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Reversal of apomorphine locomotor sensitization by a single post-conditioning trial treatment with a low autoreceptor dose of apomorphine: A memory re-consolidation approach

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ABSTRACT

Sensitization is a common feature of psychostimulants and sensitization effects are generally considered to be linked to the addictive properties of these drugs. We used a conventional paired/unpaired Pavlovian protocol to induce a context specific sensitization to the locomotor stimulant effect of a high dose of apomorphine (2.0 mg/kg). Two days following a 5 session sensitization induction phase, a brief 5 min non-drug test for conditioning was conducted. Only the paired groups exhibited locomotor stimulant conditioned response effects. Immediately following this brief test for conditioning, the paired and the unpaired groups received injections of 0.05 mg/kg apomorphine, 2.0 mg/kg apomorphine or vehicle designed to differentially impact memory re-consolidation of the conditioning. Two days later, all groups received a sensitization challenge test with 2.0 mg/kg apomorphine. The 2.0 mg/kg apomorphine post-trial treatment potentiated sensitization while the 0.05 mg/kg eliminated sensitization. These effects were only observed in the paired groups. The activation of dopaminergic systems by the high dose of apomorphine strengthened the drug/environment association whereas the inhibition of dopamine activity by the low autoreceptor dose eliminated this association. The results point to the importance of conditioning to context specific sensitization and targeting memory re-consolidation of conditioning as a paradigm to modify sensitization.

1. Introduction

Psychoactive drugs can have complex effects upon behavior that can vary substantially for a particular drug depending upon dose, context and prior history. Even when drug dose and environmental context are held constant, the behavioral impact of a drug can change markedly with repeated exposure to the same drug dose treatment. For a number of psychostimulant drugs, particularly drugs with high drug abuse liability such as cocaine and amphetamine, a positive behavioral feedback occurs. With repeated intermittent psychostimulant drug treatments, the magnitude of the behavioral response elicited by the drug typically increases; and, in a non-drug test, the behavioral response is increased in the same direction as the drug response. This behavioral sensitization drug effect has been repeatedly demonstrated (Borowski and Kuhn, 1991; Heidbreder and Shippenberg, 1994; Mattingly et al., 1994; Carey and Gui, 1998; Bloise et al., 2007; Braga et al., 2009a,b; Dias et al., 2010; Filip et al., 2010; Matos et al., 2010) and is generally considered an important contributor to the addictive potency of psychostimulant drugs such as cocaine (e.g., Robinson and Berridge, 1993; Carey and Damianopoulos, 2006). Thus, it would appear that psychostimulant behavioral sensitization effects are a composite of the conditioned effects plus an increased unconditioned drug response (Peris et al., 1990; Pert et al., 1990; Henry and White, 1991; Zeigler et al., 1991; Kalivas et al., 1992; Robinson and Berridge, 1993; Jodogne et al., 1994; Carey et al., 2005a; Braga et al., 2009a,b; Dias et al., 2010). The quantitative contribution of Pavlovian conditioned drug effects to behavioral sensitization, nonetheless, remains rather obscure (Einat et al., 1996). Often but certainly not always the behavioral sensitization effects are context specific (Einat et al., 1996) and this link to associated test environment stimuli is sometimes the only basis for implicating Pavlovian conditioning mechanisms to sensitization effects. Clearly, however, context specificity links sensitization to an associative process as the same protocol that induces sensitization effects also generates Pavlovian conditioned drug responses to the contextual stimuli.

In Pavlovian conditioned drug effects, the conditioned response occurs in the presence of the conditioned stimuli with the absence of drug treatment. In contrast, for sensitization, both the drug treatment and conditioned stimuli are present. Carey and co-workers (2005b) along with others (e.g., Murray and Bevins, 2007) have pointed out that drug treatments such as cocaine provide drug stimuli that can contribute to the contextual stimulus complex. This latter consideration is pertinent to the issue of the contribution of Pavlovian conditioned

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contextual stimuli to sensitization. If the Pavlovian conditioned contextual stimuli were critical to the induction of sensitization effects, then the conditioned response could simply be eliminated or substantially attenuated by extinction resulting in elimination or diminution of the sensitization effect. Extinction of a cocaine conditioned response, however, has little or no impact upon the cocaine sensitization response (Carey and Gui, 1998). While this result would seemingly, discount the relevance of Pavlovian conditioned stimuli to sensitization effects, it is also long known from animal conditioning studies that Pavlovian conditioning is a primitive involuntary learning process that, as shown in other areas of study (e.g., fear conditioning), is long lasting and highly resistant to extinction (Pavlov, 1928). That is, the behavioral expression of the conditioned response may be suppressed by an extinction protocol but, nonetheless, it can be reinstated either spontaneously after a period of non-testing or by disinhibition with stress or other highly arousing stimuli (Pavlov, 1928). Thus, when a drug sensitization test is administered after an extinction protocol, the drug treatment could disinhibit the extinction either by the introduction of the activating drug effects or by the drug generated interoceptive stimuli. This type of disinhibition is akin to the "priming" effect in instrumental conditioning wherein an extinguished drug self-administration response is reinstated by administering the drug prior to a non-drug or non-reinforced test series. In this case, the "priming" effect of the drug treatment can be seen as a disinhibition of extinction by drug induced activation effects and/or by the introduction of drug stimuli which had been present during the drug self-administration.

The relevant observation here is that extinction of the expression of the conditioning in a non-drug test is not a sufficient basis for discounting the contribution of the Pavlovian conditioned stimuli to the sensitization effect. Rather, the lack of impact of an extinction procedure on sensitization is consistent with the fact that extinction is an ineffective procedure for inducing enduring changes in cue reactivity in drug addiction to achieve effective elimination of drug craving, drug taking or relapse.

Recently Carey and co-workers (2008) reconceptualized Pavlovian conditioning from a CS–CR formulation to a CS–memory trace–CR (CS–MT–CR) process framework formulation. Importantly, recent studies which have pointed to a new perspective regarding the plasticity of an established memory trace suggest that viewing the CS as activating a memory trace that is expressed as the CR, can offer an alternative strategy to diminish or eliminate the CS–CR. Previously, a brief dynamic phase in the formation of a memory trace was thought to exist only during the formation of a new memory trace and only then could an intervention prevent or enhance memory formation. This understanding of the consolidation aspect of memory has been recognized for some time (Duncan, 1949; McGaugh, 1966) in a variety of learning paradigms in which manipulations ranging from electroconvulsive shock (ECS) (Duncan, 1949), protein synthesis inhibitors (Flood et al., 1973; Flood et al., 1975; Davis and Squire, 1984; Litvin and Anokhin, 2000; Lattal and Abel, 2004; Gold, 2006) to glucose (Messier, 2004; Salinas and Gold, 2005) administered during a short post-trial temporal interval can block or enhance the retention of a learned response. In contrast, these same treatments administered after a longer temporal interval (e.g. 2 h), have no effect upon retention of the learning experience. Thus, a period of memory vulnerability followed by invulnerability has led to a general consensus that, following a brief post-trial time interval, memory traces become consolidated and incorporated into the physical structure of the brain. Recent analyses of memory, nonetheless, have suggested that the memory trace is not hard wired; but, memory traces, each time they are reactivated, once again become labile and sensitive to modification (Przybyzlawski and Sara, 1997; Nader et al., 2000; Berman and Dudai, 2001; Eisenberg et al., 2003; Nader, 2003; Pedeira and Maldonado, 2003; Dubiec et al., 2002; Dudai, 2004; McGaugh, 2004; Eisenhardt and Menzel, 2007). This alternative conceptualization creates the possibility that an established memory can be re-activated by cue re-exposure and then modified. This viewpoint of the memory trace differs from the perspective in which the original memory trace persists even alongside new memory traces that compete with the expression of the original memory trace. In fact, recent studies have shown that post-trial amnesic treatments that follow shortly after non-drug re-exposure of animals to cues associated with cocaine can impair the drug memory traces (Lee et al., 2006).

Apomorphine behavioral stimulant effects are initiated by increases in dopamine D1 and D2 receptor activities widely in the brain. Seemingly, with repeated high dose apomorphine treatments there is a widespread dopaminergic activation in the hippocampus and cortex in the presence of contextual stimuli and this association is strengthened and reinforced by the concurrent increased activation of dopaminergic reward areas in the brain (Robledo et al., 1992). This increase in dopamine post-synaptic activity evoked by apomorphine paired to contextual stimuli may facilitate the mnemonic system to enable apomorphine treatments to forge potent and lasting associational effects to contextual cues. In the present study we administer a high dose of apomorphine (2.0 mg/kg) to induce behavioral sensitization to the locomotor stimulant effects of the apomorphine treatment. Subsequently we conduct a brief (5 min.) non-drug test to evoke the conditioned apomorphine locomotor stimulant response and thereby activate the memory trace of the association between the test environment and apomorphine. Immediately after this re-consolidation trial we administer treatments to inhibit/enhance dopaminergic activity. Conveniently, apomorphine can also be used to serve this purpose. Apomorphine at high dose levels (>0.5 mg/kg) activates dopamine post-synaptic receptors and functions as a behavioral stimulant but, at low doses (<0.1 mg/kg) apomorphine preferentially is an agonist at dopamine D2 autoreceptors and inhibits DA neurons and suppresses locomotor behavior. Accordingly, we administer either low (0.05 mg/kg) or high (2.0 mg/kg) apomorphine treatments immediately after a brief conditioning trial so that the post-trial drug treatments occur at a time when the memory trace is being re-consolidated and therefore vulnerable to modification. Subsequent to this post-trial treatment protocol we assess its impact on the previously established apomorphine sensitization in a challenge test. The present report details the results of the investigation.

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 15:00 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 (SONY, model IR575M), mounted 60 cm above the arena was used to record behavioral data. Locomotion, measured as distance traveled (m), was automatically analyzed 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. Experimental procedure

The experiments were conducted following an experimental protocol from Braga et al. (2009a, 2009b). First, all rats received three 30 min habituation sessions (habituation phase), conducted on consecutive days. The habituation protocol was conducted so that a stable baseline of the locomotor behavior 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 next day, the animals were assigned to groups equated on baselines and were submitted to the pharmacological treatment phase, in which there were three basic treatment groups: a paired group, an unpaired group and a vehicle treatment group. In the paired group, rats received 2.0 mg/kg apomorphine (APO-2.0-P; n = 20) 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 = 20), 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 = 26) was treated in the same way as the paired group except that the animals received vehicle prior to being placed in the experimental arena. These treatments were administered for 5 consecutive days, one trial per day and served as the induction phase in which locomotion was recorded for 30 min. The induction phase was designed to establish an apomorphine sensitization response selectively in the paired apomorphine treatment groups. After a period of 2 days without injections or behavioral testing (withdrawal period), there was a conditioning test in which the animals received an injection of saline prior to being placed into the test environment and locomotion was recorded for 5 min. Immediately after the 5 minute conditioning test, the animals received their re-consolidation pharmacological treatment. For that, the paired, unpaired and vehicle groups were divided into three subgroups in which one subgroup received 2.0 mg/kg apomorphine, another subgroup received 0.05 mg/kg apomorphine and the other subgroup received vehicle. Two days later, there was a sensitization challenge test, in which the paired and unpaired groups received 2.0 mg/kg apomorphine. The experimental groups were categorized in terms of 3 treatments: (a) their induction treatment, (b) their re-consolidation treatment and (c) their drug treatment in the 2.0 mg/kg apomorphine challenge test. The three APO paired (P) subgroups were: APO-2.0 + APO-2.0 + APO-2.0, (n = 7); APO-2.0 + APO-0.05 + APO-2.0, (n = 6) and APO-2.0 + VEH + APO-2.0, (n = 7). The three APO unpaired (UP) subgroups were: APO-2.0-UP + APO-2.0 + APO-2.0, (n = 7); APO-2.0-UP + APO-0.05 + APO-2.0, (n = 6) and APO-2.0-UP + VEH + APO-2.0, (n = 7). The four vehicle (VEH) subgroups were: VEH + APO-2.0 + VEH, (n = 7); VEH + APO-0.05 + VEH, (n = 6); VEH + VEH + VEH, (n = 7) and VEH + VEH + APO-2.0; (n = 6). The treatment protocols are summarized and presented in Table 1.

2.5. Statistics

In the induction phase, a repeated two-way analysis of variance (ANOVA) was used to analyze the locomotor data to determine the group effect, day effect, as well as the interactions between variables. When a significant effect of group versus day interaction was recorded, data were further analyzed 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 test and sensitization test were analyzed using a one-way ANOVA. Wherever indicated by the ANOVA (group effects with p-values <0.05), possible differences among groups were analyzed by Duncan's multiple range test.

3. Results

Prior to the start of experimentation, the animals underwent a three-day habituation procedure. The statistical analyses using a one-way ANOVA indicated a significant decrease in locomotion over days [$F(2, 195) = 198.50$; $p<0.01$] as expected for the development of habituation to a novel environment (Cerbone and Sadile, 1994). 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 among the treatment groups ($p>0.05$) in any experiment.

Fig. 1 shows the results of the induction treatment phase where separate groups received either vehicle or apomorphine (2.0 mg/kg) paired/unpaired for 5 consecutive days. A repeated two-way ANOVA of the vehicle/apomorphine drug treatment showed an interaction of group × days [$F(16, 244) = 27.72$; $p<0.01$], an effect of groups [$F(4, 61) = 63.14$; $p<0.01$] and an effect of days of treatment [$F(4, 244) = 94.03$; $p<0.01$]. A one-way ANOVA followed by Duncan's multiple range test to further analyze the interaction of group × days, showed that from days 1–5, the APO-2.0-P groups (i.e., APO-2.0-P + VEH + APO-2.0, APO-2.0-P + APO-0.05 + APO-2.0 and APO-2.0-P +

Table 1
Treatment design for experiments.

Initial groups	Induction phase		Conditioning trial	Reconsolidation treatment	Sensitization challenge test	Final groups
	Arena	Home				
VEH (n = 26)	VEH	VEH	SAL	VEH	VEH	VEH + VEH + VEH (n = 7)
				APO-0.05	VEH	VEH + APO-0.05 + VEH (n = 6)
				APO-2.0	VEH	VEH + APO-2.0 + VEH (n = 7)
				VEH	APO-2.0	VEH + VEH + APO-2.0 (n = 6)
APO-2.0-UP (n = 20)	VEH	APO-2.0	SAL	VEH	APO-2.0	APO-2.0-UP + VEH + APO-2.0 (n = 7)
				APO-0.05	APO-2.0	APO-2.0-UP + APO-0.05 + APO-2.0 (n = 6)
				APO-2.0	APO-2.0	APO-2.0-UP + APO-2.0 + APO-2.0 (n = 7)
APO-2.0-P (n = 20)	APO-2.0	VEH	SAL	VEH	APO-2.0	APO-2.0-P + VEH + APO-2.0 (n = 7)
				APO-0.05	APO-2.0	APO-2.0-P + APO-0.05 + APO-2.0 (n = 6)
				APO-2.0	APO-2.0	APO-2.0-P + APO-2.0 + APO-2.0 (n = 7)

APO-2.0 = apomorphine 2.0 mg/kg; APO-0.05 = apomorphine 0.05 mg/kg; VEH = vehicle; UP = unpaired; P = paired.

APO-2.0 + APO-2.0) had higher locomotor levels than the VEH and the APO-2.0-UP groups ($p<0.05$). Pertinent to the issue of development of locomotor sensitization, an analysis (one-way ANOVA followed by Duncan's test) carried out for the APO-2.0-P groups across the days of administration showed higher locomotor activity on days 5 and 4 than day 1 ($p<0.05$).

Fig. 2 presents the locomotor activity scores for the vehicle, APO-2.0-unpaired and APO-2.0-paired groups during the 5 min conditioning test. A one-way ANOVA showed that there was a difference among the experimental groups [$F(2, 63) = 70.0$; $p<0.01$] and the Duncan's test showed that the APO-2.0-P group had higher locomotor levels than all other groups ($p<0.05$).

Fig. 3 presents the locomotor activity scores for groups during the sensitization test. A one-way ANOVA showed that there was a difference among the experimental groups [$F(9, 56) = 19.51$; $p<0.01$] and the Duncan's test showed that the APO-2.0-P + APO-2.0 + APO-2.0 group had higher locomotor levels than all groups ($p<0.05$). The APO-2.0-P + VEH + APO-2.0 showed higher locomotion than all the other groups ($p<0.05$), except the APO-2.0-P + APO-2.0 + APO-2.0 group.

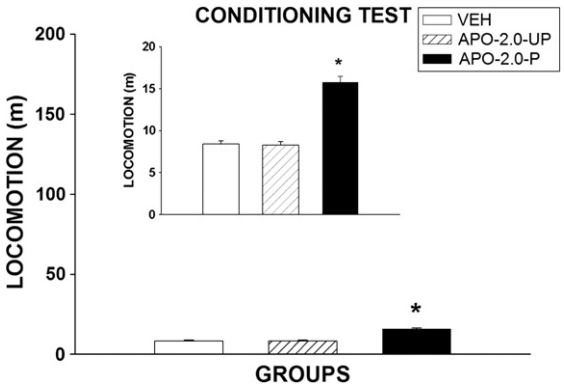

Fig. 2. Means and S. E. M. of effects of locomotor activity during the 5 min conditioning test. * denotes higher locomotor activity for the APO-2.0-P groups than the VEH and APO-2.0-UP groups ($p<0.05$; ANOVA followed by Duncan's multiple range test). The inset presents the same data on a different scale more in line with the low level of locomotion in the 5 min non-drug test.

4. Discussion

In the present study the high dose apomorphine treatment reliably induced a behavioral sensitization effect expressed as hyper-locomotion. This phenomenon has been demonstrated previously in a number of publications (Damianopoulos and Carey, 1993; Mattingly et al., 1997; Busidan and Dow-Edwards, 1999; Bloise et al., 2007; Braga et al., 2009a,b; Dias et al., 2010; Matos et al., 2010). While the role of conditioning and context specificity is not always evident in apomorphine sensitization studies (Damianopoulos and Carey, 1994; Mattingly et al., 1988) in the present study a paired/unpaired Pavlovian conditioning protocol was used so that our finding of sensitization and conditioned apomorphine effects selectively in the paired groups indicated that the effects were context specific and that associational processes were crucial for the effects. In the brief 5 min non-drug conditioning test the paired apomorphine group was the only group that exhibited a conditioned locomotor stimulant effect. This result is a prerequisite condition for the post-trial treatments to have a potential effect upon memory re-consolidation. In this brief 5 min test the conditioned response effect was robust statistically but yet there was only a very small fraction of the apomorphine sensitization response. The key feature of this conditioned effect lies not in the magnitude of the conditioned response but rather that it is a behavioral manifestation of the activation of the memory trace by the test environment cues of the high dose apomorphine association to the test environment. The impact on sensitization of the post-trial treatments was assessed in a subsequent high dose apomorphine sensitization challenge test. In this challenge test the sensitization effects were found selectively in the paired groups and there were prominent bidirectional effects of the post-trial treatments. The dopamine activating high dose apomorphine post-trial treatment resulted in a potentiation of sensitization in the challenge test whereas, the dopamine inhibitory low dose apomorphine post-trial treatment eliminated sensitization in the challenge test. Consistent with a memory based interpretation of the post-trial treatments was the finding that neither of these post-trial treatments given to the unpaired groups had an effect on the challenge test. This finding is in line with the fact that the post-trial treatments were in fact unpaired treatments and therefore not linked to the test environment cues. From a memory re-consolidation perspective however, the post-trial treatment drug effects occurred during the transient re-consolidation of the memory trace activated by placement in the test environment. The selectivity of the post-trial treatments to the paired groups is consistent with the proposition that these post-trial treatments impacted memory re-consolidation and enhanced and inhibited, respectively the associational bond between the test environment and the high dose apomorphine hyper-locomotion sensitization effect.

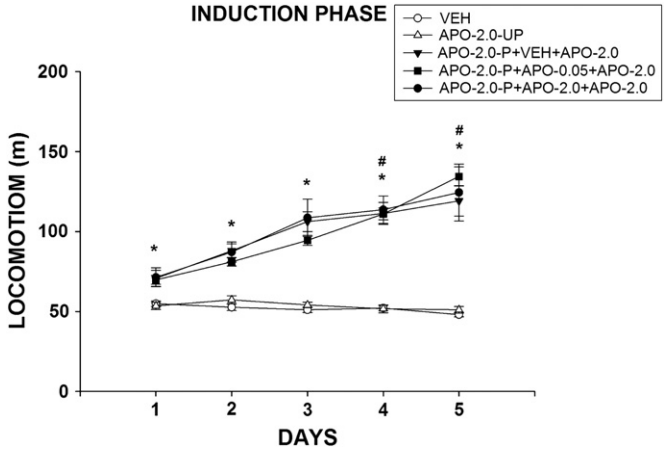

Fig. 1. Means and S. E. M. of effects of administration of apomorphine of 2.0 mg/kg on locomotion during the induction phase. * denotes higher locomotor activity than the other groups. # denotes higher locomotor activity on the 4th and 5th days than the 1st day for the APO-2.0-P groups ($p<0.05$; ANOVA followed by Duncan's multiple range test).

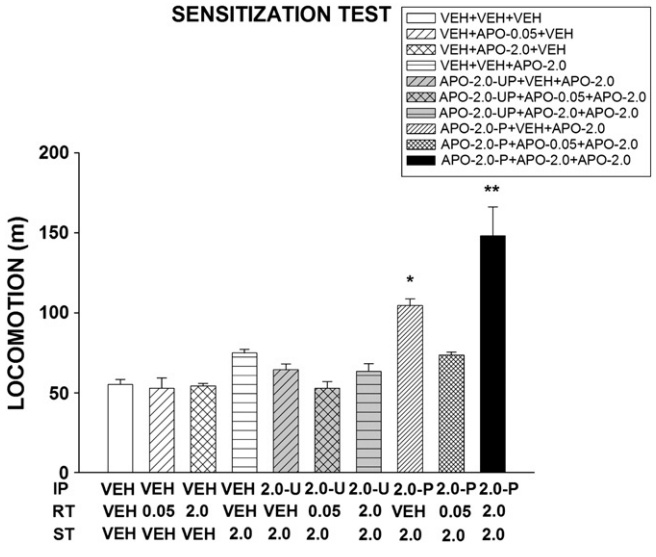

Fig. 3. Means and S. E. M. of effects of administration of 2.0 mg/kg apomorphine on locomotion during the sensitization test. ** denotes higher locomotor activity than all the other groups. *denotes higher locomotor activity than all the other groups, except the APO-2.0-P + APO-2.0 + APO-2.0 group ($p<0.05$; ANOVA followed by Duncan's multiple range test). IP = induction phase; RT = re-consolidation treatment; ST = sensitization test; U = unpaired; P = paired; 2.0 = apomorphine 2.0 mg/kg; 0.05 = apomorphine 0.05 mg/kg.

Apomorphine is a prominent dopaminergic drug and it is not altogether surprising that present results implicate dopamine in the re-consolidation process for this specific paradigm. Whether the post-trial apomorphine treatments can impact re-consolidation processes more broadly, however, necessitate the application of these treatments to sensitization effects induced by drugs other than apomorphine including dopaminergic as well as non-dopaminergic drugs that can induce sensitization and conditioned effects. A key element in the present study is that the low dose apomorphine treatment that reversed the psychostimulant sensitization was administered after a brief exposure to the contextual stimuli. Consequently, such a reversal treatment (i.e. low dose apomorphine) does not entail further exposure to the high dose apomorphine treatment. This feature of a drug memory re-consolidation paradigm that does not entail further exposure to the psychostimulant drug treatment that induced sensitization is clearly desired in a therapeutic clinical setting. Furthermore, a drug treatment such as a low dose of apomorphine given in a post-trial memory re-consolidation paradigm is short acting and can be given episodically and not chronically. While apomorphine at a high dose reliably can induce sensitization and conditioning effects in rodent behavior it is not considered an addictive drug for humans. Accordingly, it will be important to evaluate the strategy employed in the present study using psychostimulant drugs with high addictive potential such as cocaine to induce conditioned and sensitization effects and then to determine if inhibition of dopamine activity immediately after a brief re-consolidation trial with a low dose of apomorphine can also reverse sensitization effects induced by highly addictive psychostimulant drugs such as cocaine. By directing the experimental attention to the associational component of sensitization not only are sensitization drug effects addressed but in addition so is cue activated craving.

Sensitization is a common feature of psychostimulant drugs such as amphetamine and cocaine and it has been generally thought that this sensitization effect is related to the addictive properties of these drugs (e.g. Stewart and Badiani, 1993; Morgan et al., 2006). Indeed, the neuroadaptive changes underlying sensitization are thought to resemble those responsible for addiction. Therefore, an understanding of the mechanisms of sensitization is of importance in that such information could facilitate the development of improved treatments for addiction. The occurrence of sensitization has been attributed to a variety of neurobiological and behavioral factors such as accumulation of drug metabolites, increases and/or decreases in drug affected receptors, and habituation to contextual cues and associative conditioning to environmental cues (Stewart and Vezina, 1991; Stewart and Badiani, 1993; Mattingly et al., 1997; Heyne and Wolffgramm, 1998; Tirelli and Heidbreder, 1999; Zavala et al., 2000; Crombag et al., 2001; Anagnostaras et al., 2002; Tirelli et al., 2005; Uslaner et al., 2006). Given the critical importance of context specificity to sensitization effects, associative processes need to be incorporated into any account of sensitization. In that a typical sensitization protocol is essentially a Pavlovian drug conditioning protocol, it is not surprising that the contextual cues can acquire conditioned stimulus properties and in a non-drug test evoke a conditioned drug response. The occurrence of a conditioned drug response not only provides a positive link of the associative connection between the drug treatment and the environmental context in which the drug is experienced but it also provides an opportunity to experimentally interact with the associative bond in the absence of the psychostimulant drug treatment. Guided by this formulation, we suggest that the approach employed in the present study offers a new paradigm to modify sensitization effects for conditioned and sensitized drug response to a psychostimulant drug with well-defined neurotransmitter mechanisms that is, conducting a brief non-drug test to evoke the conditioned drug response and then immediately following this brief exposure to the contextual cues by administration of a post-trial drug treatment that is inhibitory in relation to the neurotransmitters activated by the psychostimulant drug. In the present study an autoreceptor preferring low dose of apomorphine was used to suppress dopaminergic activity in conjunction with the re-consolidation of the conditioned association between the high dose apomorphine treatment and the test environment. This needs to be differentiated from a counter-conditioning protocol wherein the drug treatment (e.g. a low dose of apomorphine) is given before placement in the test environment. In fact, a counter-conditioning procedure using a low dose of apomorphine does not attenuate a sensitized apomorphine locomotor stimulant response (Braga et al., 2009b).

The post-trial strategy could readily be applied to psychostimulant drugs with high addictive potential such as cocaine. In the case of cocaine, the post-trial drug treatments to be administered after a brief exposure to the conditioned cocaine cues would be the ones that activate dopamine and/or serotonin autoreceptors to suppress activity in these respective neuronal transmitter systems contemporaneously with memory trace activation by the contextual cues. In this way, the neurotransmitter systems that initiate and maintain the drug memory trace will be inactivated and consequently the linkage of these neurotransmitter systems to the contextual cues potentially can be reversed and eliminated. Application of this novel approach to the associational effects induced by addictive drugs offers the possibility of the development of behavioral and pharmaceutical tools to address critical treatment resistant issues such as craving and relapse. Clearly, drug development that fails to address the associational dimension can only be a behavioral masking pharmaceutical treatment requiring chronic dosing. On the other hand, interfering with the drug associational effects that are so critical to craving and relapse using well-established memory disruptive manipulations such as protein synthesis inhibitors does not have a clinical utility. In the present study a strategy was employed that was directed at the associational component of sensitization using a pharmaceutical with a well-delineated mechanism of action that does not alter memory processes generally but rather can be targeted to a specific association.

Clearly, this approach is only of relevance and applicable in drug sensitization circumstances wherein a drug environment association is manifested in a non-drug test in which the conditioned drug response is elicited by environmental cues. It is the evocation of the conditioned drug response that creates the crucial opportunity to apply post-trial treatments that can interact with and modify the drug/environment memory re-consolidation. This post-trial approach is not applicable when the sensitization drug is administered during sensitization induction since the association is not being re-consolidated or if the sensitization protocol does not generate a conditioned drug response to non-drug cues. In that major treatment issues in drug abuse involve activation of drug like reactions and craving by non-drug cues the post-trial strategy may have substantial clinical significance.

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