given a 3 day rest to allow for drug clearance. Part 3 was the test day for CPP, when rats were injected with vehicle and then allowed free access to both sides of the cage for 30 min. CPP were concluded to have occurred if rats spent more time on the side previously associated with the drug, relative to the pretest time. Part 4 was another conditioning phase, identical in procedure to Part 2, but only 4 days long. Rats received drug on days 1 and 3 and vehicle on days 2 and 4. Part 4 was followed by another 3 days of drug clearance before Part 5, a test for conditioned locomotion, rearing, and defecation. Part 5 was conducted by injecting each rat with vehicle and then restricting it to the "drug-associated" side of the cage (the side that the rat occupied during drug days) for 30 min. Part 6 was another conditioning phase and was identical to Part 4. Part 7 was the test for behavioural sensitization, and was performed on the day immediately after Part 6. In this test, all animals were injected with a challenge dose of 5 mg/kg DEP and then resticted to the "drug-associated" side of the cage for 30 min.

Forty-eight hours after the sensitization test, the rats were decapitated and the whole brain was removed. Analysis of levels of NE, DA, 5-HT, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA) included homogenizing whole rat brain in ice-cold 0.1 N perchloric acid containing 10 mg% EDTA and 50 μM ascorbic acid and centrifuging to remove the precipitated protein (12). Aliquots of the resultant supernatant were injected onto a reversed-phase HPLC system with a Waters 510 pump coupled to a Waters 710 B WISP injector system. The HPLC system was equipped with an electrochemical detector (Waters 460) set at 0.8 V. The mobile phase consisted of 63 mM NaH_2PO_4, 0.73 mM sodium octyl sulfate, 0.37 nM disodium EDTA, and 10% acetonitrile. The pH value of the mobile phase was adjusted to 3.0 with 85% phosphoric acid. The mobile phase was then filtered through a type-HA filter (0.45 μm), deaerated by stirring under vacuum for 15 min, and pumped at a flow rate of 0.8 ml/min through an Econosphere C20 column (dimensions: 4.6 × 250 mm; particle size: 5 μm). In addition, a precolumn with the same stationary phase as the analytical column was employed, and standard curves were prepared for each analytical run.

Statistics

The place preference data were analyzed by the Wilcoxon matched-pairs signed-ranks test because the place preference data were not normally distributed. They were found to have a flat or rectangular distribution, with a kurtosis of -0.82 ± 0.68 and a skew of -0.19 ± 0.347. The locomotion, rearing, and defecation data were assessed by ANOVA. Locomotion, rearing, and defecation had two independent factors, side (two levels: drug compartment or vehicle compartment) and drug dose (four levels: 0, 10, 20, or 40). There was also a repeated factor [days with two or 12 levels, depending on whether a test day (2) or the conditioning days (12) were being analyzed]. ANOVA with more than two repeated factors was subjected to a variety of multivariate tests of significance to correct for unreliability due to a lack of homogeneity of covariances, as is standard procedure with the statistical software used [Statistical Package for the Social Sciences for the PC (SPSSPC)]. Significant ANOVA results are reported only when verified by these additional tests. Significant main effects and interactions were followed by individual comparisons by the *F*-test for multiple comparisons, with the critical level of significance at $p < 0.05$.

RESULTS

The Wilcoxon matched-pairs signed-ranks test indicated that only the 10-mg/kg dose of DEP increased the time spent in the "drug" compartment during the conditioning test compared to the time spent in the same compartment on the fifth pretest ($Z = -2.118$, two-tailed $p = 0.034$). The 0 ($Z = -0.7845$, $p = 0.432$), 20 ($Z = -1.412$, $p = 0.158$), and 40 ($Z = -0.267$, $p = 0.790$) -mg/kg doses did not produce CPP or conditioned place aversion (Fig. 1). Table 1 shows pretest and test times spent in the drug compartment.

ANOVA showed a dose × compartment × day interaction for locomotion during the Part 2 conditioning [$F(33, 473) = 2.50$, $p < 0.001$] (Figs. 2 and 3), and individual comparisons showed that the 10-mg/kg dose produced significantly more locomotion in rats than all other doses on all drug days; also, more locomotion was observed in this group on drug days compared to vehicle days ($p < 0.05$). The 40-mg/kg group had less locomotor activity than vehicle on all but 4 drug days, and less locomotion was observed in this group on drug days than on vehicle days. Rearing during Part 2 was associated with a significant dose × compartment × day interaction [$F(33, 473) = 1.67$, $p < 0.013$] (Figs. 4 and 5). Individual comparisons revealed that the 20- and 40-mg/kg groups had significantly less rearing than the vehicle group on all drug days, and less rearing was observed in these groups on drug days than on vehicle days. The 10-mg/kg group displayed significantly less rearing than vehicle on all drug days with the exception of the first, and significantly less rearing on drug days than on vehicle days. The 20- and 40-mg/kg doses also resulted in a significant increase in defecation over that ob-

TABLE 2

MEAN (±SEM) LEVELS (ng/g TISSUE) OF NE, DA, 5-HT, AND METABOLITES HVA, DOPAC, AND 5-HIAA IN WHOLE RAT BRAIN AFTER 12 TREATMENTS OF DIETHYLPROPION OVER 36 DAYS

	Vehicle	10 mg/kg	20 mg/kg	40 mg/kg
NE	393 ± 21	357 ± 16	385 ± 15	403 ± 19
DA	744 ± 15	696 ± 14	704 ± 19	702 ± 23
5-HT	376 ± 20	361 ± 16	337 ± 11	346 ± 21
HVA	64 ± 7	68 ± 10	55 ± 6	53 ± 8
DOPAC	75 ± 3	76 ± 3	79 ± 2	81 ± 4
5-HIAA	404 ± 53	468 ± 63	373 ± 48	328 ± 42

served after the 0- and 10-mg/kg doses, and there was a dose × day interaction [$F(3, 43) = 10.78, p < 0.001$].

In the test for conditioned behaviours, locomotion, rearing, and defecation on the vehicle side of the cage on the vehicle day preceding the test were compared to behaviours on the drug side of the cages on the following day. For the conditioning test, all animals were injected with vehicle and then confined to the "drug-associated" compartment of the boxes. ANOVA revealed a dose × day interaction for locomotor activity (Fig. 6) [$F(3, 43) = 3.32, p < 0.028$], rearing (Fig. 7) [$F(3, 43) = 4.28, p < 0.01$], and defecation (Fig. 8) [$F(3, 43) = 7.55, p < 0.001$]. Individual comparisons showed that only the 10-mg/kg dose resulted in conditioned locomotion. The 10- and 40-mg/kg groups both exhibited an increase in rearing although a drug injection of either dose itself significantly decreased rearing. Only the 40-mg/kg group showed a conditioned increase in defecation.

Individual comparisons of locomotor activity in the 10-mg/kg group from the 1st to the 12th drug day showed that sensitization occurred in the latter stages of the experiment in comparison to the initial drug days (Fig. 2). ANOVA of the sensitization test for the 5-mg/kg challenge injection of DEP given to all groups, comparing the last drug day to the challenge injection day, revealed a dose × day interaction for locomotion (Fig. 9) [$F(3, 43) = 5.42, p < 0.003$], rearing (Fig. 10) [$F(3, 43) = 6.99, p < 0.001$], and defecation (Fig. 11) [$F(3, 43) = 4.62, p < 0.007$]. Following the 5-mg/kg challenge injection, the 20- and 40-mg/kg dose groups had significantly greater locomotor activity than on the last drug day, and the 10-mg/kg group had an equal amount of activity in comparison to the last drug day. All three drug groups displayed a significantly greater amount of rearing in response to the challenge injection than with the usual dose, and rats previously receiving vehicle had significantly less rearing following the challenge injection. The challenge injection resulted in significantly less defecation in the 40-mg/kg group, and all other groups were unaffected.

The rats were weighed every 2nd day during conditioning and testing, for a total of 13 weighings during the entire 36 days. ANOVA of the weight data gave a dose × days interaction (Fig. 12) [$F(36, 516) = 4.15, p < 0.001$]. Individual comparisons showed that the 40-mg/kg group weighed significantly less than the other groups from day 14–36, and that the 20-mg/kg group weighed less than the 0- and 10-mg/kg groups from day 32–36.

ANOVA of whole-brain levels of NE, DA, 5-HT, or the metabolites HVA, DOPAC, and 5-HIAA, showed that there were no significant differences from control levels (Table 2).

DISCUSSION

The results of this study are the first demonstration of the production of stimulant-like conditioned effects and behavioural sensitization within an animal model for the centrally acting appetite-suppressing drug, DEP. DEP produced behavioural effects at the 10-mg/kg dose that were similar to low doses of psychomotor stimulants such as cocaine and amphetamine, and had an anorectic effect at the 20- and 40-mg/kg doses. The 10-mg/kg dose resulted in CPP, conditioned locomotion, and behavioural sensitization, whereas the 20- and 40-mg/kg doses did not, although the 40-mg/kg dose produced conditioned defecation. However, the two highest doses did produce behavioural sensitization in locomotion and rearing in response to a 5-mg/kg challenge injection. Animals receiving 20 or 40 mg/kg of DEP gained significantly less weight than the other groups, and this effect became more pronounced as the days passed. These two highest doses also significantly increased defecation, suggesting that the decreased weight gain associated with these two doses could be attributed to either gastrointestinal effects or decreased food intake. Repeated treatments with DEP had no effect on whole-brain levels of the neurotransmitters NE, DA, and 5-HT, or the metabolites HVA, DOPAC, and 5-HIAA, in brains after cessation of drug treatment, indicating that DEP is probably not toxic to monoamine neurons at doses of 10, 20, or 40 mg/kg in the rat, using the present treatment regimen. This issue is important because of the recent suggestion that DEP may produce irreversible damage to 5-HT neurons (4).

These results are in agreement with the findings of others that central stimulants induce conditioned behaviours (25, 31,34) and behavioural sensitization (1,27,32). Conditioned defecation, as observed with DEP (40 mg/kg), has also been observed with AMP (1.5 mg/kg) (9).

In the present experiment, doses that decreased weight gain (20 and 40 mg/kg) also decreased spontaneous locomotor activity and rearing, possibly due to an increase in stereotypy. These doses did not produce conditioned locomotor activity, although behavioural sensitization in response to a lower dose of DEP (5 mg/kg) did occur after pretreatment with all three doses of DEP. Whereas in humans, stimulant-like effects appear to occur at high doses and anorectic effects at low doses (7,8,14,16,33), the results of the present experiment imply the opposite effect in rats, at least in some respects. The low dose (10 mg/kg) produced typical psychomotor stimulant effects such as increased locomotion, CPP, conditioned increases in locomotor activity, and behavioural sensitization of locomotion and rearing. The two higher doses (20 and 40 mg/kg) resulted in decreased weight gain, although in response to a challenge injection of DEP (5 mg/kg), sensitization of locomotion and rearing also occurred at the two highest doses.

Sensitization to stimulants has been proposed as an animal model of stimulant psychoses (1,27,32). Psychosis resulting from DEP abuse have been reported in humans (7,8,16). The present observation that DEP can also produce behavioural sensitization strengthens the relationship between stimulant psychosis and behavioural sensitization. The results suggest that DEP has stimulant-like properties that resemble those induced by AMP and cocaine.

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REFERENCES

1. Angrist, B. Psychoses induced by central nervous system stimulants and related drugs. In: Creese, I., ed. Stimulants: Neurochemical, behavioural, and clinical perspectives. New York: Raven Press; 1983:1-30.
2. Beninger, R. J.; Hahn, B. Pimozide blocks establishment but not expression of amphetamine-produced environment-specific conditioning. Science 220:1304–1306; 1983.
3. Beninger, R. J.; Herz, R. S. Pimozide blocks establishment but not expression of cocaine-produced environment-specific conditioning. Life Sci. 38:1425–1431; 1986.

4. Byrne, A.; Zibin, T. Neurotoxicity associated with diethylpropion abuse. Am. J. Psychiatry (submitted).
5. Cahen, R.; Montagne, S.; Pessonier, J. Pharmacological study of 2-diethylaminopropiophenone. IV. Central sympathomimetic action. Therapie 17:395–403; 1962.
6. Carabillo, E. A. Jr. USA Drug Abuse Warning Network. In: Garattini, S.; Samanin, R., eds. Central mechanisms of anorectic drugs. New York: Raven Press; 1978:461–471.
7. Clein, L. J.; Benady, D. R. Case of diethylpropion addiction. BMJ 2:456; 1962.
8. Cox, T. C.; Jacobs, M. R.; LeBlanc, A. E.; Marshman, J. A. Diethylpropion. In: Cox, T. C.; Jacobs, M. R.; LeBlanc, A. E.; Marshman, J. A., eds. Drugs and drug abuse. Toronto: Alcoholism and Drug Addiction Research Foundation; 1983:238–242.
9. DiLullo, S. L.; Martin-Iverson, M. T. Presynaptic dopaminergic neurotransmission mediates amphetamine-induced unconditioned but not amphetamine-conditioned locomotion and defecation in the rat. Brain Res. 568:45–54, 1991.
10. DiLullo, S. L.; Martin-Iverson, M. T. Evidence for presynaptic dopamine mechanisms underlying amphetamine-conditioned locomotion. Brain Res. 578:161–167; 1992.
11. Garattini, S.; Borroni, E.; Mennini, T.; Samanin, R. Differences and similarities among anorectic agents. In: Garattini, S.; Samanin, R., eds. Central mechanisms of anorectic drugs. New York: Raven Press; 1978:127–143.
12. Goodnough, D. B.; Baker, G. B. Tranylcypromine does not enhance the effects of amytriptyline on 5-HT_2 receptors in rat cerebral cortex. J. Pharm. Sci. 83:100–103; 1994.
13. Gotestam, K. G. An animal model to test the abuse potential of anorectic drugs. In: Garattini, S.; Samanin, R., eds. Central mechanisms of anorectic drugs. New York: Raven Press; 1978: 329–342.
14. Hoekenga, M. T.; O'Dillon, R. H.; Leyland, H. M. A comprehensive review of diethylpropion hydrochloride. In: Garattini, S.; Samanin, R., eds. Central mechanisms of anorectic drugs. New York: Raven Press; 1978:391–404.
15. Johanson, C. E.; Schuster, C. R. A comparison of cocaine and diethylpropion under two different schedules of drug presentation. Presentation at conference on contemporary issues in stimulant research, Duke University Medical Center, 10–12 Nov. 1975. (Cited in Hoekenga et al.; 1978.)
16. Kuenssberg, E. V. Diethylpropion (correspondence). BMJ 2:729–730; 1962.
17. Kutscher, C. L. Action of fenfluramine, phenylpropanolamine, phentermine and diethylpropion on acoustic startle in rats. Pharmacol. Biochem. Behav. 27:749–752; 1987.
18. LeDourec, J. C.; Schmitt, H. Pharmacologic comparison of seven anorectic drugs. Therapie 19:831–841; 1964.
19. Martin-Iverson, M. T.; Ortmann, R.; Fibiger, H. C. Place preference conditioning with methylphenidate and nomifensine. Brain Res. 332:59–67; 1985.
20. Mithani, S.; Martin-Iverson, M. T.; Phillips, A. G.; Fibiger, H. C. The effects of haloperidol on amphetamine- and methylphenidate-conditioned place preferences and locomotor activity. Psychopharmacology 90:247–252; 1986.
21. Muntaner, C.; Cascella, N. G.; Kumor, K. M.; Nagoshi, C.; Herning, R.; Jaffe, J. Placebo responses to cocaine administration in humans: Effects of prior administrations and verbal instructions. Psychopharmacology 99:282–286; 1989.
22. O'Brien, C. P.; Childress, A. R.; Arndt, I. O.; McLennan, A. T.; Woody, G. E.;, Maany, I. Pharmacological and behavioral treatment of cocaine dependence: Controlled studies. J. Clin. Psychiatry 49:17–22; 1988.
23. Offermeier, J.; Potgieter, B. The possible central stimulant mechanisms of some appetite suppressants. S. Afr. Med. J. 46:72–77; 1972.
24. Offermeier, J.; duPreez, H. G. Effects of anorectics on uptake and release of monoamines in synaptosomes. In: Garattini, S.; Samanin, R., eds. Central mechanisms of anorectic drugs. New York: Raven Press; 1978:217–231.
25. Pickens, R. W.; Crowder, W. F. Effects of CS-US interval on conditioning of drug response with assessment of speed of conditioning. Psychopharmacologia (Berlin) 11:88–94, 1967.
26. Reicher, M. A.; Holman, E. W. Location preference and flavor aversion reinforced by amphetamine in rats. Anim. Learn. Behav. 5:343–346, 1977.
27. Robinson, T. E.; Becker, J. B. Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. Brain Res. Rev. 11:157–198; 1986.
28. Ross, S. B. Dopamine can be released by two mechanisms differentially affected by the dopamine transport inhibitor nomifensine. J. Pharmacol. Exp. Ther. 208:195–202; 1979.
29. Samanin, R.; Bernasconi, S.; Garattini, S. The effect of selective lesioning of brain catecholamine-containing neurons on the activity of various anorectics in the rat. Eur. J. Pharmacol. 34:373–375; 1975.
30. Scheel-Kruger, J. Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines of the brain. Eur. J. Pharmacol. 14: 47–59; 1971.
31. Schiff, S. R. Conditioned dopaminergic activity. Biol. Psychiatry 17:135–155; 1982.
32. Segal, D. S.; Schuckit, M. A. Animal models of stimulant-induced psychosis. In: Creese, I., ed. Stimulants: Neurochemical, behavioural, and clinical perspectives. New York: Raven Press; 1983:131–167.
33. Silverstone, T. Appetite suppressants. Drugs 43:820–836; 1992.
34. Spyraki, C.; Fibiger, H. C.; Phillips, A. G. Cocaine-induced place preference conditioning: Lack of effect of neuroleptics and 6-hydroxydopamine lesions. Brain Res. 253:195–203; 1982.
35. Sterner, N.; Widbom, K. Central stimulant properties of four anorexigenic drugs. Acta Pharm. Suec. 4:37–50; 1967.
36. Sullivan, A. C.; Comai, K. Pharmacological treatment of obesity. Int. J. Obesity 2:167–189; 1978.
37. Swerdlow, N. R.; Gilbert, D.; Koob, G. F. Conditioned drug effects on spatial preference: Critical evaluation. In: Boulton, A. A.; Baker, G. B.; Greenshaw, A. J., eds. Neuromethods. Vol. 13: Psychopharmacology. Clifton, NJ: Humana Press; 1989:399–446.
38. Weissman, A.; Koe, B. K.; Tenen, S. S. Anti-amphetamine effects following inhibition of tyrosine hydroxylase. J. Pharmacol. Exp. Ther. 151:339–352; 1966.