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Behavioral sensitization produced by a single administration of apomorphine: Implications for the role of Pavlovian conditioning in the mediation of context-specific sensitization

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Abstract

The present study examined the minimal number of exposures to the D1/D2 agonist apomorphine capable of producing behavioral sensitization. Rats received one (experiment 1) or two administrations on two successive days (experiment 2) of apomorphine (0.5 and 2.0 mg/kg) paired or unpaired to an open-field environment. After 2 days of drug withdrawal, the rats received a challenge injection with the same dose of apomorphine (sensitization test) and locomotion, rearing and sniffing were measured. The results of the first experiment showed that locomotor sensitization occurred after a single acute exposure to apomorphine and that 0.5 and 2.0 mg/kg treatments were equally effective. This sensitization effect was context-specific and was limited to locomotion. The second experiment revealed a differential dose effect on the sensitization test. Two treatments with 2.0 mg/kg potentiated locomotor sensitization as compared with a single treatment but two treatments with 0.5 mg/kg did not increase the sensitization effect more than the single 0.5 mg/kg treatment. This result indicates an interaction between drug dose and frequency of drug treatment for the induction of apomorphine locomotor sensitization. In that the sensitization effects are considered to be a core contributor to psychostimulant addiction, the present findings are of importance to understanding addiction because they indicate that sensitization processes can be initiated with a single drug experience and amplified with exposure to higher drug dosage levels.

Keywords: Behavioral sensitization; Context-specific sensitization; Apomorphine; Locomotion; Rearing; Sniffing; High dose; Moderate dose

1. Introduction

The intensity of locomotor activity elicited by psychomotor stimulant drugs can be augmented following repeated administration of the drug, a phenomenon termed sensitization (Damianopoulos and Carey, 1993; Post and Rose, 1976; Robinson and Becker, 1986; Segal et al., 1981; Stewart and Badiani, 1993). In fact, it has been shown that sensitization is also produced using a low number or even a single administration of a psychomotor stimulant (Battisti et al., 1999; Mattingly and Gotsick, 1989; Post et al., 1987). Recently, it has been shown that a single exposure to a psychomotor stimulant such as cocaine induced long-term potentiation in dopamine neurons, which suggests that synaptic plasticity may be involved in the early stages of the development of drug addiction (Ungless et al., 2001).

Studies have shown that the development of sensitization to the locomotor effects of psychostimulants is strongly influenced by the environmental context in which a stimulant drug is administered (Anagnostaras and Robinson, 1996; Badiani et al., 1997; Battisti et al., 1999; Crombag et al., 1996; Jodogne et al., 1994). Sensitization to locomotor stimulant effects may not be expressed or may be reduced when the environment used for sensitization testing is different from that in which rats were previously administered with the drug (Battisti et al., 1999). Although there is compelling evidence that sensitization involves a strong context-dependent component, sensitization with high drug dose levels is less clearly linked to context (Anagnostaras and Robinson, 1996; Battisti et al., 2000; Beinfeld, 2002;

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Carlezon et al., 1995; Mattingly et al., 2000; Todtenkopf and Carlezon, 2006; Todtenkopf et al., 2002) suggesting context-independent mechanisms. It is important to note, however, that even in studies where sensitization is weakly linked to test environment cues, there can still be linkage to interoceptive drug stimulus cues since drugs also have stimulus properties (Carey et al., 2005a; Overton, 1977; Revusky and Reilly, 1990; Siegel, 1988; Taukulis, 1996; Todtenkopf and Carlezon, 2006). If the stimulus context is broadened to include both the environmental stimuli plus the interoceptive drug stimuli in a compound stimulus, then, context-independent sensitization becomes problematic. Importantly, however, the presence of all the stimulus elements of the compound stimulus becomes the critical factor for the expression of sensitization effects in any test for sensitization (Carey et al., 2005b).

The present experiments were designed to examine the minimal number of exposures to a drug capable of producing behavioral sensitization. In order to evaluate the influence of context, a conventional paired/unpaired Pavlovian conditioning protocol was used, where the paired and unpaired groups received the same number of drug injections with only the sequence of the injections being altered. To evaluate the minimal number of administrations which were capable of producing sensitization, two experiments were conducted; the first with a single administration of the drug and the second with two administrations of the drug on two successive treatment days. The change in the behavioral activity elicited by the initial drug treatment versus the behavioral activity expressed to the same drug treatment in a subsequent test provided the measurement for sensitization effects. In addition, the effect of dopaminergic receptor stimulation level on sensitization was also evaluated using a moderate (0.5 mg/kg) and a high (2.0 mg/kg) dose of the D1/D2 agonist apomorphine.

2. Materials and methods

2.1. Subjects

Male Wistar albino rats, provided by the State University of North Fluminense, initially weighing 250–300 g, were housed in individual plastic cages (25×18×17 cm) until the end of experiment. Food and water were freely available at all times. The vivarium was maintained at a constant temperature (22±1 °C), and a 12/12 h light/dark cycle (lights on at 0700 h and off at 1900 h). All experiment occurred between 7:00 and 12:00 h. For 7 days prior to all experimental procedures each animal was weighed and handled daily for 5 min. All experiments were conducted in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus and measurement of behavior

The behavioral measurements were conducted in a black open-field chamber (60×60×45 cm). A closed-circuit video-camera (DISISEC, model IR575M), mounted 50 cm above the arena was used for the purpose of recording behavioral data. The complete test procedure was conducted automatically without the presence of the experimenter in the test room. The behavioral data for locomotion (measured as number of crossings), rearing and sniffing were observed during a 35 min period in the test environment. For crossing, the experimental arena floor was divided into eight equal-sized squares and the number of times that the rat passed from one square to another with its four paws was recorded. Rearing responses were scored when both forelimbs were raised off the floor onto the wall or into the air. Sniffing was measured as the time (seconds) that the animal showed a rapid flaring and contracting of the nostrils accompanied by movements of whiskers with the nose making contact or not with the wall and floor. Behavioral activity was analysed by a trained observer who was unaware of the treatment under test. All behavioral testings were conducted under dim red light (approximately 4 lx) to enhance the contrast between the white subject and dark background of the test chamber. Masking noise (approximately 60 dB) was provided by a fan and an air conditioning unit located in the experimental room. The fan was turned on immediately prior to placing the animal in the experimental arena and turned off upon removal of the animal from the experimental arena.

2.3. Drugs

Apomorphine-HCl (Sigma, St. Louis, MO, USA) was dissolved in 0.1% ascorbate/saline and was injected subcutaneously in the nape of the neck at doses of 0.5 or 2.0 mg/kg using a volume of 1.0 ml/kg body weight. Drug solutions were freshly prepared before each experiment.

2.4. Design and procedures

The experiments were conducted following a modified experimental protocol from Dias et al. (2006). First, all rats received three 35 min habituation sessions (habituation phase), conducted on consecutive days. The animals were administered saline and immediately placed in the experimental arena and activity was measured. On the next day, the animals were randomly assigned in groups and were submitted to the pharmacological treatments. Basically, there were three treatment groups: a paired group, an unpaired group and a vehicle treatment group. In the paired group, rats received administration of apomorphine (APO; 0.5 or 2.0 mg/kg) immediately before being placed into the test environment and vehicle administration 30 min after removal from the test environment. In the unpaired group, rats received administration of vehicle immediately before being placed into the test environment and apomorphine 30 min after being removed from the test environment. The vehicle treatment group was treated the same way as the paired group except that it received vehicle prior to being placed into the experimental arena. The animals were tested for 35 min in the test environment. The treatments were administered for 1 day (experiment 1) and for 2 consecutive days (experiment 2). After a period of drug withdrawal of 2 days, the sensitization test was conducted. In the first experiment, the groups during the pharmacological treatment phase were vehicle ($n=29$), APO-0.5-paired ($n=6$), APO-0.5-

unpaired ($n=5$), APO-2.0-paired ($n=9$) and APO-2.0-unpaired ($n=7$). On the sensitization test, the vehicle group was subdivided into three subgroups: one vehicle group ($n=15$); one APO 0.5 mg/kg ($n=7$) group; and one APO 2.0 mg/kg ($n=7$) group. The APO-paired and unpaired groups received the same APO treatment (0.5 mg/kg or 2.0 mg/kg). In experiment 2, the groups during the acute treatment phase were vehicle ($n=28$), APO-0.5-paired ($n=8$), APO-0.5-unpaired ($n=6$), APO-2.0-paired ($n=8$) and APO-2.0-unpaired ($n=8$). For the sensitization test, the vehicle group was subdivided into three subgroups which received vehicle ($n=11$), VEH-APO-0.5 ($n=8$) or VEH-APO-2.O ($n=9$). Again the APO-paired/unpaired groups received the APO dose treatments in the sensitization test that received in the treatment phase.

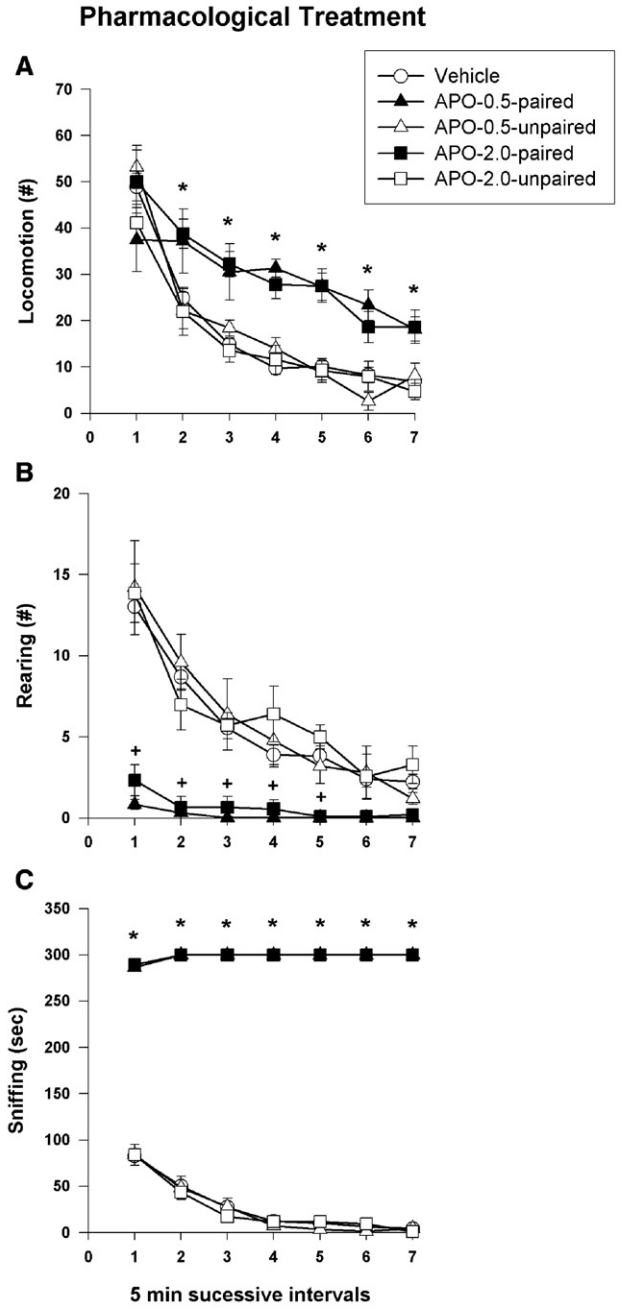

Fig. 1. Means and S. E. M. for locomotion (A), rearing (B) and sniffing (C) during the pharmacological treatment phase of the experiment 1. The panels present the within-session scores during seven successive 5-min intervals. * denotes that APO-paired groups showed a higher activity than the other groups. $^{+}$ denotes that APO-paired groups showed a lower activity than the other groups ($p<0.05$; ANOVA followed by Duncan's multiple range test).

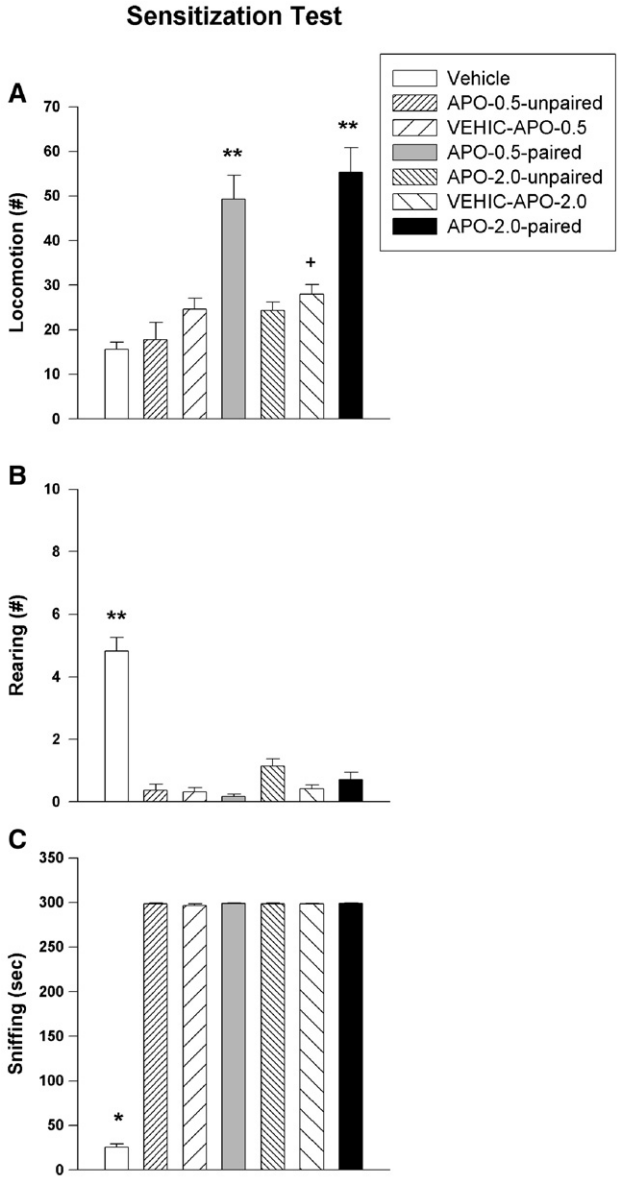

Fig. 2. Means and S. E. M. for locomotion (A), rearing (B) and sniffing (C) during the sensitization test of experiment 1. ** denotes higher activity than the other groups. * denotes lower activity than the other groups. $^{+}$ denotes higher activity than the vehicle group ($p<0.05$; ANOVA followed by Duncan's multiple range test).

2.5. Statistics

In order to make within-treatment assessments of the behavioral activity data, the total time of the test (35 min) was divided into 7 intervals of 5 min duration. The behavioral data of the acute treatment phase and sensitization test of experiment 1 were analysed by repeated two-way ANOVA, consisting of between-subject factor of group and a repeated-measurements

factor, interval, to provide a direct comparison of all groups. The sensitization tests for experiments 1 and 2 were evaluated with a one-way ANOVA. In addition, locomotion sensitization effects were evaluated using two-way ANOVAs in which locomotion scores on the acute test were compared to scores on the sensitization test for each group in each experiment. In order to make specific group comparisons, post-hoc Duncan's multiple range tests were performed. When a significant effect of group versus interval interaction or group versus day interaction was recorded, data were further analysed by one-way ANOVA followed by the Duncan post-hoc test. $p<0.05$ as the criterion for statistical significance.

3. Results

3.1. Pre-treatment habituation effects

Prior to the initiation of each experiment, a three-day habituation procedure was conducted and the locomotion was measured. In experiment 1, the statistical analyses with a repeated two-way ANOVA indicated a significant interaction day × interval ($F_{12,\ 990}=3.21$, $p<0.01$), effect of interval ($F_{6,\ 990}=489.65$; $p<0.01$), and effect of days ($F_{2,\ 165}=23.04$; $p<0.01$). The Duncan's test showed that day 1 had higher locomotion activity than day 2 ($p<0.05$) and day 3 ($p<0.05$). In experiment 2, the results showed an interaction day × interval effect ($F_{12,\ 1026}=3.91$, $p<0.01$), a significant effect of interval ($F_{6,\ 1026}=637.5$; $p<0.01$), and a significant effect of days ($F_{2,\ 171}=2730.2$; $p<0.01$). The Duncan's test showed that day 1 had a higher locomotion activity than day 2 ($p<0.05$) and day 3 ($p<0.05$). Therefore, in both experiments, the locomotion activity declined with repeated testing ($p<0.05$) as expected for the development of habituation to a novel environment (Cerbone and Sadile, 1994). Importantly, prior to the initiation of the conditioning protocol, there were no differences among the treatment groups ($p>0.05$) in any experiment.

3.2. Experiment 1

Fig. 1 shows the mean activity scores obtained during the pharmacological treatment phase. For locomotion (Fig. 1A), the statistical analyses with a repeated two-way ANOVA indicated a statistically significant interaction group × interval ($F_{24,\ 306}=3.03$, $p<0.01$), a significant effect of interval ($F_{6,\ 306}=78.26$; $p<0.01$), and a significant effect of group ($F_{4,\ 51}=431.93$; $p<0.01$). To further analyse the group × interval interaction, the one-way ANOVA followed by Duncan's multiple range test showed that for the first interval, there were no differences

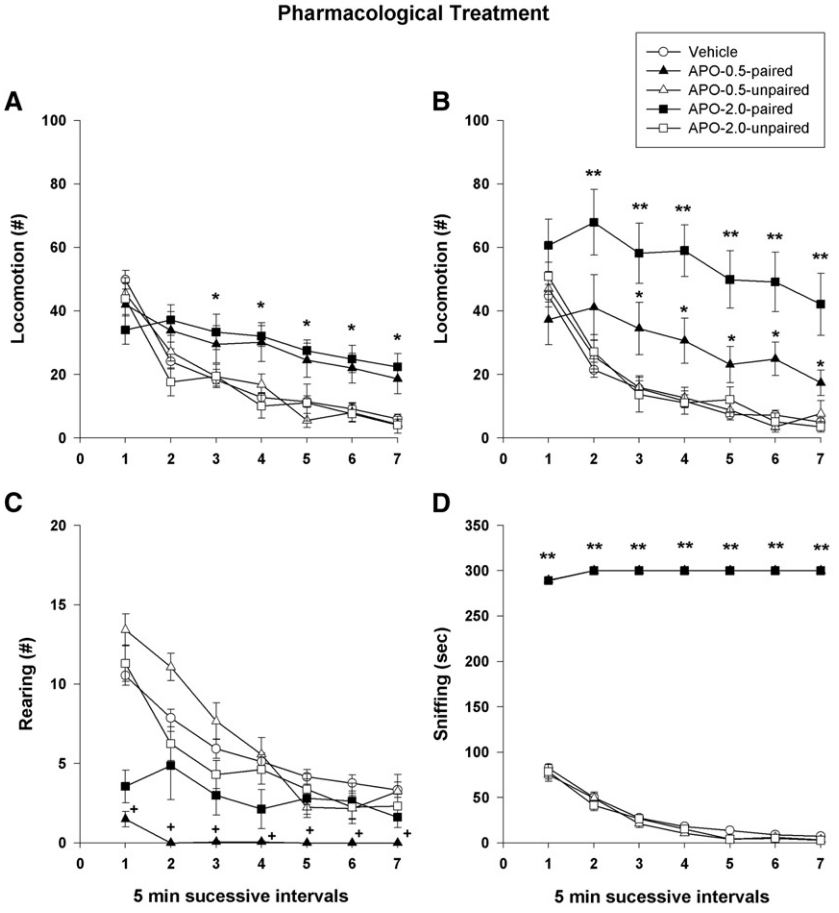

Fig. 3. Means and S. E. M. for locomotion from day 1 (A) and day 2 (B), rearing (C) and sniffing (D) during the pharmacological treatment in experiment 2. The panels present the within-session scores in seven successive 5-min intervals. ** denotes higher activity than the other groups. * denotes higher activity than the vehicle and unpaired groups. $^{+}$ denotes lower activity than the vehicle and unpaired groups ($p<0.05$; ANOVA followed by Duncan's multiple range test).

among the groups ($F_{4,\ 51}=1.12$; $p=0.36$). However, from the 2nd interval until the 7th interval, APO-0.5-paired and APO-2.0-paired groups showed a higher locomotion activity than the other groups ($p<0.05$). The results also showed that locomotion activity decreased for all groups during the session, i.e., the locomotion activity during the 1st interval was significantly higher than the activity during the 7th interval ($p<0.05$).

For rearing (Fig. 1B), the results showed that there was a significant interaction group × interval ($F_{24,\ 306}=4.13$, $p<0.01$), a significant effect of interval ($F_{6,\ 306}=30.82$; $p<0.01$), and a significant effect of group ($F_{4,\ 51}=25.32$; $p<0.01$). To further analyse the group versus interval interaction, a one-way ANOVA followed by Duncan's multiple range test showed that from the 1st interval until the 5th interval, the APO-0.5-paired and APO-2.0-paired groups showed a lower number of rearing responses than the other groups ($p<0.05$). However, during the 6th interval and the 7th interval there were no differences among the groups ($p>0.05$). The results also showed that the rearing activity decreased across the session for all groups, i.e., the rearing activity during the 1st interval was significantly higher than the activity during the 7th interval ($p<0.05$), except for APO-2.0-paired group that did not show difference across the session ($p>0.05$).

For sniffing (Fig. 1C), the results showed that there was an interaction group × interval effect ($F_{24,\ 306}=27.21$, $p<0.01$), a significant effect of interval ($F_{6,\ 306}=89.32$; $p<0.01$), and a significant effect of group ($F_{4,\ 51}=5307.0$; $p<0.01$). The results showed that from the 1st interval until the end of the experiment, the APO-0.5-paired and APO-2.0-paired groups showed a higher sniffing time than the other groups ($p<0.05$). The results also showed that there was a different pattern of sniffing activity among the groups across the session interval. That is, the vehicle, APO-0.5-unpaired and APO-2.0-unpaired groups decreased sniffing activity across the session, i.e., the sniffing activity during the 1st interval was significantly higher than during the 7th interval ($p<0.05$) whereas for the APO-0.5-paired and APO-2.0-paired groups there was a general increase in sniffing activity during all experimental sessions ($p<0.05$).

Fig. 2 shows the mean total activity scores obtained on the sensitization test day. For locomotion (Fig. 2A), the one-way ANOVA ($F_{6,\ 385}=22.40$; $p<0.01$) followed by Duncan's test showed that apomorphine paired groups showed a higher locomotion than the other groups ($p<0.05$) and the VEH-APO-2.0 group showed a higher locomotion than the vehicle group ($p<0.05$). The results also showed that the vehicle group showed a higher number of rearing responses ($F_{6,\ 385}=36.40$; $p<0.01$; Fig. 2B) and a lower sniffing time ($F_{6,\ 385}=3115.23$; $p<0.01$; Fig. 2C) than the other groups ($p<0.05$).

3.3. Experiment 2

Fig. 3 shows the mean total activity scores obtained during the pharmacological treatment phase. For locomotion, the statistical analyses with a repeated two-way ANOVA indicated a significant interaction group × day effect ($F_{4,\ 106}=5.73$; $p<0.01$), an interaction group × interval effect ($F_{24,\ 636}=5.64$, $p<0.01$), a significant effect of group ($F_{4,\ 106}=23.0$; $p<0.01$), a significant effect of day ($F_{1,\ 106}=5.02$; $p<0.05$) and a significant effect of interval ($F_{6,\ 636}=105.27$; $p<0.01$). However, there was no interaction interval × day ($F_{6,\ 636}=0.7$; $p>0.05$) and no interaction interval × group × day ($F2_{4,\ 636}=0.6$; $p>0.05$). To further analyse the interaction group × interval on the first day of the pharmacological treatment (Fig. 3A), a one-way ANOVA was used followed by Duncan's multiple test. The results showed that the experimental groups did not show statistical differences at the 1st interval. However, the locomotion activity during the 1st interval was higher than during other intervals ($p<0.05$). In the 2nd interval, the APO-2.0-paired and APO-0.5-paired were different from vehicle group. However, from the 3rd interval until the 7th interval, the APO-2.0-paired and APO-0.5-paired

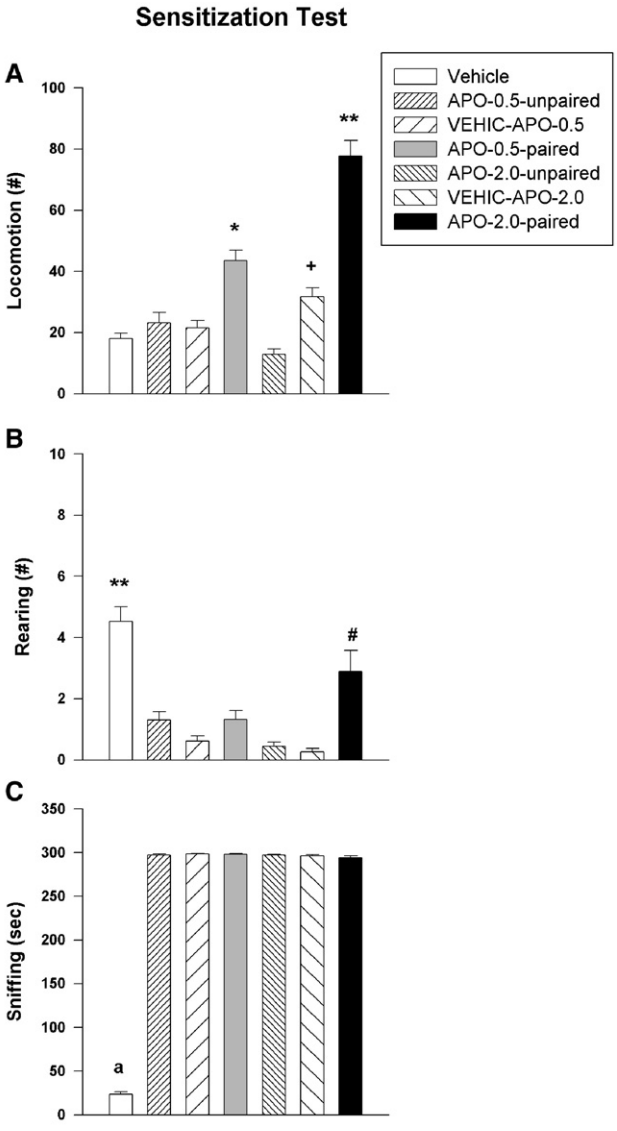

Fig. 4. Means and S. E. M. for locomotion (A), rearing (B) and sniffing (C) during the sensitization test day of experiment 2. ** denotes higher activity than the other groups. * denotes higher activity than the vehicle, VEH-APO and unpaired groups. $^{+}$ denotes higher activity than the vehicle, VEH-APO-0.5 and unpaired groups. $^{\#}$ denotes higher activity than APO-0.5-paired, unpaired and VEH-APO groups. ^{a} denotes lower activity than the other groups ($p<0.05$; ANOVA followed by Duncan's multiple range test).

groups showed higher locomotion than the other groups ($p<0.05$).

On the second day of the pharmacological treatment (Fig. 3B), the results showed that at the 1st interval there were no differences among the experimental groups but locomotion at the 1st interval was higher than at the other intervals ($p<0.05$). From the 2nd interval until the 7th interval, the APO-2.0-paired group showed higher locomotion activity than the other groups ($p<0.05$). On the other hand, the APO-0.5-paired group showed higher locomotion than vehicle group at the 2nd interval, but from the 3rd interval until the 7th interval the locomotion activity for the APO-0.5-paired group was higher than the vehicle, APO-2.0-unpaired and APO-0.5-unpaired groups ($p<0.05$).

For rearing responses (Fig. 3C), the statistical analyses with a repeated two-way ANOVA indicated a significant interaction group × interval ($F_{24,\ 636}=5.48$, $p<0.01$), a significant effect of group ($F_{4,\ 106}=16.65$; $p<0.01$) and a significant effect of interval ($F_{6,\ 636}=50.53$; $p<0.01$). However, there was no effect of day ($F_{1,\ 106}=1.95$; $p>0.05$), no interaction group × day ($F_{4,\ 106}=2.36$; $p>0.05$), no interaction interval × day ($F_{6,\ 636}=0.95$; $p>0.05$) and no interaction interval × group × day ($F2_{4,\ 636}=0.88$; $p>0.05$). To further analyse the interaction group × interval, a one-way ANOVA followed by Duncan's multiple test was used and showed that from the 1st interval until the 7th interval, the APO-0.5-paired group had the lower number of rearing responses than the other groups ($p<0.05$) except in the 1st, 4th, 5th, 6th and 7th intervals where that group was not different from the APO-2.0-paired group.

For sniffing (Fig. 3D), the statistical analyses with a repeated two-way ANOVA indicated a significant interaction group × interval ($F_{24,\ 636}=25.33$, $p<0.01$), a significant interaction interval × day ($F_{6,\ 636}=2.15$; $p<0.05$), a significant effect of interval ($F_{6,\ 636}=90.07$; $p<0.01$), and a significant effect of group ($F_{4,\ 106}=3832.5$; $p<0.01$). However, there was no effect of day ($F_{1,\ 106}=1.00$; $p>0.05$), no interaction group × day ($F_{4,\ 106}=0.16$; $p<0.05$), and no interaction interval × group × day ($F_{24,\ 636}=0.61$; $p>0.05$). To further analyse the group × interval interaction, a one-way ANOVA followed by Duncan's multiple test was used and showed that from the 1st interval until the 7th interval the APO-0.5-paired and APO-2.0-paired groups showed a higher time of sniffing than the other groups ($p<0.05$).

Fig. 4 shows the mean total activity scores obtained on the sensitization test. For locomotion (Fig. 4A), the one-way ANOVA showed that there was a group effect ($F_{6,\ 399}=53.0$; $p<0.01$) and Duncan's test showed that the APO-2.0-paired group had a higher locomotion than the other groups ($p<0.05$). The APO-0.5-paired group had higher locomotion than the vehicle, unpaired and VEH-APO groups ($p<0.05$). The VEH-APO-2.0 group showed higher locomotion activity than the vehicle, unpaired and VEH-APO-0.5 groups ($p<0.05$). For rearing (Fig. 4B), the results showed that there was effect of the group ($F_{6,\ 399}=19.32$; $p<0.01$) and Duncan's test showed that the vehicle group has the highest number of rearing responses than the other groups ($p<0.05$) and the APO-2.0-paired group showed higher rearing activity than the APO-0.5-paired, unpaired and VEH-APO groups ($p<0.05$). For sniffing (Fig. 4C), the results showed that there was an effect of the group ($F_{6,\ 399}=3342.2$; $p<0.01$) and Duncan's test showed that the vehicle group had a lower sniffing time than the all groups ($p<0.05$).

Fig. 5 shows the mean of locomotor activity during the first pharmacological treatment days and the sensitization tests for experiment 1 and 2. For experiment 1 (Fig. 5A), the two-way ANOVA showed that there was interaction group × day ($F_{3,\ 370}=4.52$; $p<0.01$), a significant effect of group ($F_{3,\ 370}=24.61$; $p<0.01$) and a significant effect of day ($F_{1,\ 370}=24.76$; $p<0.01$). The one-way ANOVA followed by Duncan's test

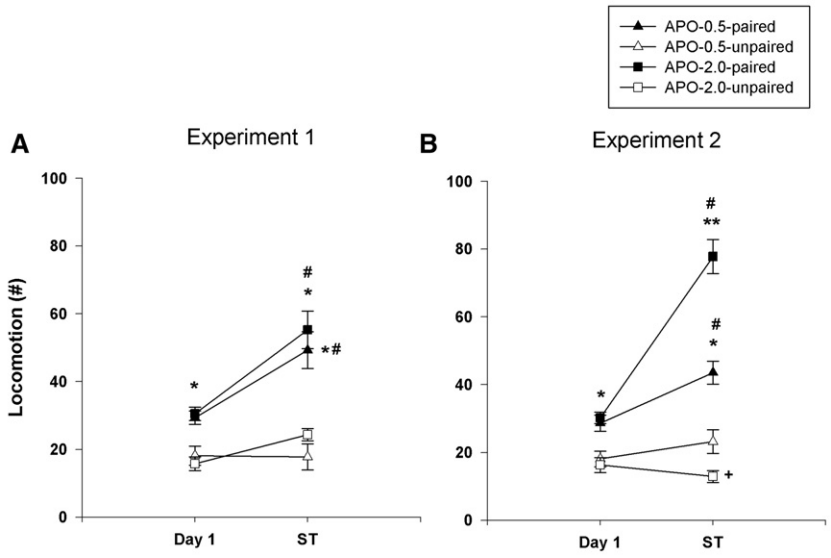

Fig. 5. Means and S. E. M. for locomotion during the first day of pharmacological treatment and during the sensitization test (ST) of experiment 1 (A) and 2 (B) for the APO-paired and APO-unpaired groups. * denotes higher locomotor activity than the unpaired groups. ** denotes higher locomotor activity than the other groups. # denotes higher locomotor activity when comparing results for day 1 and ST. + denotes lower locomotor activity than the other groups ($p<0.05$; ANOVA followed by Duncan's multiple range test).

showed that on the treatment day ($F_{3,\ 185}=12.7$; $p<0.01$) the APO-0.5-paired and APO-2.0-paired showed higher locomotion than the unpaired groups ($p<0.05$). On the sensitization test day, the one-way ANOVA ($F_{3,\ 185}=14.96$; $p<0.01$) followed by Duncan's test showed that the APO-0.5-paired and APO-2.0-paired groups showed higher locomotion than the unpaired groups ($p<0.05$). For experiment 2 (Fig. 5B), the two-way ANOVA showed that there was interaction group × day ($F_{3,\ 412}=29.13$; $p<0.01$), a group effect ($F_{3,\ 412}=71.75$; $p<0.01$) and effect of day ($F_{1,\ 412}=56.93$; $p<0.01$). The one-way ANOVA followed by Duncan's test showed that on the first treatment day ($F_{3,\ 206}=11.10$; $p<0.01$) the APO-0.5-paired and APO-2.0-paired showed higher locomotion than the unpaired groups ($p<0.05$). On the sensitization test day, the one-way ANOVA ($F_{3,\ 206}=64.32$; $p<0.01$) followed by Duncan's test showed the APO-2.0-paired group had higher locomotion than all other groups ($p<0.05$), the APO-0.5-paired group showed higher locomotion than the unpaired groups ($p<0.05$) and the APO-2.0-unpaired group showed the lowest locomotion activity of all groups ($p<0.05$).

4. Discussion

The results of the first experiment demonstrate that sensitization to the locomotor stimulant effect of apomorphine occurs after a single acute exposure of the drug. Importantly, the sensitization effect was context-specific in that the same apomorphine treatment given outside the test environment produced no sensitization. The sensitization that was observed was evident only as an effect on locomotor behavior. Other behavioral measures, such as sniffing and rearing, gave no indication of sensitization. With each of these additional measures, however, ceiling and floor effects were apparent. The initial apomorphine treatment completely inhibited rearing behavior (floor effect); but it enhanced sniffing behavior to a level of virtually continuous sniffing (ceiling effect). These additional behavioral measurements which were not effective for detecting sensitization effects were, nonetheless, useful in validating the efficacy of the apomorphine treatment across all groups. Importantly, these behavioral measurements indicate that apomorphine effects on behavior are not simply locomotor activation effects but rather a radical reorganization of behavior. This behavioral reorganization effect has been examined in detail previously (Damianopoulos and Carey, 1993).

While the results of the first experiment showed that the 0.5 and 2.0 mg/kg apomorphine treatments were equally effective in the initial treatment and sensitization test, the second experiment revealed a differential dose effect on the sensitization test. Two treatments with the higher apomorphine dose potentiated locomotor sensitization and elicited some rearing behavior. The rearing activity in the high dose APO group was modest and still less than the vehicle group. Thus, there was a diminution of the inhibitory effect of apomorphine upon rearing behavior in the high dose APO group rather than an enhancement of rearing. In the two experiments, the 0.5 and 2.0 mg/kg apomorphine treatments had qualitatively similar and quantitatively equivalent effects on the first drug treatment test. Consequently, the differences observed in the sensitization tests between experiment 1 and experiment 2 cannot be attributed to initial differences in drug dose effects in the two experiments. Moreover, on the sensitization test, the locomotor sensitization effect in experiment 2 for the 0.5 mg/kg apomorphine group was essentially the same as the sensitization effects for the 0.5 mg/kg apomorphine group of experiment 1. Thus, the additional apomorphine treatment in experiment 2 did not increase the sensitization effect in the 0.5 mg/kg apomorphine group. The additional apomorphine treatment, however, selectively potentiated the sensitization effect in the 2.0 mg/kg dose group. This differential result indicates an interaction between drug dose or drug stimulation intensity and frequency of drug treatment for the induction of locomotor sensitization effects.

Implicit in the occurrence of context-specific drug sensitization is that it is mediated by a learning/conditioning process. In a conventional Pavlovian conditioning framework, the environmental context is the conditioned stimulus (CS) and the drug treatment is the unconditioned stimulus (US). When these two events occur contiguously an association forms such that the CS alone can evoke the response to the US without the US being present. Applied to the sensitization phenomenon, this type of process has not proven to be a significant contributor. There are a number of reports (Carey and Gui, 1998; Carey et al., 1999; Damianopoulos and Carey, 1993; Mattingly et al., 1988a,b) in which sensitization effects were robust but the efficacy of the environmental CS by itself to elicit the US response was slight. Thus, simply adding of the UR effects contributed by the environmental CS to those of the initial US drug response cannot account for the increased drug responsivity; i.e., for the sensitization effect. On the other hand, the environmental context is critical as shown by the fact that when the drug US is administered in a different environment (e.g., the home cage) the sensitization effect does not transfer to another test environment.

Previously we have argued that the CS in drug conditioning must be broadened to include as a compound stimulus both the environmental stimulus context and the interoceptive stimulus cues evoked by the drug US (Carey et al., 2005b; Damianopoulos and Carey, 1993). This expansion of contextual cues allows context-specific sensitization phenomena to remain within the domain of Pavlovian conditioning. From this new perspective, the failure of the environmental stimulus component of the contextual CS by itself to elicit a substantial US drug response would be expected. That is, the environmental contextual stimuli represent only a part of the contextual CS. How large a part would depend upon the intensity of the drug induced stimuli.

Another important finding in the present report is that the context-specific sensitization effect only required one pairing of the test environment to the drug administration. Recently we have suggested (Carey et al., 2005b) that stimulus salience can be an important contributor to the rapid acquisition of psychostimulant sensitization effects. In instances such as the present experiments, the drug treatment administered in conjunction with placement in the test environment represents the first time the animal is exposed to the drug treatment. Consequently, the interoceptive drug stimuli generated by the

apomorphine treatment are highly novel and salient. It has been shown that salience of a CS is an important factor in Pavlovian conditioning (Damianopoulos, 1987; Frey and Sears, 1978; Mackintosh, 1975). The more salient the CS, the more rapid the conditioning to the CS. Thus, placing context-specific sensitization in a Pavlovian conditioning framework wherein the CS is comprised of the test environment plus the novel interoceptive drug cues makes for a highly salient compound stimulus conducive to rapid conditioning (Jarbe et al., 1981). When one trial sensitization effects are placed in this Pavlovian perspective, a testable protocol can be readily developed to elucidate and resolve this issue of the relationship of conditioning to sensitization. That is, a one trial sensitization procedure can be used as was employed in the present study. If separate groups are used which have degrees of drug pre-exposures (e.g., 0, 1, 5 or 10 drug pre-exposures in a home cage or in a third environment), the prediction from the stimulus salience model would be that the context sensitization would decrease as the number of drug pre-exposures increases. In contrast, models of sensitization which involve changes in brain function would predict just the opposite, namely that sensitization effects would be enhanced. Thus, the present results provide a testing paradigm to address critical issues in drug sensitization.

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