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Diethylpropion produces psychostimulant and reward effects

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ABSTRACT

Diethylpropion (DEP) is a stimulant drug widely used for weight control in Brazil and other American countries. However, its effects on behavior and addiction potential are not yet well known. Data suggest that sensitization resulting from pre-exposure to psychostimulants could be a possible risk factor in subsequent drug addiction. The purpose of this investigation was to verify whether pre-exposure to DEP would sensitize rats to the motor activating effect and to the rewarding value of DEP. Two experiments were conducted. In both experiments rats were pre-exposed to DEP (20 mg/kg) or vehicle for 7 consecutive days. The acute effect of DEP (0.0, 1.0, 2.5 or 5.0 mg/kg) on motor activity (Experiment 1) and induction of Conditioned Place Preference—CPP (Experiment 2) were then measured. Results from Experiment 1 showed that 2.5 and 5.0 mg/kg DEP increased motor activity. Sensitization of this motor effect was observed. In Experiment 2, the doses of 2.5 and 5.0 mg/kg DEP induced CPP, indicating their rewarding value. However, no sensitization effect was observed. The results suggest that DEP at low doses has psychostimulant and rewarding properties. It is recommended that more effort should be dedicated to elucidating DEP abuse potential.

1. Introduction

Diethylpropion—DEP-(2-[diethylamino] propiophenone), also known as amfepramone, is an amphetamine-like anorectic stimulant drug widely used in Brazil and other American countries for weight control and medical treatment of obesity (Behar, 2002; International Narcotics Control Board, 2007; Lima et al., 1998; Noto et al., 2002). DEP misuse has been reported among adults and secondary school students, is often abused by drug addicts and is frequently targeted by drug traffickers (Dal Pizzol et al., 2006; Galduróz et al., 2005; International Narcotics Control Board, 2006; Nappo et al., 2002).

DEP has neural effects similar to amphetamine (AMPH): enhancement of norepinephrine (NE) and dopamine (DA) release and inhibition of reuptake of these neurotransmitters (Da Silva and Cordellini, 2003; Samanin and Garattini, 1993). However, DEP is less potent than AMPH in producing these effects (Offermeier and du Preez, 1978). DEP also enhances serotonin (5-HT) neurotransmission, but to a lesser extent than for NE and DA neurotransmission (Garantini et al., 1978). Anorectic properties of DEP seem to be related to its DA and NE effects (Samanin and Garattini, 1993), whereas its motor and reinforcing properties have been associated to DA and 5-HT effects (Gevaerd et al., 1999a; Mello et al., 2005; Planeta and DeLucia, 1998).

In spite of its widespread use, no sufficient data are available on the behavioral effects of DEP. It has been reported that moderate doses of DEP (5.0–15.0 mg/kg) increase motor activity (Da Silva and Cordellini, 2003; Gevaerd et al., 1999b; Reimer et al., 1995) and that 7.5 mg/kg DEP produces similar indices of motor activity as 2.5 mg/kg of AMPH (Garantini et al., 1978). At higher doses (25.0, 50.0 and 100.0 mg/kg), DEP produced stereotyped movements and EEG activity resembling those observed after high doses of AMPH (Safta et al., 1976). In operant paradigms, Rhesus monkeys reinforced by food delivery under fixed-ratio (FR) and differential reinforcement of low rate (DRL) schedules demonstrate decreased ratio responding when treated with DEP, as well as with cocaine (COC) or AMPH (Johanson and Uhlenhuth, 1978).

DEP abuse potential is also scarcely known. There are some studies in rats showing that 2.0 mg/kg DEP infusion will substitute a 0.25 mg/kg self-administered intravenous (i.v.) infusion of AMPH (Gotestam and Andersson, 1975a,b). Non-human primates also self-administered DEP and, when unlimited access was provided, high levels of drug intake were observed (Griffiths et al., 1978a,b; Johanson, 1978). Giving the opportunity to choose between Vehicle, DEP and COC, rhesus monkeys preferred DEP to vehicle, and COC to DEP. However, when DEP doses were increased, preference for COC was reduced (Johanson and Schuster, 1977). Similar results were obtained when humans were given a choice between vehicle, DEP and AMPH: the participants preferred DEP to vehicle, and AMPH to DEP, but when the DEP dose was increased both drugs were equally chosen (Johanson and

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Uhlenhuth, 1978). Using the conditioned place preference (CPP) paradigm, it has been shown that 10.0 and 15.0 mg/kg of DEP, but not 20.0 or 40.0 mg/kg DEP, induced CPP in rats (Gevaerd et al., 1999a; Mello et al., 2005; Planeta and DeLucia, 1998; Reimer et al., 1995). To our knowledge, lower doses of DEP have not yet been tested under this paradigm.

Sensitization to the rewarding effects of drugs has been proposed as one of the mechanisms of drug abuse (Robinson, 1993; Schenk and Davidson, 1998). Specifically, data suggest that sensitization is involved in acquisition and reinstatement of self-administration (Koob and Le Moal, 1997; Robinson and Berridge, 2001). Even though it has been reported that pre-exposure to 20.0 and 40.0 mg/kg of DEP sensitized the effect of this drug on motor activity (Reimer et al., 1995), to our knowledge there are no data reporting the effect of pre-exposure to DEP on its rewarding effects. Therefore, the current study assessed whether low doses of DEP would have motor stimulant effects and could acquire rewarding properties according to the CPP paradigm. Besides, it was evaluated if pre-exposure to DEP sensitized the subjects to the drug's effect on motor activity and CPP.

2. Methods

2.1. Subjects

Naive male Wistar rats were obtained from the Instituto Butantan—Central Biotery (São Paulo, SP) with a mean initial weight of 270 g. The animals were housed two per cage in semi-transparent plastic home-cages (414×344×168 mm). The lights in the colony room were on from 7:00 a.m. to 7:00 p.m. During the experiment food and water were freely available.

All experimental procedures involving the subjects followed the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Apparatus

Activity tests were conducted in a Plexiglas activity chamber (432×432×305 mm) manufactured by Med Associates (ENV-515, St. Albans, VT). An array of three 16×16×16 photodetectors, spaced 25 mm apart, was used to detect motor activity. The CPP procedure was conducted in a Plexiglas two-compartment place preference insert manufactured by Med Associates (ENV-517, St. Albans, VT), covering the entire area of the activity chamber. One compartment of the insert had a stainless-steel wire mesh floor (16-mm wire with 127-mm openings) and its walls were covered in white film, while the other compartment had stainless-steel bars floor (48-mm rods placed 16 mm apart) and walls covered in black film. The lid was covered with black standard cardboard. The compartments were separated by an arched doorway with a manual guillotine door. The array of three 16×16×16 photodetectors was used to measure the amount of time that the subjects spent in each area.

2.3. Drug

DEP (Galena Quimica e Farmacêutica, São Paulo, SP) was dissolved in a 0.9% NaCl solution. NaCl solution was also used in all vehicle and 0.0 mg/kg DEP injections. All injections were given intraperitoneally (i.p.) at a volume of 1.0 ml/mg.

2.4. Procedure

2.4.1. Experiment 1: motor activity

Experiment 1 tested the effect of acute low doses of DEP on motor activity and evaluated if this effect was altered by pre-exposure to the drug. The overall design is summarized in Table 1. The experiment was carried out in two steps. (1) *Pre-exposure*. Rats were randomly assigned to one of the 8 groups shown in Table 1. Animals in PE 0.0, PE 1.0, PE 2.5, and PE 5.0 groups (PE groups) received daily injections of 20.0 mg/kg of DEP for 7 consecutive days. Animals in NPE 0.0, NPE 1.0, NPE 2.5, and NPE 5.0 (NPE groups) received injections of vehicle on the same days (days 1–7). After the injections animals remained in their home cages for a further 7 days without treatment (days 8–14). The whole Pre-exposure procedure was conducted in the colony room. (2) *Motor Activity*. On days 15 and 16 each rat was placed in the activity chamber for 15 min, and no measures were taken. On day 17 (*Vehicle*) each animal received a vehicle injection, was returned to its home cage and after a 15-min waiting period was placed in the activity chamber for another 15 min. On day 18 (*Challenge*) the procedure of day 17 was repeated, except that animals in PE 0.0 and in NPE 0.0 were injected with 0.0 mg/kg DEP; animals in PE 1.0 and in NPE 1.0 were injected with 1.0 mg/kg DEP; animals in PE 2.5 and in NPE 2.5 were injected with 2.5 mg/kg DEP, and animals in PE 5.0 and in NPE 5.0 were injected with 5.0 mg/kg DEP. On every Motor Activity day rats were taken from the colony room and transported to the experimental room, where treatments were initiated after a mandatory 10-min acclimatization period to the room.

Table 1
Treatment groups for motor activity (Experiment 1)

Group	Treatment days					
	1–7[a]	8–14[a]	15–16[b]	17[b]	18[b]	*n*
NPE 0.0	Vehicle	–	–	Vehicle	Diethylpropion 0.0 mg/kg	5
PE 0.0	Diethylpropion 20 mg/kg	–	–	Vehicle	Diethylpropion 0.0 mg/kg	5
NPE 1.0	Vehicle	–	–	Vehicle	Diethylpropion 1.0 mg/kg	5
PE 1.0	Diethylpropion 20 mg/kg	–	–	Vehicle	Diethylpropion 1.0 mg/kg	5
NPE 2.5	Vehicle	–	–	Vehicle	Diethylpropion 2.5 mg/kg	5
PE 2.5	Diethylpropion 20 mg/kg	–	–	Vehicle	Diethylpropion 2.5 mg/kg	5
NPE 5.0	Vehicle	–	–	Vehicle	Diethylpropion 5.0 mg/kg	6
PE 5.0	Diethylpropion 20 mg/kg	–	–	Vehicle	Diethylpropion 5.0 mg/kg	6

[a] Days 1–7 and 8–14 were conducted in the home cage. On days 1–7 animals were pre-exposed to Diethylpropion or Vehicle. On days 8–14 animals remained without treatment.
[b] Days 15–16, 17 and 18 were conducted in the activity chamber. On days 15–16 animals were placed in the activity chamber for 15 min. On day 17 animals were given Vehicle and placed in the activity chamber for 15 min. On day 18 animals were challenged with Diethylpropion or Vehicle and placed in the activity chamber for 15 min.

2.4.2. Experiment 2: Conditioned Place Preference

Experiment 2 assessed the effect of low doses of DEP in the CPP paradigm, and evaluated if drug-induced CPP was changed by pre-exposure to the drug. The experiment was carried out in two steps. (1) *Pre-exposure*. Rats were randomly assigned to one of the 8 groups shown in Table 2. The procedure of days 1–7 was the same as described in Experiment 1. After the injections animals remained 4 days without any treatment (days 8–11). (2) *Conditioned Place Preference*. An unbiased CPP procedure was used, consisting of three phases: Preconditioning, Conditioning and Post-conditioning. In Preconditioning (days 12–14), animals were placed for 15 min in the CPP apparatus and free access to both compartments was allowed. A first measure of time spent in each compartment was taken on day 14 (PRE). At this point, two subjects were removed from the experiment (one for PE 2.5 group and one from PE 5.0 group) because they showed a strong preference for one side of the chamber. These subjects were not included in Table 2. In Conditioning (days 15–18), eight sessions were run, two sessions per day. In the first session of the day, each rat received a vehicle injection before being confined for 30 min to one

Table 2
Treatment groups for Conditioned Place Preference (Experiment 2)

Group	Treatment days					
	1–7[a]	8–11[a]	12–14[b]	15–18[b]	19[b]	*n*
NPE 0.0	Vehicle	–	–	Vehicle/Diethylpropion 0.0 mg/kg	–	5
PE 0.0	Diethylpropion 20 mg/kg	–	–	Vehicle/Diethylpropion 0.0 mg/kg	–	5
NPE 1.0	Vehicle	–	–	Vehicle/Diethylpropion 1.0 mg/kg	–	9
PE 1.0	Diethylpropion 20 mg/kg	–	–	Vehicle/Diethylpropion 1.0 mg/kg	–	9
NPE 2.5	Vehicle	–	–	Vehicle/Diethylpropion 2.5 mg/kg	–	9
PE 2.5	Diethylpropion 20 mg/kg	–	–	Vehicle/Diethylpropion 2.5 mg/kg	–	8
NPE 5.0	Vehicle	–	–	Vehicle/Diethylpropion 5.0 mg/kg	–	9
PE 5.0	Diethylpropion 20 mg/kg	–	–	Vehicle/Diethylpropion 5.0 mg/kg	–	8

[a] Days 1–7 and 8–14 were conducted in home cage. On days 1–7 animals were pre-exposed to Diethylpropion or vehicle. On days 8–11 animals remained without treatment.
[b] Days 15–16, 17 and 18 were conducted in the CPP apparatus. On days 12–14 and 19 animals were placed in the CPP apparatus for 15 min and free access to both compartments was allowed. On days 15–18 vehicle was paired with one compartment and Diethylpropion was paired with the other compartment.

compartment of the chamber (vehicle-paired compartment). Immediately after this, in the second session, each rat was injected with DEP and confined for 30 min in the other compartment (drug-paired compartment). Animals in PE 0.0 and in NPE 0.0 were injected with 0.0 mg/kg DEP; animals in PE 1.0 and in NPE 1.0 were injected with 1.0 mg/kg DEP; animals in PE 2.5 and in NPE 2.5 were injected with 2.5 mg/kg DEP, and animals in PE 5.0 and in NPE 5.0 were injected with 5.0 mg/kg DEP. Half of the animals of each group receiving DEP were placed in the white compartment and the other half in the black compartment. Post-conditioning was run on day 19 (POST), conducting the same PRE procedure.

2.5. Data analysis

Med-Associates Activity Monitor (ver. 4.31) software was used to obtain motor activity and place preference measures. This software allows a distinction between repetitive interruptions of the same photobeam and interruptions of adjacent photobeams; the latter was used as a measure of motor activity. Time spent in vehicle-paired and drug-paired compartments was recorded for each rat on days 14 (PRE) and 19 (POST) in order to determine CPP. Since measures obtained from vehicle- and drug-paired compartments were complementary, the latter were used for the statistical analysis. Measurements from the first minute of each session were excluded from the analysis because this time is typically characterized by exploratory behavior not related to the experimental variables (Garcia-Mijares and Silva, in press).

In Experiment 1, the dependent variable Motor Activity was expressed as the difference in number of photobeam interruptions between Vehicle and Challenge Day. In Experiment 2, the dependent variable Preference was expressed as the difference in time spent in the drug-paired compartment between *PRE*-conditioning and *POST*-conditioning sessions. These variables were first analyzed by two-way ANOVA using drug DOSE (0.0, 1.0, 2.5 and 5.0 mg/kg of DEP) and pre-exposure GROUP (PE and NPE) as between-subjects factor. When significant effects were found for DOSE, The post-hoc Dunnet's test was performed using 0.0 mg/kg of DEP as the control category.

Limits of 90% confidence intervals (CI) of the standardized effects sizes (Cohen's f^2 for ANOVA effects and Cohen's f for post-hoc comparisons effects) are reported. Cohen's suggestion for interpretation of effect sizes was used as indicative of the strength of the effects (Cohen, 1992).

3. Results

Means (±SE) of weights on pre-exposure days from animals in Experiments 1 and 2 are shown in Fig. 1. The data of the NPE groups showed the typical weight gain over time described for young adult rats in ad lib feeding condition (Tomanari et al., 2003). Conversely, rats treated with 20.0 mg/kg DEP (PE group) apparently did not gain weight during the pre-treatment days. It could also be noted in this Figure that four days after DEP withdrawal, rats from the PE group gained weight.

3.1. Experiment 1: motor activity

Fig. 2 shows the means (±SE) of motor activity, expressed as the difference in photobeam interruptions between *Vehicle* and *Challenge* Day for (a) pre-exposure groups NPE and PE, (b) DEP doses (0.0, 1.0, 2.5 and 5.0 mg/kg) and (c) pre-exposure groups at each drug dose. As indicated in Fig. 2a, ANOVA results were significant for differences in motor activity between pre-exposure groups ($F_{(1,34)}=5.71$; $p=0.02$). The 90% CI of the standardized effect size parameter (f^2) ranged from 0.02 to 0.40, indicating that the effect of pre-exposure to DEP on motor activity varied from small to strong. Therefore, although DEP pre-exposure resulted in a true effect, a larger sample size is needed to accurately assess its magnitude.

As shown in Fig. 2b, a significant main effect on motor activity was obtained for DEP doses ($F_{(3,34)}=13.90$; $p=0.00$). The 90% CI for the size of the dose effect ranged from 0.42 to 1.46, indicating that motor activity strongly depended on drug dose. Dunnet's post-hoc test showed that administration of 2.5 and 5.0 mg/kg of DEP significantly enhanced motor activity ($p=0.00$ for both DEP doses compared to 0.0 mg/kg). This effect was robust for both DEP doses as indicated by the limits of the 90% CI of the effect sizes (1.07–2.50 for 2.5 mg/kg and 0.82–2.07 for 5.0 mg/kg). No significant differences in motor activity were observed between 1.0 mg/kg DEP and vehicle ($p=0.96$). However, as the upper limit of the 90% CI of the effect size (0.00–0.50) did not exclude the possibility of a true effect, the data from this dose was inconclusive.

Fig. 2c shows that the PE group had higher means at every DEP dose than the NPE group, suggesting a shift of the dose–response curve to the left. This is consistent with the ANOVA results that showed no interaction effect between pre-exposure groups and drug dose ($F_{(3,34)}=1418$; $p=0.255$). The 90% CI (0.0–0.014) of Cohen's parameter f^2 also indicated that the interaction effect is indeed very weak.

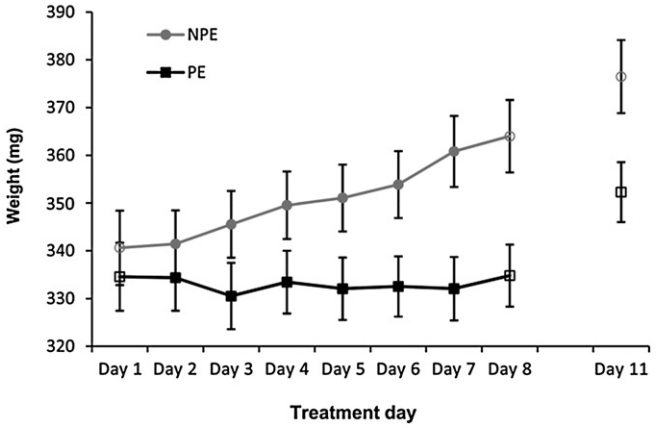

Fig. 1. Effect of DEP on body weight. Lines represent means (±SE) weights (mg) for each pre-exposure group NPE and PE. Closed marks represent weights on pre-exposure days. Open marks represent weights on the first (day 1) and after (day 8 and day 11) the last day of drug administration. On pre-exposure days animals from the NPE group were injected with vehicle and animals from PE group were injected with 20.0 mg/kg DEP.

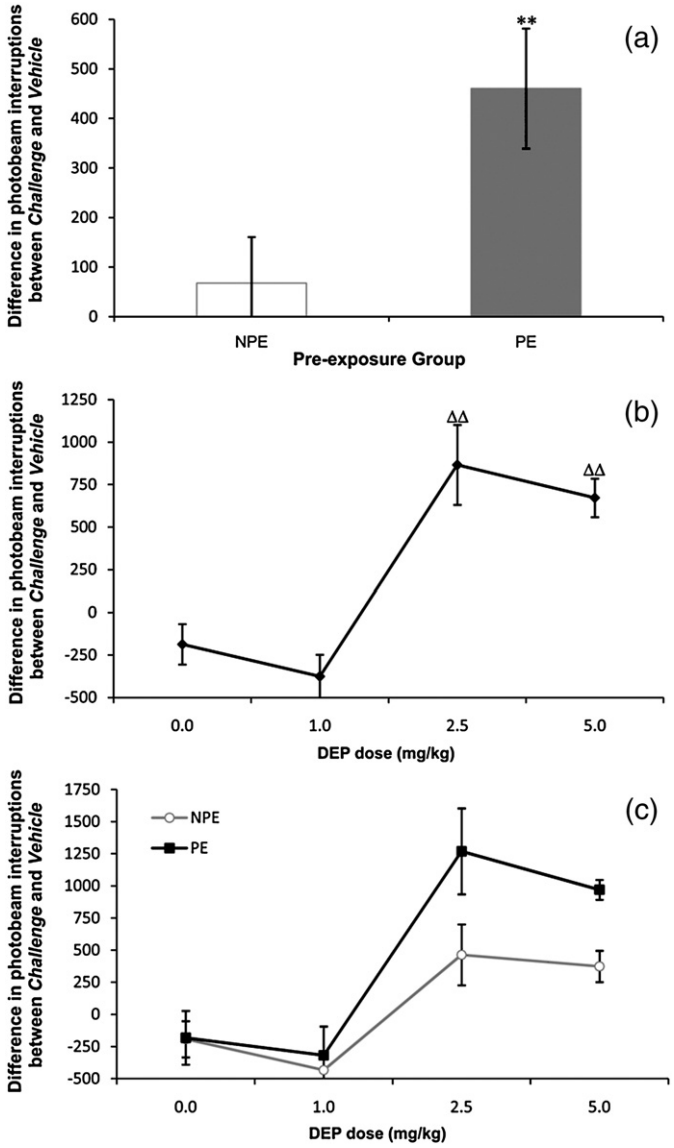

Fig. 2. Effect of DEP on motor activity. Left ordinates represent means (±SE) of the difference in photobeam interruptions between day 17 (*Vehicle*) and day 18 (*Challenge*) for (a) each pre-exposure group (NPE and PE), (b) each challenge DEP dose (0.0, 1.0, 2.5, 5.0 mg/kg) and (c) each pre-exposure group at each challenge DEP dose. Before testing, animals in NPE groups were pretreated with vehicle while rats in PE groups were pretreated with 20.0 mg/kg DEP, for seven consecutive days. (a) $^{*}p<0.05$ for significant main differences between groups in two-way ANOVA, (b) $^{\Delta\Delta}p<0.01$ for differences between drug doses in Dunnet's post-hoc test using 0.0 mg/kg as control.

3.2. Experiment 2: Conditioned Place Preference

The data on Preference values (difference in time spent in the drug-paired compartment between *PRE*-conditioning and *POST*-conditioning sessions) are shown in Fig. 3. Fig. 3a shows Preference means (±SE) of each pre-exposure group (NPE and PE). No group effect was evidenced by the ANOVA ($F_{(1,54)}=0.33$; $p=0.57$), nor by the limits of the 90% CI of the size effect parameter f^2(0.00–0.02).

As can be seen in Fig. 3b, changes in preference were dependent on DEP doses ($F_{(3,54)}=2.99$; $p=0.04$). The 90% CI of the f^2 parameter ranged from 0.05–0.26 indicating that the data are compatible with small to medium drug dose effect on changes in preference. Dunnet`s post-hoc test revealed that 2.5 and 5.0 mg/kg ($p=0.02$ and $p=0.04$, respectively), but not 1.0 mg/kg ($p=0.52$) of DEP, enhanced preference for the drug-paired compartment (i.e., induced CPP). The 90% CI for the size effects of DEP doses suggested a medium to strong effect for 5.0 mg/kg DEP (0.17–1.14) and a small to strong effect for 2.5 mg/kg DEP (0.11–1.08). On the other hand, the range of the 90% CI of size effect of DEP 1.0 mg/kg was wide (0–0.18) and included the possibility of a small association between this dose and Preference.

Fig. 3c shows Preference means (±SE) of each pre-exposure groups NPE and PE for each DEP dose (0.0, 1.0, 2.5 and 5.0 mg/kg). The ANOVA revealed that the interaction effect between these variables was not significant ($F_{(1,54)}=0.33$; $p=0.57$). The limits of the 90% CI of the effect size (0–0.03) indicate that this effect is not substantial.

It is noteworthy that the mean differences between time spent in the drug-paired compartment on *PRE* and *POST*-conditioning days were 67.24 s (±22.09) and 98.89 s (±23.66) for 2.5 and 5.0 mg/kg DEP, respectively (Fig. 3b). As this represents just 8.0% and 11.8% of the *PRE*-conditioning value, we may question whether a difference of

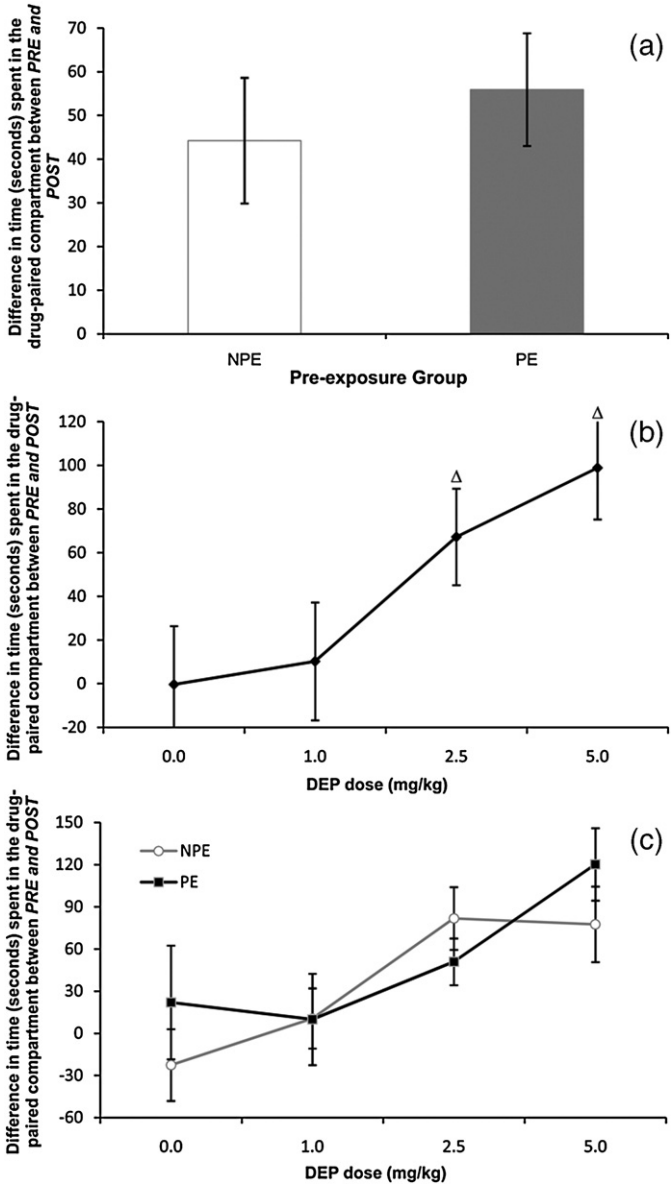

Fig. 3. CPP induced by DEP. Left ordinates represent means (±SE) of the difference in time spent in the drug-paired compartment between *PRE*-conditioning and *POST*-conditioning sessions for (a) each pre-exposure group (NPE and PE), (b) each challenge DEP dose (0.0, 1.0, 2.5, 5.0 mg/kg) and (c) each pre-exposure group at each challenge DEP dose. Before testing, animals in NPE groups were pretreated with vehicle while rats in PE groups were pretreated with 20.0 mg/kg DEP, for seven consecutive days. (b) $^{\Delta}p<0.05$ for differences between drug doses in Dunnet's post-hoc test using 0.0 mg/kg as control.

approximately 1 min constitutes a preference. In order to elucidate this point, a minute to minute descriptive analysis of time spent in each compartment throughout the whole session for each DEP dose, on *PRE* and *POST*-conditioning days, was carried out as shown in Fig. 4. As can be observed in this figure, what seems to be altered by conditioning to DEP is the second per minute distribution in each compartment. Specifically, at 5.0 mg/kg animals spent more time (seconds/minute) in the drug-paired than in the vehicle-paired compartment (Mean=36.8 s±4.6 SE) at almost every minute of the *POST*-conditioning session (12 min out of 15). This pattern was not observed during the *PRE*-conditioning session at 5.0 mg/kg, nor in *PRE* or *POST*-conditioning sessions at 1.0 and 0.0 mg/kg DEP doses.

4. Discussion

The aims of the first experiment were to test acute effects of low doses of DEP on motor activity, and sensitization of this behavior after pre-exposure to DEP. Our results showed that 5.0 mg/kg of DEP

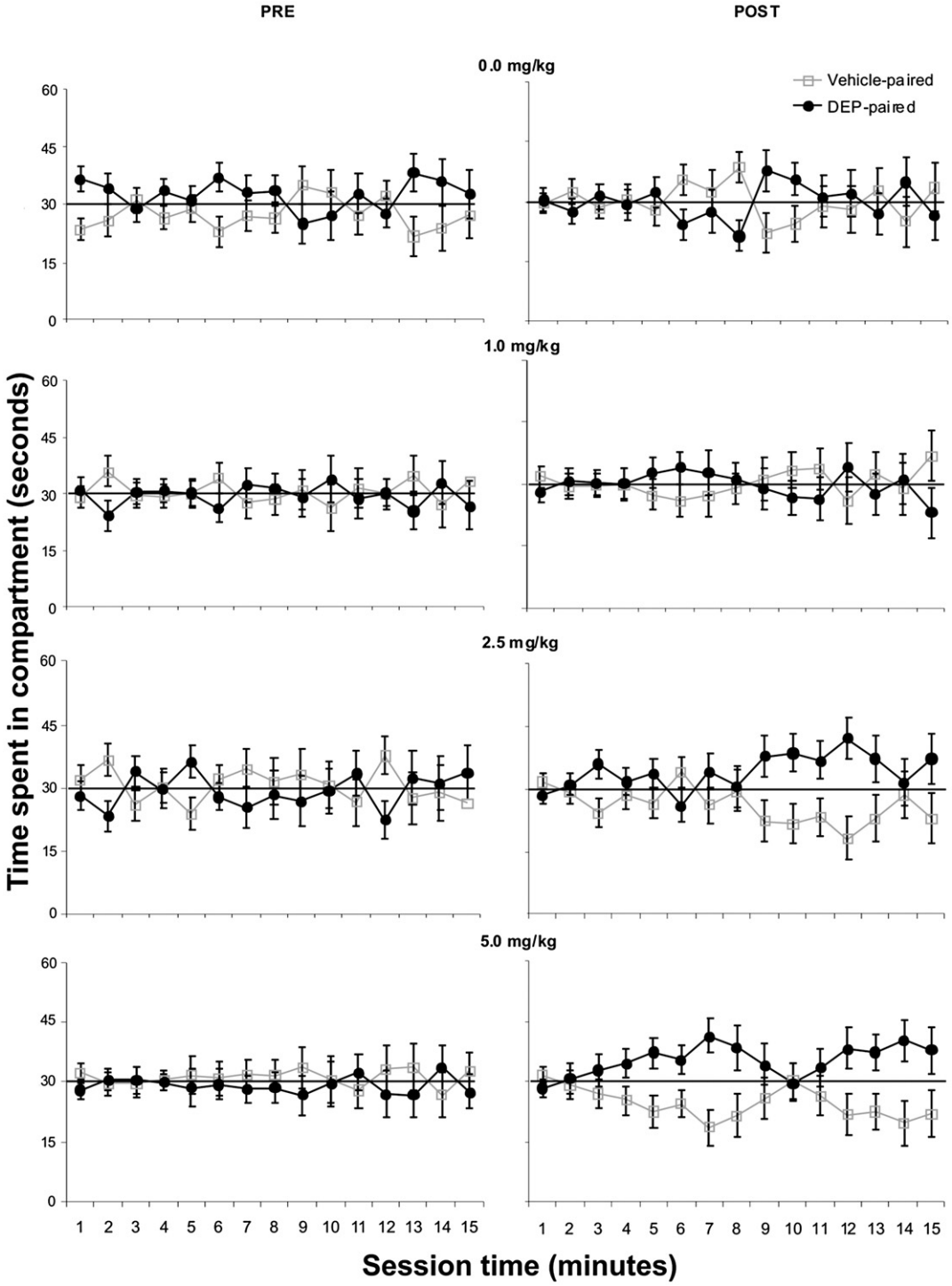

Fig. 4. Minute-to-minute CPP induced by DEP. Lines represent time (seconds per minute) spent in each compartment (vehicle-paired and DEP-paired) throughout the whole preconditioning and post-conditioning sessions, for each conditioning DEP dose (0.0, 1.0, 2.5, 5.0 mg/kg).

enhanced motor activity, consistent with previous reports of the stimulant effect of 5.0 mg/kg of DEP (Garantini et al., 1978; Reimer et al., 1995). In addition, we report that 2.5 mg/kg DEP also had a stimulant effect on motor activity.

Conversely, the data obtained from the lower doses of 1.0 mg/kg DEP were not conclusive, as the estimate limits for CI of the effect size were compatible with the possibility of either nonexistent or strong effect. Thus additional data are needed to allow the effect of this dose to be estimated with greater precision.

It was also seen in Experiment 1 that animals pre-exposed to DEP were sensitized to its motor effects. This is in agreement with the vast body of data on sensitization showing increased motor activity after pre-exposure to stimulants (see Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000 for review) and indirectly complement the data which supports that DEP has neural effects similar to amphetamine (Da Silva and Cordellini, 2003; Samanin and Garattini, 1993). However, it is important to point out that our data did not reveal with precision the magnitude of the DEP-induced sensitization of motor activity, that could vary from small to strong.

In Experiment 2, the rewarding properties of acute low doses of DEP along with the effect of pre-exposure to DEP on these properties were examined. Experiments using the CPP paradigm have demonstrated that 10.0 and 15.0 mg/kg DEP enhance the time spent in a drug-paired compartment, suggesting that they had acquired rewarding properties (Planeta and DeLucia, 1998; Reimer et al., 1995). The present results suggest that the lower doses of 2.5 and 5.0 mg/kg DEP also produce CPP, but not the dose of 1.0 mg/kg.

The failure to observe CPP at 1.0 mg/kg could suggest the development of Latent Inhibition (LI) on the habituation days, since it has been reported that LI develops after repeated pre-exposure to the drug-paired chamber (Martin-Iverson and Reimer, 1996). However, even if the three-day habituation in the CPP chamber had induced LI, it is likely that this effect would have been prevented by the administration of DEP in the pre-exposure and conditioning sessions. DEP is an amphetamine-like drug, and it is well established that LI is disrupted when amphetamine and related drugs are administered on conditioning days of the LI procedure (Joseph et al., 2000; Weiner and Feldon, 1997) and that this disrupting effect is more likely to be observed when animals are repeatedly treated with the drug (Joseph et al., 2000; Tenn et al., 2005; Young et al., 2005).

Thus, since we observed CPP even for the two highest DEP doses tested, we conclude that LI learning probably has a weak role in determining the present results, indicating that no LI (at least not a strong LI) was produced by pre-exposure in the experimental chamber.

It is also important to highlight that data from the 1.0 mg/kg DEP dose were inconclusive, so that the non-significant results cannot be interpreted that this dose has no reward properties.

A minute-to-minute analysis of CPP at 2.5 and 5.0 mg/kg DEP showed that animals did not display an exclusive preference for any compartment at any minute of the place preference test. Instead, they seem to have distributed their time between both compartments so as to spend more seconds per minute in the drug-paired than in the vehicle-paired compartment. A few explanations could be addressed for this "non-exclusive" preference. First, testing in CPP paradigm is conducted under extinction, and a typical extinction effect is an increase in response variability. Second, the CPP paradigm can be seen as a choice procedure, and choice studies have demonstrated that the distribution of response frequency and time allocation is a function of the rewarding value of alternatives (Baum, 1979, 1975; Herrnstein and Vaughan, 1980; Heyman, 1996). Thus, the results obtained in the minute to minute analysis strengthens the suggestion that the rewarding value of the drug-paired compartment at 2.5 and 5.0 mg/kg DEP was higher than the rewarding value of the vehicle-paired compartment.

Pre-exposure to DEP was not able to sensitize the animals to the conditioned preference effects of the drug. Yet, the drug doses that increased motor activity proved to be the same as those inducing preference in the CPP model. This is consistent with results obtained in other studies indicating that stimulant doses which enhance motor activity are frequently the same doses as induce CPP, and is coherent with the suggestion that motor and rewarding effects of stimulant drugs are probably mediated by the same dopaminergic pathways (Bedingfield et al., 1997; Vezina, 2004; Vezina et al., 2007; Wise and Bozarth, 1987). However, sensitization was observed for the effects of DEP on motor activity, but not for CPP. This is in agreement with recent studies that found that the effect of stimulants on CPP can be dissociated from stimulant effects on locomotion and are mediated by different dopamine processes (Aujla and Beninger, 2003; Cunningham et al., 2002; Gerdjikov et al., 2004; Janhunen et al., 2005; Kelley and Rowan, 2004; Nocjar and Panksepp, 2002). Furthermore, differences in the dopaminergic regulation of reward and motor effects of stimulants have also been shown in self-administration protocols (Sellings and Clarke, 2003; Sellings et al., 2006a,b).

There is another possibility for the lack of sensitization to DEP observed: the CPP paradigm would not have detected an enhanced rewarding effect of DEP. It is known that dose–response curves for drug-induced CPP are sometimes difficult to demonstrate (Swerdlow et al., 1989), indicating that CPP paradigm could have low sensitivity to discriminate between active doses. Since sensitization to a drug effect could be defined as a shift of dose–response curve to the left, it had been claimed that CPP is not an adequate model to detect augmentation of drug rewarding effects (Bardo and Bevins, 2000). However, there is a considerable body of data showing sensitization to rewarding effects of various abused drugs. In these experiments, sensitization is typically verified when drug doses (low doses) that normally failed to induced CPP, successfully increased the preference for the drug-paired compartment after pre-exposure to the drug. Although Experiment 2 results strongly indicated that pre-exposure to DEP did not enhance the CPP produced by the doses tested, it is worth pointing out that the data from the lowest dose of 1.0 mg/kg was inconclusive. Therefore, the possibility of observing sensitization to DEP when replicating the 1.0 mg/kg challenge dose cannot be ruled out.

Finally, experiments carried out in our laboratory revealed that, under equal withdrawal interval lengths, pre-exposure to caffeine sensitized rats to the reinforcing value of DEP when this parameter was evaluated by self-administration in a progressive ratio schedule, but not when it was tested under the CPP paradigm (Garcia-Mijares, 2005). Other experiments have also yielded divergent results when the CPP and the self-administration models were used to assess sensitization to drug rewarding effects. For example, adolescent rats that were pre-exposed to methylphenidate did not show enhanced COC-induced CPP (Andersen et al., 2002), but did show increased COC self-administration (Brandon et al., 2001). It has been suggested (Brandon et al., 2001; Garcia-Mijares and Silva, in press) that CPP and progressive ratio self-administration procedures involve different mechanisms of reward which could account for the discrepant results obtained in some experiments that employed both procedures to assess sensitization to drug reward. Briefly, in CPP procedures, cues associated to drugs by virtue of Pavlovian conditioning, facilitate drug-seeking instrumental behavior, whereas in progressive ratio schedules a direct association between instrumental behavior and reward is established. In this regard, CPP procedures could be seen as a drug-seeking process, whereas the progressive ratio model would be akin to a self-administration process. Some experiments have shown that the neuronal mechanisms that mediate drug seeking are somewhat different from those that mediate drug self-administration (Shalev et al., 2002). Therefore, further studies using other behavioral models are required to elucidate the effects of pre-exposure to DEP on its rewarding properties.

In summary, our results suggest that DEP has psychostimulant and rewarding properties at low doses, and that DEP pre-exposure sensitized the subjects to the drug effect on motor activity. Considering that the rewarding effect of a drug has been consistently linked to its

abuse potential and that prescription and intake of DEP in Brazil are increasing (Chiapetti and Serbena, 2007; Nappo et al., 2002), the present data indicate that more research should be devoted towards better elucidating DEP abuse potential, and that its medical prescription should be carefully monitored until such potential is well established.

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