Pharmacology, Biochemistry and Behavior 96 (2010) 317–324

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Apomorphine conditioning and sensitization: The paired/unpaired treatment order as a new major determinant of drug conditioned and sensitization effects

Liana Wermelinger de Matos [a], Robert J. Carey [b], Marinete Pinheiro Carrera [a,*]

[a] Behavioral Pharmacology Group, Laboratory of Animal Morphology and Pathology, State University of North Fluminense Darcy Ribeiro, Avenida Alberto Lamego, 2000, Campos dos Goytacazes, 28013-600, RJ, Brazil
[b] Research and Development (151), VA Medical Center and SUNY Upstate Medical University, 800 Irving Avenue, Syracuse, NY 13210, USA

ARTICLE INFO

Article history:
Received 24 February 2010
Received in revised form 14 May 2010
Accepted 23 May 2010
Available online 1 June 2010

Keywords:
Sensitization
Pavlovian paired/unpaired protocol
Conditioning
Counter-conditioning procedure
Extinction
Anti-habituation process
Apomorphine

ABSTRACT

Repeated treatments with psychostimulant drugs generate behavioral sensitization. In the present study we employed a paired/unpaired protocol to assess the effects of repeated apomorphine (2.0 mg/kg) treatments upon locomotion behavior. In the first experiment we assessed the effects of conditioning upon apomorphine sensitization. Neither the extinction of the conditioned response nor a counter-conditioning procedure in which we paired an inhibitory treatment (apomorphine 0.05 mg/kg) with the previously established conditioned stimulus modified the sensitization response. In the second experiment, we administered the paired/unpaired protocol in two phases. In the second phase, we reversed the paired/unpaired protocol. Following the first phase, the paired group alone exhibited conditioned locomotion in the vehicle test and a sensitization response. In the second phase, the initial unpaired group which received 5 paired apomorphine trials during the reversal phase did not develop a conditioned response but developed a potentiated sensitization response. This disassociation of the conditioned response from the sensitization response is attributed to an apomorphine anti-habituation effect that can generate a false positive Pavlovian conditioned response effect. The potentiated sensitization response induced by the treatment reversal protocol points to an important role for the sequential experience of the paired/unpaired protocol in behavioral sensitization.

1. Introduction

Repeated treatment with dopamine agonist stimulant drugs reliably induces sensitization effects (Damianopoulos and Carey, 1993; Post and Rose, 1976; Robinson and Becker, 1986; Segal et al., 1981; Stewart and Badiani, 1993). This behavioral sensitization effect is multifaceted in that it can include changes in neurotransmitter receptor sensitivity as well as learning/plasticity mechanisms such as Pavlovian conditioning (Adams et al., 2000; Crombag et al., 2000; Hinson and Poulos, 1981; Post et al., 1992; Stewart and Badiani, 1993). To date, the contribution and interplay of these factors have remained elusive. In terms of changes in receptor sensitivity, the issue can be complex even if the drug treatment is selective to one neurotransmitter system. In the case of the dopaminergic system, there are multiple postsynaptic receptors and, in addition, there are inhibitory autoreceptors (Aghajanian and Bunney, 1973; Kebabian and Calne, 1979; Missale et al., 1998; Sibley and Monsma, 1992; Skirboll et al., 1979). From the perspective of dopaminergic receptor effects, an enhanced behavioral stimulant drug response could occur as a result of an increase in postsynaptic receptor sensitivity or as a result of subsensitivity of the inhibitory autoreceptors. In addition to increasing the magnitude of the behavioral response to the same drug dose, repeated treatments with dopaminergic stimulant drugs also generate conditioned drug responses (Carey and Gui, 1998; Hinson and Poulos, 1981; Möller et al., 1987; Schiff, 1982).

As has been shown in several recent reports (Braga et al., 2009a, 2009b; Keller et al., 2002; Mattingly et al., 1997; Pinheiro Carrera et al., 1998) with the dopamine agonist apomorphine, both the conditioned and sensitized behavioral responses can be induced using a Pavlovian conditioning protocol (Braga et al., 2009a, 2009b). In the Pavlovian protocol, the same drug treatment is given to different groups, with treatments paired or unpaired to the test environment. It has been reported (Braga et al., 2009a, b; Dias et al., 2010) that the apomorphine hyperlocomotion effect expressed either as a conditioned response or as a sensitized response occurs only in the paired treatment groups. The same repeated apomorphine treatments administered unpaired to the test environment are without effect upon either conditioned or sensitized responses. This dependence of the conditioned and sensitized apomorphine hyperlocomotion in response to contextual cues establishes a linkage of both conditioned and sensitized responses to learning processes. Once the conditioned and sensitized responses have been induced it has been shown for other dopaminergic drugs (i.e., cocaine) that the conditioned response can be extinguished, although the sensitized response remains intact and is unaffected by the extinction

* Corresponding author. Behavioral Pharmacology Group, Laboratory of Animal Morphology and Pathology, State University of North Fluminense, Avenida Alberto Lamego, 2000, Campos dos Goytacazes, 28013-600, RJ, Brazil. Fax: +55 22 27397197.
E-mail address: marinete@uenf.br (M.P. Carrera).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.05.025

of the conditioned response (Carey and Gui, 1998). Altogether these and a number of other findings (Anagnostaras and Robinson, 1996; Anagnostaras et al., 2002; Braga et al., 2009b; Carey and Gui, 1998; Crombag et al., 2000; Hotsenpiller and Wolf, 2002; Tirelli et al., 2005) demonstrate that the sensitized response cannot be accounted for by the summation of the conditioned and behavioral drug response. In the present study, we expand on this issue of the relationship between the conditioned response and sensitized unconditioned behavioral drug response using the dopamine agonist apomorphine. To induce conditioning and sensitization effects, we employed the Pavlovian paired/unpaired treatment protocol.

Following the induction of a conditioned and a sensitized apomorphine behavioral response of hyperlocomotion with repeated apomorphine treatments (2.0 mg/kg), we instituted an extinction procedure involving repeated vehicle treatments in the test environment and subsequently assessed apomorphine conditioning and sensitization. We also included a counter-conditioning procedure in which we substituted low dose (0.05 mg/kg) apomorphine in the place of vehicle in the extinction treatment. Whereas the 2.0 mg/kg apomorphine treatment induces hyperlocomotion, the low dose apomorphine treatment, which preferentially activates dopamine autoreceptors (Carey et al., 2008a), inhibits locomotion. In addition to addressing the impact of extinction and counter-conditioning upon apomorphine conditioning and sensitization, we also investigated the issue of the unpaired treatment component in the Pavlovian drug conditioning protocol. In several studies our group (Braga et al., 2009a, 2009b) suggested that repeated unpaired apomorphine (2.0 mg/kg) treatments have no effect upon subsequent tests for conditioning and sensitization. In such experiments, typically, the conditioning and sensitization tests are the terminal phase of the experiment. In the present study, however, after we first conducted the paired/unpaired apomorphine (2.0 mg/kg) protocol and tested for conditioning and sensitization, we then conducted a reversal experiment in which the paired/unpaired treatment groups had their treatments reversed. Seemingly, if the unpaired treatments are without effect, then the profile of effects in the conditioning and sensitization tests of the paired and unpaired groups following the reversal treatment procedure should be the mirror image of their performance which followed the initial induction phase of the experiment. This report details the results of these experiments and points to the need to reconsider the impact of an unpaired treatment protocol.

2. Materials and methods

2.1. Subjects

Male Wistar albino rats provided by the State University of North Fluminense, initially weighing 200–300 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±2 °C), and a 12/12 h light/dark cycle (lights on at 0700 h and off at 1900 h). All experiment occurred between 8:00 and 18:00 h. For 7 days prior to all experimental procedures each animal was weighed and handled daily for 5 min. All experimental procedures were conducted in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and were approved by the ethical committee of the State University of North Fluminense.

2.2. Apparatus and measurement of behaviour

The behavioral measurements were conducted in a black open field chamber (60×60×45 cm). A closed-circuit video-camera (SONY, model IR575M), mounted 60 cm above the arena was used to record behavioral data. Locomotion, measured as distance travelled (m), was automatically analysed by EthoVision (Noldus, The Netherlands). The complete test procedure was conducted automatically without the presence of the experimenter in the test room. All behavioral testing was conducted under dim red light to avoid the possible aversive quality of white light and to enhance the contrast between the white subject and dark background of the test chamber. The testing under red light conditions is less stressful and also favors locomotor activation as the rats are transferred from the ambient light of the vivarium to the red light of the testing room (Nasello et al., 1998). Masking noise was provided by a fan located in the experimental room that 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 (i.e., test chamber).

2.3. Drugs

Apomorphine–HCl (Sigma, St. Louis, MO, USA) was dissolved in 0.1% ascorbate/saline (2.0 mg/ml) and was injected subcutaneously in the nape of the neck at a dose of 2.0 and 0.05 mg/kg. A 0.1% ascorbate/saline solution was used as vehicle for the apomorphine experiments. All doses were administered in a volume of 1.0 ml/kg body weight. Drug solutions were freshly prepared before each experiment.

2.4. Experiment 1: effect of extinction and a low autoreceptor dose of apomorphine on reversal of a conditioned and sensitized locomotor response produced by a high postsynaptic dose of apomorphine

The experiments were conducted following an experimental protocol from Braga et al. (2009a,b). In general, the experiment consisted of two phases: an induction phase and an extinction phase. Each phase was separated by a withdrawal period of 7 days. In the first phase there was a pharmacological/vehicle treatment, followed by a conditioning test after a withdrawal period of 2 days, then, after a second withdrawal period of 2 days there was a sensitization test. Initially all rats received three 40 min habituation sessions (habituation phase) conducted on consecutive days. The habituation protocol was conducted so that a stable baseline of locomotor behaviour could be established prior to the start of the drug treatments. The animals were administered with saline and placed in the experimental arena and locomotor activity was measured. On the following day, the animals were assigned to groups equated on baselines and were submitted to the pharmacological treatment of the induction phase, in which there were three basic treatment groups: a paired group, an unpaired group and a vehicle treatment group. In the paired group (APO-2.0-P; $n=18$), rats received apomorphine 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 (APO-2.0-UP; $n=6$), rats received vehicle immediately before being placed into the test environment and apomorphine 2.0 mg/kg 30 min after being removed from the test environment. The vehicle group (VEH; $n=6$) was treated in the same way as the paired group except that the animals received vehicle prior to being placed in the experimental arena. Treatments were administered on 5 consecutive days. This was the drug treatment phase and it was designed to monitor possible changes in the behavioral response to repeated drug treatment. After a period of 2 days without injections or behavioral testing (withdrawal period), the animals received an injection of saline prior to being placed into the test environment (conditioning test). Following a second withdrawal period (2 days), the apomorphine challenge test was performed in which the animals from paired and unpaired groups received 2.0 mg/kg apomorphine. The vehicle group received vehicle. Following a withdrawal period of 7 days, the next treatment phase was initiated. In this phase, the animals from the APO-2.0-P group were divided into three sub-groups in which one sub-group received 0.05 mg/kg apomorphine (APO-2.0-P + 0.05-P; $n=6$); the second sub-group received 0.05 mg/kg apomorphine not associated to the experimental arena (APO-2.0-P + 0.05-UP; $n=6$) and the third sub-group received vehicle associated to the experimental arena (APO-2.0-P + VEH-P; $n=6$). The animals from the APO-

Table 1
Treatment design for experiments 1 and 2.

Induction phase					Extinction/reverse phase				
Groups	Pharmacological treatment		CT	ST	Groups	Pharmacological treatment		CT	ST
	Arena	Home-cage				Arena	Home-Cage		
Experiment 1									
VEH ($n=6$)	VEH	VEH	VEH	VEH	VEH + VEH ($n=6$)	VEH	VEH	VEH	VEH
APO-2.0-UP ($n=6$)	VEH	2.0	VEH	2.0	APO-2.0-UP + 0.05-UP ($n=6$)	VEH	0.05	VEH	2.0
APO-2.0-P ($n=18$)	2.0	VEH	VEH	2.0	APO-2.0-P + VEH-P ($n=6$)	VEH	VEH	VEH	2.0
					APO-2.0-P + 0.05-UP ($n=6$)	VEH	0.05	VEH	2.0
					APO-2.0-P + 0.05-P ($n=6$)	0.05	VEH	VEH	2.0
Experiment 2									
VEH ($n=6$)	VEH	VEH	VEH	VEH	VEH + VEH ($n=6$)	VEH	VEH	VEH	VEH
APO-2.0-UP ($n=6$)	VEH	2.0	2.0	2.0	APO-2.0-UP + 2.0-P ($n=6$)	2.0	VEH	VEH	2.0
APO-2.0-P ($n=12$)	2.0	VEH	VEH	2.0	APO-2.0-P + VEH-P ($n=6$)	VEH	VEH	VEH	2.0
					APO-2.0-P + 2.0-UP ($n=6$)	VEH	2.0	VEH	2.0

APO = apomorphine; 2.0 = apomorphine 2.0 mg/kg; 0.05 = apomorphine 0.05 mg/kg; VEH = vehicle; UP = unpaired; P = paired; CT = conditioning test; ST = sensitization test.

2.0-UP group received 0.05 mg/kg apomorphine unpaired (APO-2.0-UP + 0.05-UP; $n=6$) and the vehicle group received vehicle (VEH–VEH). The treatment protocols are summarized and presented in Table 1.

2.5. Experiment 2: effect of the paired/unpaired protocol changes on the reversal of locomotor conditioned and sensitized responses produced by apomorphine

In this experiment, the same initial experimental protocol as experiment 1 was used. In the induction phase, there were three experimental groups: a paired group (APO-P; $n=12$), an unpaired group (APO-UP; $n=6$) and a vehicle treatment group (VEH; $n=6$). In the pharmacological treatment of the reverse phase, the APO-P group was divided into two sub-groups in which one sub-group received vehicle associated to the experimental arena (APO-P + VEH-P; $n=6$) and the other sub-group was subjected to the unpaired protocol, receiving vehicle immediately before being placed into the test environment and 2.0 mg/kg apomorphine 30 min after being removed from the test environment (APO-P + 2.0-UP; $n=6$). The animals from the APO-UP group in the reverse phase were subjected to the paired protocol (2.0 mg/kg apomorphine immediately before being placed into the test environment and vehicle 30 min after being removed from the test environment (APO-UP + 2.0-P). The vehicle group received only vehicle (VEH–VEH). The treatment protocols are summarized and presented in Table 1.

2.6. Statistics

For the 5-day drug treatment phases, a repeated two-way analysis of variance (ANOVA) was used to analyse the locomotor data to determine the group effect, day effect, as well as the interactions between variables. When a significant effect of group vs. 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. The behavioral data obtained from the conditioning and apomorphine challenge tests were analysed using a one-way ANOVA. Wherever indicated by the ANOVA (group effects with p-values<0.05), specific differences among groups were analysed by Duncan's multiple range test.

3. Results

Prior to the start of experimentation, the animals underwent to a three-day habituation procedure. The statistical analysis using a one-way ANOVA indicated a significant decrease in locomotion over days ($F_{(2,\ 123)}=20.88$; $p<0.01$ as expected for the development of habituation to a novel environment (Cerbone and Sadile, 1994)). The Duncan's test showed that day 1 had higher locomotor activity than day 2 and day 3 ($p<0.05$) (data not shown) and day 2 had higher locomotor activity than day 3 ($p<0.05$) (data not shown). Importantly, prior to the initiation of the conditioning protocol, there were no differences ($p>0.05$) among the treatment groups in any experiment.

3.1. Experiment 1: effect of extinction and low autoreceptor dose of apomorphine on conditioned and sensitized locomotor response produced by a high postsynaptic dose of apomorphine

Fig. 1 shows the mean locomotor activity scores during the induction phase for the pharmacological treatment, conditioning test and sensitization test. For the pharmacological treatment phase (Fig. 1A), a repeated two-way ANOVA indicated that there was an effect of groups [$F_{(2,\ 27)}=17.60$; $p<0.01$], an interaction of group × days [$F_{(8,\ 108)}=9.72$; $p<0.01$] but no effect of days of treatment [$F_{(4,\ 108)}=1.43$; $p>0.05$]. A one-way ANOVA followed by Duncan's multiple range test to further analyse the interaction group × days showed that from days 1 to 5, the APO-2.0-P group had higher locomotor activity than all other groups ($p<0.05$). There was no difference between the VEH and APO-2.0-UP groups ($p>0.05$). The results also showed that for the APO-P group, the fourth and fifth days of administration had higher locomotor activity than the first and second days of pharmacological treatment ($p<0.05$), showing the development of a locomotor sensitized response. For the conditioning test (Fig. 1B), a one-way ANOVA showed that there was a difference among the experimental groups [$F_{(2,\ 27)}=20.14$; $p<0.01$] and the Duncan test showed that the APO-2.0-P group had higher locomotor activity than the VEH and APO-2.0-UP groups ($p<0.05$). For the sensitization test (Fig. 1C), a one-way ANOVA showed that there was a difference among the experimental groups [$F_{(2,\ 27)}=11.57$; $p<0.01$] and the Duncan's multiple range test showed that the APO-2.0-P group had higher locomotor activity than all other groups ($p<0.05$). There was no difference between the VEH and APO-2.0-UP groups ($p>0.05$).

Fig. 2 shows the mean locomotor activity scores during the extinction/counter-conditioning and the subsequent conditioning test and sensitization test. In this phase, the animals from the APO-2.0-P group were divided into three sub-groups in which one sub-group received 0.05 mg/kg apomorphine associated to the experimental arena during the extinction/counter-conditioning phase (APO-2.0-P + 0.05-P; $n=6$); the other sub-group received 0.05 mg/kg apomorphine not associated to the experimental arena (APO-2.0-P + 0.05-UP; $n=6$) and the last sub-group received vehicle associated to the experimental arena (APO-2.0-P + VEH-P; $n=6$). The animals from the APO-2.0-UP group received 0.05 mg/kg apomorphine not associated to the experimental arena (APO-2.0-UP + 0.05-UP; $n=6$) and the vehicle group received vehicle (VEH–VEH). For the extinction/counter-conditioning phase

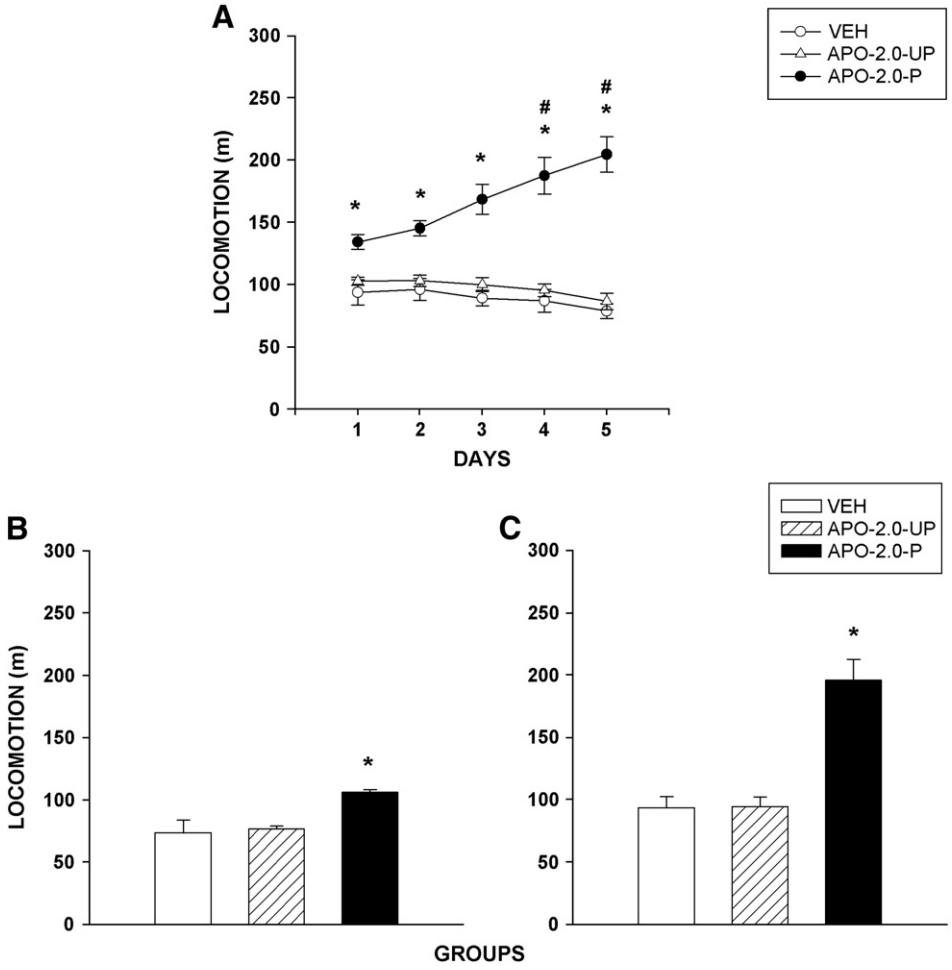

Fig. 1. Means and S.E.M. of effects of administration of apomorphine 2.0 mg/kg on locomotion during the induction phase of the experiment 1 for pharmacological treatment (A), conditioning test (B) and sensitization test (C). * denotes higher locomotor activity than the other groups. # denotes that for the APO-2.0-paired group the locomotor activity on the 4th and 5th days were higher than the 1st and 2nd days ($p<0.05$; ANOVA followed by Duncan's multiple range test).

(Fig. 2A), a repeated two-way ANOVA indicated that there was only an effect of groups [$F_{(4,\ 25)}=5.0$; $p<0.01$]. There was no effect of days of treatment [$F_{(4,\ 100)}=2.0$; $p>0.05$] and no interaction of group × days [$F_{(16,\ 100)}=0.8$; $p>0.05$]. The Duncan's test showed that the APO-2.0-P + 0.05-P group had lower locomotor activity than the other groups ($p<0.05$). There was no difference among the VEH–VEH, APO-2.0-P + 0.05-UP, APO-2.0-P + VEH-P and APO-2.0-UP + 0.05-UP groups ($p>0.05$). For the conditioning test (Fig. 2B), a one-way ANOVA showed that there was no difference among the experimental groups [$F_{(4,\ 29)}=0.5$; $p>0.05$]. For the sensitization test (Fig. 2C), a one-way ANOVA showed that there was a difference among the experimental groups [$F_{(4,\ 25)}=5.71$; $p<0.01$] and the Duncan's multiple range test showed that the APO-2.0-P + 0.05-P, APO-2.0-P + 0.05-UP and APO-2.0-P + VEH-P groups had higher locomotor activity than VEH–VEH and APO-2.0-UP + 0.05-UP groups ($p<0.05$).

3.2. Experiment 2: effect of the paired/unpaired protocol changes on the reversal of locomotor conditioned and sensitized responses produced by apomorphine

Fig. 3 shows the mean locomotor activity scores during the induction phase for the pharmacological treatment, conditioning test and sensitization test. For the pharmacological treatment phase (Fig. 3A), a repeated two-way ANOVA indicated that there was an interaction of group × days [$F_{(8,\ 84)}=9.75$; $p<0.01$], an effect of groups [$F_{(2,\ 21)}=18.83$; $p<0.01$] and an effect of days of treatment [$F_{(4,\ 84)}=7.0$; $p<0.01$]. A one-way ANOVA followed by Duncan's multiple range test to further analyse the interaction group × days showed that from days 1 to 5, the APO-P group had higher locomotor activity than the VEH and APO-UP groups ($p<0.05$). For the conditioning test (Fig. 3B), a one-way ANOVA showed that there was a difference among the experimental groups [$F_{(2,\ 23)}=10.80$; $p<0.01$] and the Duncan test showed that the APO-P group had higher locomotor activity than the VEH and APO-UP groups ($p<0.05$). For the sensitization test (Fig. 3C), a one-way ANOVA showed that there was a difference among the experimental groups [$F_{(2,\ 23)}=20.74$; $p<0.01$] and the Duncan's multiple range test showed that the APO-P group had higher locomotor activity than all other groups ($p<0.05$). There was no difference between the VEH and APO-UP groups ($p>0.05$).

Fig. 4 shows the mean locomotor activity scores during the reverse phase for the pharmacological treatment, conditioning test and sensitization test. In this phase, the animals from the APO-P group were divided into two sub-groups in which one sub-group received vehicle associated to the experimental arena (APO-P + VEH-P; $n=6$) and the other sub-group was subjected to the unpaired protocol, receiving vehicle immediately before being placed into the test environment and 2.0 mg/kg apomorphine 30 min after being removed from the test environment (APO-P + 2.0-UP; $n=6$). The animals from the APO-UP group were subjected to the paired protocol, in which they received 2.0 mg/kg apomorphine immediately before being placed into the test environment and vehicle 30 min after being removed from the test environment (APO-UP + 2.0-P). The vehicle group received only vehicle (VEH–VEH). For the pharmacological treatment reverse phase (Fig. 4A), a repeated two-way ANOVA

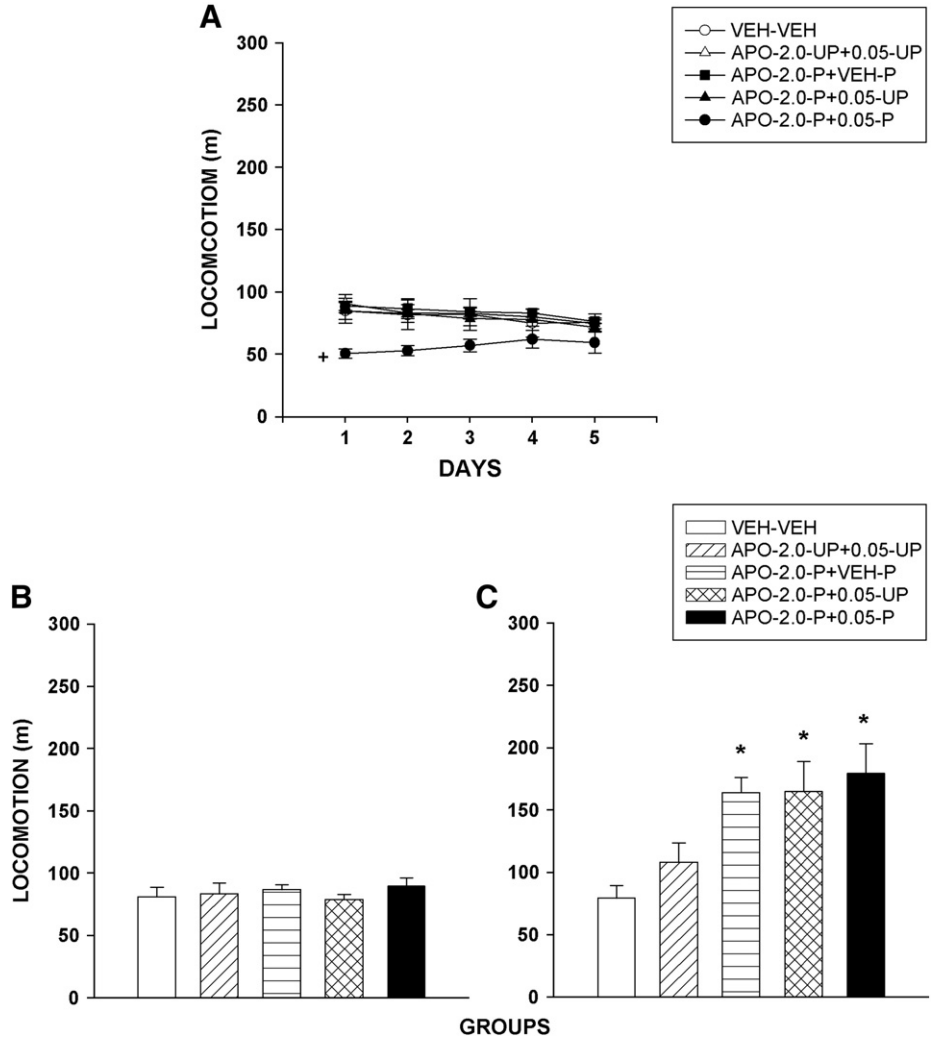

Fig. 2. Means and S.E.M. of locomotor activity during (A) the extinction/counter-conditioning phase of experiment 1, (B) conditioning test and sensitization test (C). $^{+}$ denotes lower locomotor activity than the all groups. * denotes higher locomotor activity than the VEH–VEH and APO-2.0-UP + 0.05-UP groups ($p<0.05$; ANOVA followed by Duncan's multiple range test).

indicated that there was an effect of groups [$F_{(3,\ 20)} = 48.44$; $p<0.01$], an effect of days of treatment [$F_{(4,\ 80)} = 3.2$; $p<0.05$] and interaction of group × days [$F_{(12,\ 80)} = 6.6$; $p<0.01$]. A one-way ANOVA followed by Duncan's multiple range test to further analyse the interaction group × days, showed that from days 1 to 5, the APO-UP + 2.0-P group had higher locomotor activity than the VEH–VEH, APO-P + VEH-P and APO-P + 2.0-UP groups ($p<0.05$). For the conditioning test (Fig. 4B), a one-way ANOVA showed that there was no difference among the experimental groups [$F_{(3,\ 23)} = 2.43$; $p>0.05$]. For the sensitization test (Fig. 4C), a one-way ANOVA showed that there was a difference among the experimental groups [$F_{(3,\ 23)} = 10.83$; $p<0.01$] and the Duncan's multiple range test showed that the APO-UP + 2.0-P group had higher locomotor activity than the other groups ($p<0.05$). The results also showed that the APO-P + 2.0-UP and APO-P + VEH-P groups had higher locomotor activity than VEH–VEH ($p<0.05$).

4. Discussion

In agreement with our previous report (Dias et al., 2010), we have shown once again, with a Pavlovian paired/unpaired protocol, that repeated apomorphine treatments at a dose level (2.0 mg/kg), which induces hyperlocomotion, generates a conditioned hyperlocomotion response and a sensitized apomorphine locomotion stimulant effect selectively in the paired groups. By using extinction and counter-conditioning procedures, we further showed that the conditioned locomotion stimulant response could be readily and reliably eliminated. While the counter-conditioning treatment suppressed locomotion more than extinction in the extinction phase of experiment 1 it was no more effective than extinction in eliminating the apomorphine conditioned hyper-locomotion response. In that the extinction protocol alone completely eliminated the conditioned response, this lack of a counter-conditioning effect was probably a case of a floor effect. On the other hand, the sensitized apomorphine locomotion response was insensitive to these extinction and counter-conditioning manipulations. This profound difference between conditioned and sensitized apomorphine effects was reinforced by our reversal protocol in which we found that switching the paired/unpaired treatments failed to induce a conditioned apomorphine response in the previously unpaired group but, in contrast, potentiated the apomorphine sensitization response. Altogether, these studies emphasize the necessity of differentiating conditioning and sensitization as distinct and separate phenomena.

There are two important issues raised by the present study: (a) what is the relationship between a conditioned drug response and a sensitized drug response; and (b) what is the impact of the unpaired drug treatments? In considering the conditioned/sensitized drug response issue wherein the response is locomotion, it is critical to be able to differentiate drug effects which achieve a positive effect in a

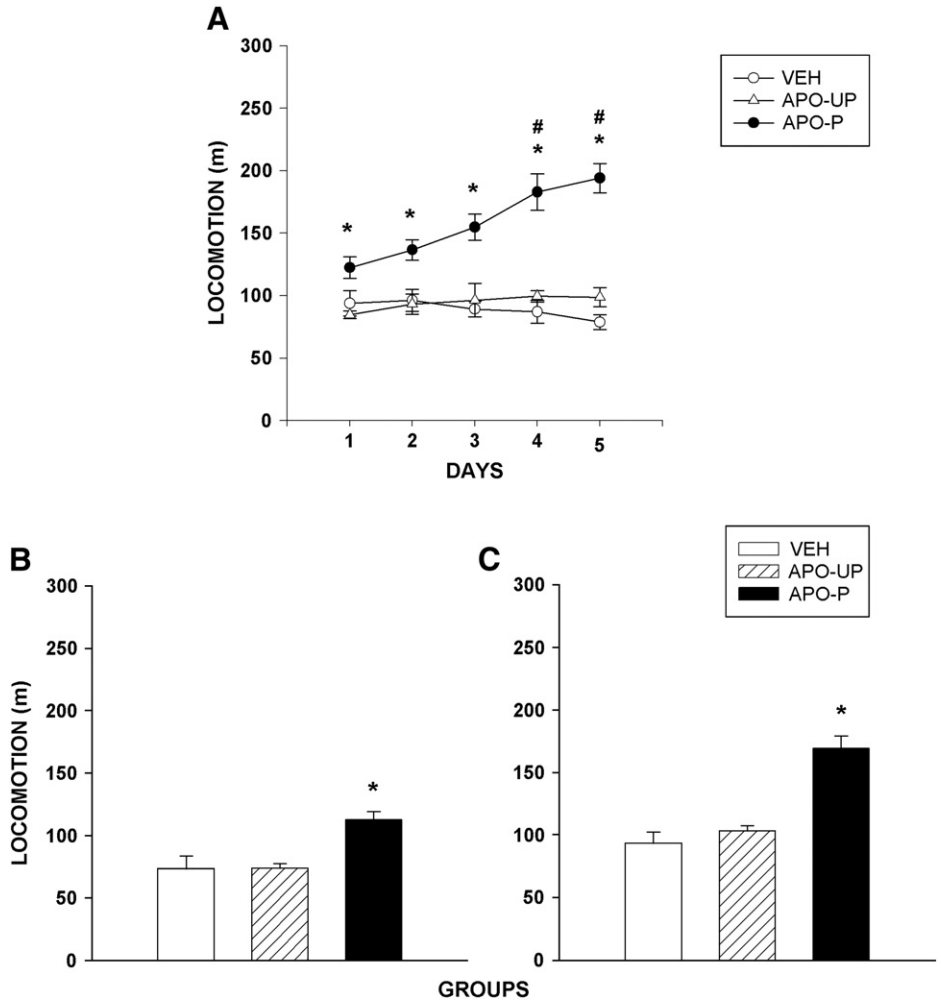

Fig. 3. Means and S.E.M. of effects of administration of apomorphine 2.0 mg/kg on locomotion during the induction phase of the experiment 2 for pharmacological treatment (A), conditioning test (B) and sensitization test (C). * denotes higher locomotor activity than the other groups. # denotes that for the APO-2.0-paired group the locomotor activity on the 4th and 5th days were higher than the 1st and 2nd days ($p<0.05$; ANOVA followed by Duncan's multiple range test).

conditioning test by decreasing a negative effect; i.e., by blocking an inhibitory behavioral effect; such as, habituation vs. a positive effect in which the conditioned drug effect is additive to the baseline locomotion activation. This critical differentiation takes on importance when the target behavior is locomotion in an open field because repeated non-drug exposure to the open field induces habituation that decreases locomotion. In the present study, the animals received three pre-exposures to the test environment as a way to induce habituation prior to the start of the experiment. While both drug and non-drug groups were equally habituated prior to the start of experimentation, the vehicle and unpaired groups received 5 additional non-drug test environment exposures whereas the drug group received the five additional exposures in the drug state. As a consequence, the non-drug groups had 5 more non-drug habituation trials than the paired drug group. In addition, the conditioning test was administered 2 days after the paired/unpaired protocol. For the non-drug and unpaired groups, the conditioning test occurred only 2 days after their 5 additional habituation sessions, whereas, for the paired drug group, the conditioning test was carried out 7 days after their previous non-drug test in the test environment. Possibly, 7 days may have been sufficient to attenuate the habituation effects. Some support for this possibility is provided by the increase in locomotion distance scores of the vehicle group from their first conditioning tests (Figs. 1B and 3B) when compared to their scores after a 7-day delay (Figs. 2B and 4B). In both instances, locomotion scores increased in the vehicle control groups after 7 days of non-drug testing and were comparable to the scores for the paired drug groups in the conditioning tests (Figs. 1B and 3B). Altogether, these findings suggest that the repeated 2.0 mg/kg apomorphine paired treatments may have simply blocked habituation and in this way led to a greater locomotion scores in the conditioning test.

Although a Pavlovian paired/unpaired protocol was employed, the conditioning test results may have produced a false positive for conditioning; and, instead, may represent an anti-habituation effect (Carey and Gui, 1997). Such a consideration is plausible given that the dose level of apomorphine (2.0 mg/kg) could have been sufficient to induce drug state dependent effects in which the information acquired in the drug state did not transfer to the non-drug conditioning test. Conceptualized as an anti-habituation effect rather than as a Pavlovian conditioned response, the present results are consistent with the lack of interaction between the conditioned and unconditioned responses. In that locomotion activation is widely used as the dependent variable in studies of behavioral sensitization induced by dopaminergic stimulant drugs, this anti-habituation/conditioning issue is not unique to apomorphine (Ahmed et al., 1995; Carey et al., 2008b; Damianopoulos and Carey, 1992). In Pavlovian conditioning, the conditioned response is a replica, albeit, partial of the unconditioned drug response. Consequently, the conditioned and unconditioned responses would be expected to at least be additive in a sensitization test. In that the evidence in the present study points to anti-habituation rather than Pavlovian conditioning as the basis for the positive outcome in the conditioning test, there would be no expectation of an additive transfer

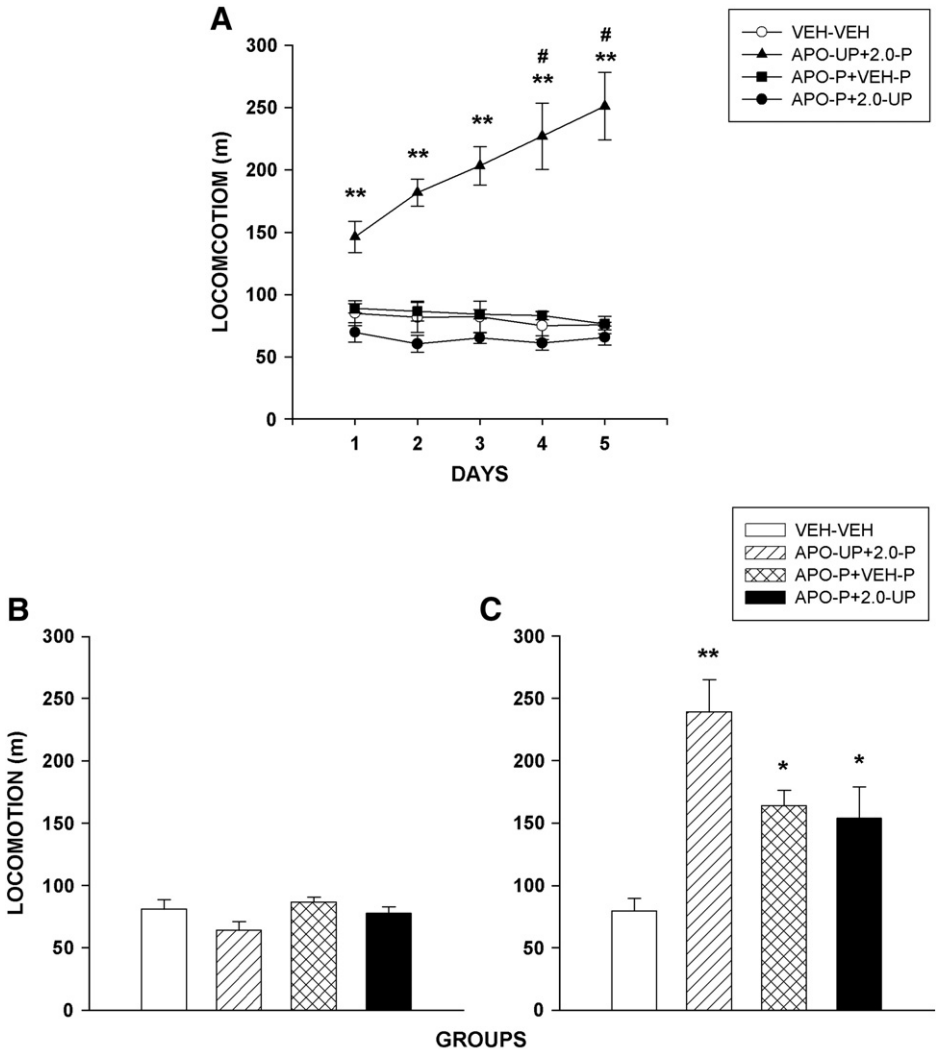

Fig. 4. Means and S.E.M. of locomotor activity during the reverse phase of the experiment 2 for pharmacological treatment (A), conditioning test (B) and sensitization test (C). ** denotes higher locomotor activity than the other groups. # denotes that for the APO-2.0-UP + 2.0-P group the locomotor activity on the 4th and 5th days were higher than the 1st and 2nd days. * denotes higher locomotor activity than the VEH–VEH group ($p<0.05$; ANOVA followed by Duncan's multiple range test).

effect from the conditioning test to the test for sensitization. This consideration of the conditioning test results as anti-habituation effects is pertinent to the asymmetry observed when the paired and unpaired treatments were reversed.

As we have argued, the initial paired/unpaired treatment protocol resulted in a positive outcome for conditioning test, which could be accounted for by an anti-habituation effect. Why then when the paired/unpaired treatments were reversed did not a positive outcome for conditioning occur in the test for conditioning? While in both cases there was the same 2-day vs. 7-day delay between the paired/unpaired groups in terms of their non-drug exposure to the test environment, there was a major difference. In the first paired/unpaired treatment cycle, the paired group had 3 non-drug exposures to the test environment, whereas, the unpaired group had 8 non-drug exposures. By the completion of the second paired/unpaired cycle (reversal protocol) both unpaired and paired groups now had 8 (9 if the first conditioning test is included) non-drug exposures to the test environment. Presumably, the substantial increase in non-drug exposures was sufficient to induce a stable habituation response to the test environment. Surprisingly, the greater non-drug test environment exposure of the unpaired group prior to the reversal treatment which served to block a positive conditioning effect (anti-habituation) in the test for conditioning, but yet, had the opposite effect and potentiated the locomotion stimulant response to apomorphine in the sensitization test. One speculative possibility to account for this differential outcome in terms of habituation processes is that the greater habituation to the environmental cues resulted in a reduction of competing behavioral responses elicited by environmental cues. Seemingly, this decrease in response competition allowed the apomorphine induced locomotor behavior to be more pronounced. The difficulty with this line of reasoning is that the unpaired group should have shown an enhanced response to apomorphine in the initial sensitization test (challenge test 1 shown in Fig.1) as compared to the apomorphine paired group in its first paired treatment test. This outcome would be expected because of the greater number of non-drug test environment exposures for the unpaired group (8) before its first paired apomorphine treatment vs. the paired group prior to its first paired apomorphine treatment (3). In fact, the results presented in Fig. 1 show just the opposite result. Additionally, the expectation would be that the paired group, following the reversal treatment, should have manifested a greater response on the second sensitization than on the first sensitization test because it would have acquired an increase in habituation as a result of the additional 5 non-drug test environment exposures it received during the unpaired treatments in the reversal phase. As is evident in comparing the sensitization results for the initial paired treatment group, the effect of the additional 5 unpaired treatment trials were just the opposite (Fig. 3 vs. Fig. 4).

The disparity between the paired/unpaired groups on the second sensitization test remains both puzzling and intriguing. Both groups had the same number of drug exposures as well as the same number of paired/unpaired experiences to the test environment. Previous studies (Braga et al., 2009c) have indicated that the locomotion stimulant effect induced by the dose level of the apomorphine (2.0 mg/kg) used in the present experiment becomes asymptotic after 5 or 6 treatments. In the present study, the reversal protocol was able to set a new higher response ceiling for the apomorphine locomotion output. A receptor based explanation for this reversal effect seems unlikely in that the drug and environment exposures were the same for both groups. Seemingly, the explanation would lie in behavioral mechanisms. It is possible that the initial repeated paired treatments in the paired group forged a response pattern (Damianopoulos and Carey, 1994), which limits the response ceiling but that the increased number of non-drug experiences to the test environment in the unpaired group created the opportunity for a more complex response pattern which in effect raises the ceiling of locomotion output. Alternatively, one might speculate on a contrast effect such that the unpaired group went from experiencing the apomorphine treatment effects in the confined quarters of the home-cage to experiencing apomorphine in the more expansive test cage; and, this was a positive contrast, whereas, the paired group had the opposite experience which was a negative contrast effect. While admittedly these are speculative notions, the profound unexpected impact of the paired/unpaired treatment sequence upon the magnitude of the sensitization response is of considerable importance and merits further experimental study. It also is important to recognize that the detection of this new behavioral factor as a determinant of the sensitization response was only possible because a paired/unpaired treatment protocol was used. Seemingly, the paired/unpaired protocol needs to be seen as essential not only for drug conditioning studies but also for drug sensitization experimentation.

Acknowledgements

This research was supported by FAPERJ (E-26/110.195/2008), VA Merit Review Grant and NIDA Grant R01 05366. L.W.M. is a recipient of a fellowship from UENF, Brazil and M.P.C. is a CNPq research fellow. We thank Gina Nunes Teixeira for technical assistance and Dr. Richard Ian Samuels for revision of the text.

References

Adams JU, Careri JM, Efferen TR, Rotrosen J. Conditioned locomotor stimulant effects of cocaine in rats do not result from interference with habituation. Psychopharmacology 2000;151:13–8.

Aghajanian GK, Bunney BS. Central dopaminergic neurons: neurophysiological identification and responses to drugs. In: Snyder S, Usdin E, editors. Frontiers in catecholamine research. New York: Pergamon Press; 1973. p. 643–8.

Ahmed SH, Cador M, Le Moal M, Stinus L. Amphetamine-induced conditioned activity in rats: comparison with novelty-induced activity and role of the basolateral amygdala. Behav Neurosci 1995;109:723–33.

Anagnostaras SG, Robinson TE. Sensitization to the psychomotor stimulant effects of amphetamine: modulation by associative learning. Behav Neurosci 1996;110:1397–414.

Anagnostaras SG, Schallert T, Robinson TE. Memory processes governing amphetamine-induced psychomotor sensitization. Neuropsychopharmacology 2002;26:703–15.

Braga PQ, Dias FR, Carey RJ, Carrera MP. Low dose apomorphine induces context-specific sensitization of hypolocomotion without conditioning: support for a new state dependent retrieval hypothesis of drug conditioning and sensitization. Pharmacol Biochem Behav 2009a;93:128–33.

Braga PQ, Dias FR, Carey RJ, Carrera MP. Behavioral sensitization to dopaminergic inhibitory and stimulatory effects induced by low vs. high dose apomorphine treatments: an unconventional dose and response reversal sensitization challenge test reveals sensitization mechanisms. Behav Brain Res 2009b;204:169–74.

Braga PQ, Galvanho JP, Bloise E, Carey RJ, Carrera MP. The expression of locomotor sensitization to apomorphine is dependent on time interval between injection and testing. Pharmacol Biochem Behav 2009c;91:278–82.

Carey R, Gui J. A simple and reliable method for the positive identification of Pavlovian conditioned cocaine effects in open-field behavior. J Neurosci Meth 1997;73:1–8.

Carey RJ, Gui J. Cocaine conditioning and cocaine sensitization: what is the relationship? Behav Brain Res 1998;92:67–76.

Carey RJ, Damianopoulos EN, Shanahan AB. Cocaine conditioned behavior: a cocaine memory trace or an anti-habituation effect. Pharmacol Biochem Behav 2008a;90:625–31.

Carey RJ, Huston JP, Müller CP. Pharmacological inhibition of dopamine and serotonin activity blocks spontaneous and cocaine-activated behaviour. Prog Brain Res 2008b;172:347–60.

Cerbone A, Sadile AG. Behavioral habituation to spatial novelty: interference and noninterference studies. Neurosci Biobehav Rev 1994;18:497–518.

Crombag HS, Badiani A, Maren S, Robinson TE. The role of contextual versus discrete drug-associated cues in promoting the induction of psychomotor sensitization to intravenous amphetamine. Behav Brain Res 2000;116:1-22.

Damianopoulos EN, Carey RJ. Conditioning, habituation and behavioral reorganization factors in chronic cocaine effects. Behav Brain Res 1992;49:149–57.

Damianopoulos EN, Carey RJ. Apomorphine sensitization effects: evidence for environmentally contingent behavioral reorganization processes. Pharmacol Biochem Behav 1993;45:655–63.

Damianopoulos EN, Carey RJ. A new method to assess Pavlovian conditioning of psychostimulant drug effects. J Neurosci Meth 1994;53:7-17.

Dias FR, Carey RJ, Carrera MP. Apomorphine-induced context specific behavioural sensitization is prevented by the D1 antagonist SCH-23390 but potentiated and uncoupled from contextual cues by the D2 antagonist sulpiride. Psychopharmacology 2010; 209:137-157-1.

Hinson RE, Poulos CX. Sensitization to the behavioral effects of cocaine: modification by Pavlovian conditioning. Pharmacol Biochem Behav 1981;15:559–62.

Hotsenpiller G, Wolf ME. Conditioned locomotion is not correlated with behavioral sensitization to cocaine: an intra-laboratory multi-sample analysis. Neuropsychopharmacology 2002;27:924–9.

Kebabian JW, Calne DB. Multiple receptors for dopamine. Nature 1979;277:93–6.

Keller S, Delius JD, Acerbo MJ. Apomorphine sensitization: evoking conditions, context dependence, effect persistence and conditioned nature. Behav Pharmacol 2002;13:189–201.

Mattingly BA, Koch C, Osborne FH, Gotsick JE. Stimulus and response factors affecting the development of behavioral sensitization to apomorphine. Psychopharmacology 1997;130:109–16.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78:189–225.

Möller HG, Nowak K, Kuschinsky K. Studies on interactions between conditioned and unconditioned behavioural responses to apomorphine in rats. Naunyn Schmiedebergs Arch Pharmacol 1987;335:673–9.

Nasello AG, Machado C, Bastos JF, Felicio LF. Sudden darkness induces a high activity–low anxiety state in male and female rats. Physiol Behav 1998;63:451–4.

Pinheiro Carrera M, Brunhara FC, Schwarting RK, Tomaz C. Drug conditioning induced by intrastriatal apomorphine administration. Brain Res 1998;790:60–6.

Post RM, Rose H. Increasing effects of repetitive cocaine administration in the rat. Nature 1976;260:731–2.

Post RM, Weiss SR, Fontana D, Pert A. Conditioned sensitization to the psychomotor stimulant cocaine. Ann NY Acad Sci 1992;654:386–99.

Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 1986;396:157–98.

Schiff SR. Conditioned dopaminergic activity. Biol Psychiatry 1982;17:135–54.

Segal DS, Geyer MA, Schuckit MA. Stimulant-induced psychosis: an evaluation of animal models. Essays Neurochem Neuropharmacol 1981;5:95-129.

Sibley DR, Monsma Jr FJ. Molecular biology of dopamine receptors. Trends Pharmacol Sci 1992;13:61–9.

Skirboll LR, Grace AA, Bunney BS. Dopamine auto- and postsynaptic receptors: electrophysiological evidence for differential sensitivity to dopamine agonists. Science 1979;206:80–2.

Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 1993;4:289–312.

Tirelli E, Michel A, Brabant C. Cocaine-conditioned activity persists for a longer time than cocaine-sensitized activity in mice: implications for the theories using Pavlovian excitatory conditioning to explain the context-specificity of sensitization. Behav Brain Res 2005;165:18–25.