Published in final edited form as:
Psychopharmacology (Berl). 2012 April ; 220(4): 731–740. doi:10.1007/s00213-011-2524-9.

Individual differences in the improvement of cocaine-induced place preference response by the 5-HT$_{2C}$ receptor antagonist SB242084 in rats

Nancy Capriles[1], **Stanley Watson Jr.**[1], and **Huda Akil**[1]
[1]Molecular and Behavioral Neuroscience Institute, The University of Michigan School of Medicine, Ann Arbor, MI, USA

Abstract

Rationale and objectives—The 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors have been shown to be differentially involved in modulating cocaine-induced behaviors. In this study we investigated the effects of the 5-HT$_{2A}$ antagonist MDL100907 (0.3 mg/kg, i.p.) and the 5-HT$_{2C}$ antagonist SB242084 (0.5 mg/kg, i.p.) on *development*, *expression*, and *recall* of cocaine-induced conditioned place preference (CPP) in (HR) high- and (LR) low-responder rats to novelty.

Results—First, we examined the effects of MDL100907 and SB242084 on *development* of cocaine-induced CPP. Our results indicated that LR, but not HR, animals conditioned with SB242084+cocaine showed a significantly higher CPP response than controls. This effect was long-lasting, as it was still present 30 days after the last conditioning session. Second, we investigated the *acute* effects of MDL-100907 and SB242084 on CPP *expression* 24h after cocaine conditioning. Again, our data showed that SB242084 significantly enhanced the expression of cocaine CPP in LR, but not, HR animals. Finally, we studied the acute effects of MDL100907 and SB242084 on CPP *recall* 30 days after cocaine conditioning. Neither MDL100907 nor SB242084 significantly affected the CPP response regardless of the rats' behavioral phenotype.

Conclusions—This is the first study investigating the contribution of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors on *development*, *expression* and *recall* of cocaine-induced CPP in the HR-LR model of individual vulnerability to drug abuse. Our results show that SB242084 differentially modulates development and expression of CPP in HR vs. LR rats, and suggest that 5-HT$_{2C}$ receptors play a key role in individual differences on cocaine reward-related learning/memory processes.

1. Introduction

The serotonin receptor family is an important modulator of the behavioral responsiveness to cocaine. To date, several serotonin receptor subtypes have been described and two receptors of the 5-HT2 family, the 5-HT$_{2A}$ and the 5-HT$_{2C}$ receptors, seem to be particularly involved in regulating cocaine-associated behaviors (Burmeister et al. 2004; Filip et al. 2006; Fletcher et al. 2002; Grottick et al. 2000). These receptors are distributed throughout the brain, including nuclei of the mesolimbic DA circuits (Clemett et al. 2000; Cornea-Hebert et al. 1999; Eberle-Wang et al. 1997) and their functions in the CNS and addiction-related behaviors have been the subject of several studies in recent years. By contrast, the third receptor of the 5HT2 family, the 5-HT$_{2B}$ receptor, is not highly expressed in the CNS and typically has minimal effects on cocaine-evoked behaviors (Filip et al. 2004, 2006; Fletcher

Correspondence: Dr N. Capriles, Molecular and Behavioral Neuroscience Institute, The University of Michigan School of Medicine, 205 Zina Pitcher Place, Ann Arbor, MI 48109-0720, USA. Tel: +1 734 936 1947; Fax: +1 734 647 4130, capriles@umich.edu.

et al. 2002). Preclinical studies have demonstrated that 5-HT_{2A} and 5-HT_{2C} receptors have different, and often opposite effects on various cocaine-induced behaviors (Bubar and Cunningham 2006, 2008; Filip et al. 2006; Fletcher et al. 2002). For example, the selective 5-HT_{2A} antagonist MDL100907 attenuates locomotor activity elicited by cocaine, whereas the selective 5-HT_{2C} antagonist SB242084 potentiates this cocaine effect (Fletcher et al. 2002). Moreover, blockade of the 5-HT_{2A} receptors by ketanserin or MDL100907 attenuates cue-induced reinstatement of cocaine-seeking behavior (Burmeister et al. 2004; Nic Dhonnchadha et al. 2009; Pockros et al. 2011) in the absence of any comparable effect induced by SB242084 (Burmeister et al. 2004). On the other hand, agonist stimulation of the 5-HT_{2C} receptors decreases both cue- and cocaine-primed reinstatement (Burbassi and Cervo 2008; Neisewander and Acosta 2007).

Addictive drugs can be both ‘rewarding’ (interpreted by the brain as intrinsically positive) and ‘reinforcing’ (behaviors associated with such drugs tend to be repeated) (Hyman and Malenka 2001; White 1989). Cocaine-induced reward can be assessed by conditioned place preference (CPP), a behavioral paradigm that is thought to model the critical role that environmental cues associated to the drug administration exert over the behavior in cocaine users (Bardo et al. 1995; Tzschentke and Schmidt 1998). The process of association between the drug and experiential aspects of drug use is driven by Pavlovian (classical) learning and nowadays there is a growing recognition and interest in understanding the biological and behavioral mechanisms underlying the encoding of drug-related memories (Nic Dhonnchadha and Cunningham 2008). A large body of evidence is also suggesting that individual differences play an important role in determining the risks to drug abuse. For example, differences in reactivity to a novel environment have been reported to predict cocaine self-administration (Piazza et al. 2000). These models have also proved to be useful in studying differential responses to pharmacological agents in relation to the treatment of drug addiction. However, whether or not 5-HT_{2A} and 5-HT_{2C} receptors contribute to individual differences in cocaine-induced CPP in rats has not been addressed.

In the present study, we classified outbred Sprague-Dawley male rats as (HR) high and (LR) low-responders based on the median split of their locomotor activity in a novel environment. The set of experiments was performed to investigate possible HR-LR differences in: 1) the effects of repeated administration of the 5-HT_{2A} antagonist MDL100907 or the 5-HT_{2C} receptor antagonist SB242084 on *development* of cocaine-induced CPP, 2) the acute effects of these antagonists on *expression* of the CPP 24h after the last conditioning trial, in the absence of cocaine, and 3) the effects of the these antagonists on *retention* of the cocaine-induced CPP 30 days after the conditioning phase.

2. Materials and methods

2.1. Animals

A total of 203 adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA), weighing approximately 225–250 g upon arrival, were used. Animals were housed two per cage in a room adjacent to the testing room, and maintained on a 12/12 h light/dark cycle (lights on at 16:00 hours). Housing was located in a temperature- and humidity-controlled environment. Rats were acclimated to the animal quarters for 1 week before any experimental procedure, and were then conditioned and tested during the dark phase of the cycle. Food and water were available *ad libitum*. Animals were treated in accordance with National Institutes of Health guidelines on laboratory animal use and care.

2.2. Drugs

Cocaine hydrochloride was obtained from Mallinckrodt Inc. (St Louis, MO). SB242084 (6-Chloro-2,3-dihydro-5-methyl-N-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-carboxyamide dihydrochloride) was purchased from Tocris (Ellisville, MO). MDL100907 (R-(+)-α-(2, 3-Dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol) was purchased from Axon Medchem (Groningen, Netherlands). Cocaine HCL was dissolved in 0.9% saline, while MDL100907 and SB242084 were daily dissolved in saline containing 8.0% hydroxypropyl-β-cyclodextrin. All drugs were injected i.p. at a volume of 1ml/kg.

2.3. Apparatus

Locomotor activity test—After 7 days of habituation to the housing conditions, animals were tested for locomotor activity during a 60 minutes exposure to the mild stress of a novel environment. Each rat was placed in a 43L ×21.5 W ×24.5 H (in cm) clear acrylic activity monitor and locomotor activity was monitored by means of two banks of photocells connected to a microprocessor. Rats that exhibited locomotor counts in the higher half of the sample were classified as HR, whereas rats that exhibited locomotor counts in the lower half of the sample were classified as LR. After animals were defined as HR and LR, assignment to individual treatments for each experiment was counterbalanced based on activity levels. This design ensured that each treatment group had animals representative of the range of locomotor activity in the HR and LR groups. One week after the locomotor activity test, HR and LR animals were trained in the place preference paradigm.

Place Preference Test—The place preference conditioning apparatus consisted of an 80L ×40W ×45H (in cm) box equally divided into two compartments by a removable door in the middle. The first compartment had a smooth clear Plexiglas floor and spots on the walls. The second compartment had a rough metallic floor and black stripes on the walls. During conditioning and testing, the entire room was illuminated by a red light from the ceiling, and a single lamp reflecting white light off one wall of the room.

The place preference conditioning consisted of three phases: pre-conditioning, conditioning and test (Mueller and Stewart 2000). During the pre-conditioning phase, all animals received a single pre-exposure test in which they were placed in the place preference apparatus and allowed to explore both compartments of the box for 15 minutes. The placement of the animals in the apparatus was counterbalanced, that is, half of the animals in each group (HR and LR) were initially placed in one side of the apparatus, while the remaining half was placed in the other side. The amount of time spent on each side was monitored and used to assess unconditioned preferences for each individual rat. The unconditioned preference for one or other compartement of the apparatus was evenly distributed across the rat population. One animal showing a strong preference (more than 85% of the session time) for a particular side was eliminated from the study. The least preferred compartment for each rat was then assigned to be the cocaine-paired environment during conditioning (biased design) (Calcagnetti and Schechter 1993; Prus et al. 2009). In addition, the number of complete transitions from one chamber to the other was recorded.

During the conditioning phase (8 days), cocaine (COC) was administered in a dose of 20 mg/kg, i.p. once every other day, immediately before the rats were placed into the assigned chamber for 30 minutes. On alternate days, rats received saline injections (SAL, 1 ml/kg) before being placed in the other chamber. The order of COC- and SAL-pairings was counterbalanced so that half of the animals in each group started the conditioning phase with cocaine while the remaining half of the group started with saline.

Twenty-four hours after the last conditioning trial, a test for CPP was given. HR and LR animals were given free access to both compartments and the amount of time spent on each side of the apparatus was monitored for 15 minutes. Each animal was initially placed in the same side of the apparatus as during the pre-conditioning phase. The difference in the time spent in the COC-paired compartment during the testing phase *versus* the pre-conditioning phase served as an index of place preference. To assess individual differences in maintenance of the CPP, two additional tests were given 15 and 30 days after the last conditioning trial. The number of complete transitions from one chamber to the other was recorded during tests as an indirect index of locomotion. No COC injections were given on the days of testing, maintaining the same procedure that was used during the Pre-conditioning phase. During the interim period between tests, animals were left undisturbed in their home-cages.

2.4. Experimental Procedures

2.4.1. Effects of repeated administration of MDL100907 or SB242084 on *development* of COC-induced CPP—

Animals were tested for CPP 1, 15 and 30 days following the last conditioning trial. Moreover, since other studies have shown that pharmacological manipulation of the 5-HT_{2A} and 5-HT_{2C} receptors results in alteration of food intake in rodents (Hayashi et al. 2005; Kennett et al. 1997; Maurel et al. 1999; Thomsen et al. 2008; Vickers et al. 2001), the potential effect of MDL100907 or SB242084, alone or followed by cocaine, on body weight was monitored through the conditioning phase (1 to 8 days).

MDL100907 treatment: Twenty-seven rats were separated according to their locomotor activity and used for this experiment. After pre-conditioning, animals in both HR and LR groups received the following treatment during the conditioning phase: MDL100907 (0.3 mg/kg, i.p.)-cocaine or vehicle (saline containing 8.0% hydroxypropyl-β-cyclodextrin, 1ml/kg)-cocaine (n=6–7 per group). Pretreatments were administered 30 minutes before cocaine (20 mg/kg, i.p.) (Fletcher et al. 2002; Nic Dhonnchadha et al. 2009).

SB242084 treatment: Twenty-nine animals were separated in HR and LR and used for this experiment. After pre-conditioning, animals in both HR and LR groups received the following treatment during the conditioning phase: SB242084 (0.5 mg/kg, i.p.)-cocaine or vehicle (saline containing 8.0% hydroxypropyl-β-cyclodextrin, 1ml/kg, i.p.)-cocaine (n=6–9 per group). Pretreatments were administered 30 minutes before cocaine (20 mg/kg, i.p.) (Burmeister et al. 2004; Fletcher et al. 2002, 2006, 2008; Nic Dhonnchadha et al. 2009).

Effects of MDL100907 or SB242084 on CPP acquisition in absence of cocaine: Forty animals were separated according to their locomotor activity and used to test the potential conditioning effects induced by MDL100907 and SB242084 when administered alone, and to control for changes in place preference that might spontaneously occur over time. After pre-conditioning, HR and LR animals were divided into three treatment groups: MDL100907 (0.3 mg/kg, i.p.)-Saline, SB242084 (0.5 mg/kg, i.p.)-Saline and Vehicle (saline containing 8.0% hydroxypropyl-β-cyclodextrin, 1ml/kg, i.p.)-Saline (n=5 per group). A fourth treatment group, vehicle (saline containing 8.0% hydroxypropyl-β-cyclodextrin, 1ml/kg, i.p.) followed by cocaine (20 mg/kg, i.p.) was also run in parallel as an internal control for the experiment (n=5 per group). In all cases, pretreatment was followed by treatment 30 minutes later.

2.4.2. Influence of MDL100907 or SB242084 on the *expression* of COC-induced CPP

Twenty-seven and thirty-five animals were separated in HR and LR according to their locomotor activity, and used to test the potential effects of MDL100907 and SB242084

respectively on expression of cocaine-induced CPP. Following pre-conditioning phase, all animals were trained in the conditioned place preference paradigm as described above.

MDL100907 treatment—Twenty-four hours after the last conditioning trial, animals in both HR and LR groups received the following drug treatment: MDL100907 (0.3 mg/kg, i.p.) or vehicle (saline containing 8.0% hydroxypropyl-β-cyclodextrin, 1ml/kg, i.p.) (n=6–8 per group) 30 minutes before the CPP Test at T1. As an indirect measure of locomotion, the number of transitions between the two chambers during the test was also monitored.

SB242084 treatment—HR and LR groups received the following drug treatment 24 h after the last conditioning trial: SB242084 (0.5 mg/kg, i.p.) or vehicle (saline containing 8.0% hydroxypropyl-β-cyclodextrin, 1ml/kg, i.p.) (n=8–11) 30 minutes before the CPP Test at T1. Locomotion activity was also monitored as detailed above.

2.4.3. Effects of MDL100907 or SB242084 on *recall* of COC-induced CPP

Nineteen and twenty-one animals were separated in HR and LR according to their locomotor activity, and used to test the potential effects of MDL100907 and SB242084 respectively on recall of cocaine-induced CPP. Following pre-conditioning phase, all animals were trained in the conditioned place preference paradigm and tested for CPP 1 and 15 days after conditioning, as described above.

MDL100907 treatment—HR and LR animals were submitted to one of the following treatment conditions 30 days after the last conditioning trial: MDL100907 (0.3 mg/kg, i.p.) or vehicle (saline containing 8.0% hydroxypropyl-β-cyclodextrin, 1 ml/kg, i.p.) (n=4–5 per group). Treatment was administered 30 minutes before the Test for CPP at day 30. A total of 3 animals, 2 HR and 1 LR, showing no COC-induced CPP on the second test (Day 15) were excluded from the experiment. Number of transitions between chambers on the day of the test was also monitored.

SB242084 treatment—Animals in the HR and LR groups received one of the following treatments 30 days after last conditioning trial: SB242084 (0.5 mg/kg, i.p.) or vehicle (saline containing 8.0% hydroxypropyl-β-cyclodextrin, 1 ml/kg, i.p.) (n=4–6 per group).

Treatment was administered 30 minutes before the Test for CPP at day 30. A total of 2 animals, 1 HR and 1 LR, showing no COC-induced CPP on the second test (Day 15) were excluded from the experiment. Locomotion activity was monitored as detailed above.

2.5. Statistical analysis

Cocaine-induced CPP was measured as the amount of time spent in the drug-paired chamber on the Test day relative to the amount of time spent in the drug-paired chamber during the Pre-conditioning phase. Means are represented as percent change in time during Test relative to Pre-conditioning.

Three-way ANOVAs with repeated measures with Phenotype, Pretreatment drug, and Test or Conditioning day as factors were used: to test the effects of MDL100907 and SB242084, alone or with cocaine, on development, expression, and recall of conditioned place preference; to test the effects of the same drugs on changes in body weight; to control for changes in place preference that might spontaneously occur over time. Two-way ANOVAs with Phenotype and Pretreatment drug as factors were performed to analyze the effects of the 5HT2 antagonists on locomotion as measured by crossings across CPP chambers during testing.

A statistically significant effect was followed by Bonferroni test for post-hoc comparisons (acceptable significance level, $p<0.05$). In addition, independent simple regressions between Locomotion score and CPP for each antagonist were also determined.

3. Results

Locomotor Activity

Locomotor activity for HR and LR animals used to investigate the effects of MDL100907 and SB242084 on development, expression and recall of cocaine-induced CPP was 796.5 ± 24.7 and 528.5 ± 27.7 counts, 744.5 ± 25.7 and 427.9 ± 27.6 counts, and 744.9 ± 28.1 and 420.1 ± 23.2 counts respectively. Locomotor activity for HR and LR animals used to investigate place preference conditioning in absence of cocaine was 692.3 ± 31.5 and 431.8 ± 18.3 counts. There were no statistically significant differences between groups of animals of the same phenotype.

Cocaine-induced CPP

Overall, the CPP protocol used in our study produced a significant conditioned place preference in both HR and LR animals. In fact, when we combined vehicle+cocaine treated animals from the experiments investigating the development of cocaine-induced CPP, and vehicle-treated animals from the experiments investigating the expression and recall of cocaine-induced CPP, statistical analysis revealed a significant Conditioning overall, $F(3, 207)=26.83$, $p<0.0001$, as well as a significant interaction between Phenotype (HR vs. LR) and Test day (Pre-conditioning, T1, T15 and T30) $F(3, 207)=2.75$, $p<0.05$. Post-hoc comparisons showed that LR animals had significantly lower CPP than individuals in the HR group on test T30 (30 days after the last conditioning trial) (Figure 1).

3.1. Influence of repeated administration of MDL100907 or SB242084 on *development* of COC-induced CPP

Figures 2A and 2B show the effects of MDL100907 or SB242084 on *development* of COC-induced CPP in HR vs. LR animals. A three-way ANOVA with repeated measures for the effects of MDL100907, where the factors were Phenotype (HR vs. LR), Pretreatment drug (MDL100907 or vehicle) and Test day (Pre-conditioning, T1, T15 and T30), revealed a significant main effect of Test day $F(3, 69)=8.06$, $p<0.001$ with no other significant main effects and no significant interactions. Post-hoc comparisons revealed a significant increase in the preference response relative to Pre-conditioning during all Test days (Figure 2A).

A three-way ANOVA with repeated measures for the effects of SB242084 on COC-induced CPP revealed a significant effect of Test day $F(3, 75)=17.37$, $p<0.001$, as well as a significant interaction of the three factors (Phenotype × Pretreatment drug × Test day) $F(3, 75)=3.36$, $p<0.05$. Post-hoc comparisons indicated that LR animals pretreated with SB242084 showed a significant increase in COC-induced CPP on Test T1 compared to animals in the same phenotype group pretreated with vehicle. This effect was still detected 30 days after the last conditioning (Figure 2B). Moreover, a regression analysis in all animals pre-treated with the 5-HT_{2C} antagonist SB242084 revealed a significant negative correlation between Locomotion score and CPP during Test T1 ($r=0.566$, $p<0.05$) and Test T30 ($r=0.656$, $p<0.01$).

The effect of SB242084 in LR animals was specific to cocaine-induced CPP. In fact, neither SB242084 nor MDL100907 evoked conditioned place preference when administered alone. Similarly, the vehicle-saline treated group in the same experiment failed to show any significant changes in place preference over time (Figure 3). By contrast, the internal control group for the experiment that was treated with vehicle followed by cocaine (20 mg/kg, i.p.)

showed a statistically significant effect $F(1, 8)=16.71$, $p<0.01$, and confirmed that animals could be conditioned under the experimental conditions used.

Finally, a three-way ANOVA for the effects on body weight induced by repeated administration of MDL100907 or SB242084 alone, showed a significant effect of Conditioning day $F(6, 198)= 7.69$, $p<0.001$, indicating that all animals showed a progressive increase in body weight over time regardless of Pretreatment drug (data not shown).

3.2. Influence of MDL100907 or SB242084 on the *expression* of COC-induced CPP

Figure 4A and Figure 4B show the effects induced by MDL100907 or SB242084 on the *expression* of the CPP associated to cocaine in HR vs. LR animals. A three-way ANOVA with repeated measures for the influence of MDL100907 on CPP expression revealed a significant effect of Test day (Pre-conditioning vs. T1) $F(1, 23)=28.98$, $p<0.001$, but not significant effects of Phenotype or Pretreatment drug, nor significant interactions (Figure 4A).

By contrast, a three-way ANOVA with repeated measures for the acute effects of SB242084 on CPP expression revealed a significant interaction of all main factors in the analysis (Phenotype × Pretreatment drug × Test day) $F(1, 31)=7.29$, $p<0.05$ (Figure 4B). Following post-hoc comparisons indicated that acute administration of SB242084 significantly enhanced the expression of the CPP in LR, but not HR, animals (Figure 4B).

The effects of the 5-HT_{2A} and 5-HT_{2C} antagonists on cocaine-induced locomotion were also indirectly determined by analysis of the number of transitions during Test T1. While MDL100907 did not have any effect on locomotion, a two-way ANOVA for the effects of SB242084 on conditioned HR vs. LR animals revealed a significant main effect of the Pretreatment drug $F(1, 31)=7.93$, $p<0.01$, indicating that overall animals injected with SB242084 had higher number of crossings between chambers than animals pretreated with vehicle.

3.3. Influence of MDL100907or SB242084 on *recall* of COC-induced CPP

Figures 5A and 5B show the effects of MDL100907 or SB242084 on *recall* of the CPP 30 days after the last conditioning trial (Test T30) in HR and LR groups. Three-way ANOVAs for MDL100907 or SB242084 revealed no significant main effects of these drugs on CPP recall. Moreover, statistical analysis of number of crossings in these animals during Test T30 indicated that locomotion was not affected by acute administration of these antagonists.

4. Discussion

Both HR and LR animals revealed a significant CPP for the place associated with cocaine administrations, and this effect was long-lasting. Our data agrees with earlier studies showing that HR and LR rats do not differ in the cocaine-conditioned response as measured by CPP (Erb and Parker 1994; Gong et al. 1996). However, the present study extends these findings by providing evidence of individual differences in the maintenance of the CPP following a long period of time after the last conditioning trial, as demonstrated by a significantly higher place preference in HR than in LR rats on Post-conditioning day 30. Our data seem to suggest that long after the drug is withdrawn, individuals that show higher preference for the drug initially are also at risk to relapse in cocaine-seeking behavior when re-exposed to the environmental cues associated with the drug experience.

One of the more remarkable findings from this study is that LR animals were overall more susceptible to the effects induced by the selective 5-HT_{2C} receptor antagonist SB242084 than HR rats. Indeed, repeated co-administration of SB242084+cocaine, during the

conditioning phase produced a significant increase in the CPP response in LR rats only. Moreover, a negative correlation between locomotion and CPP in animals pretreated with SB242084+cocaine was observed. These effects were present only when SB242084 was co-administered with cocaine. In fact, injection of SB242084 followed by saline did not to induce CPP in LR animals. These finding suggests that in our study the 5-HT_{2C} receptor blockade facilitated the acquisition of cocaine-induced place preference in LR animals by either increasing the efficacy of the cocaine rewarding actions or improving the learning conditions. It is believed that DA neuronal cell firing may encode the incentive value or the prediction of reward (Schultz 1998). Over recent years, evidence has emerged that suggests that 5-HT_{2C} receptors may modulate mesocorticolimbic DA release and thus, affect motivation and behavior (Di Matteo et al. 1999; Navailles et al. 2008). Moreover, using the HR-LR model of individual vulnerability to drugs, different laboratories have shown that increased vulnerability is associated with increases in basal and stimulated DA levels in the NAcc and striatum (Bradberry et al. 1991; Hooks et al. 1991, 1992; Piazza et al. 1991; Rouge-Pont et al. 1993, 1998). Our data seem to suggest that 5-HT_{2C} receptors may differentially modulate accumbal DA release in HR vs. LR animals, leading to an enhanced acquisition of the cocaine-conditioned response particularly in LR rats.

In addition to enhancing the hedonic value of cocaine, the inhibition of the 5-HT_{2C} receptors may have modulated the development of CPP to cocaine by altering the associative learning during drug conditioning. In support of this idea, the 5-HT_{2C} receptors seem to play a role in other forms of associative learning, such as passive avoidance and autoshaping (Meneses and Hong 1997; Misane and Ogren 2000).

Acute inhibition of 5-HT_{2C} receptors affected the expression of cocaine-induced CPP in LR animals. Recent studies have provided evidence that 5-HT_{2C} receptors control the expression of cocaine-conditioned behaviors (dela Cruz et al. 2009; Liu and Cunningham 2006) Our results, together with previously published studies, suggest a critical role played by 5-HT_{2C} receptors in improving the expression of cocaine-associated behaviors, perhaps by decreasing thresholds to motivational effects elicited by the environmental cues that were present at the time of cocaine administrations. Of importance, our study provides evidence of a differential role for the 5-HT_{2C} receptors in the brain of HR and LR rats in the motivational aspects that initiate and maintain cocaine abuse in these animals.

Acute administration of the 5-HT_{2A} antagonist MDL100907 did not produce significant main effects on expression of CPP in HR or LR groups. However, LR animals seemed to have a lower CPP response as compared to their counterpart vehicle-treated animals. It is possible that the use of different doses of cocaine and/or MDL100907 would have resulted in a decrease of cocaine-induced CPP expression in LR animals that reached significance. In the present study, we also investigated whether or not 5-HT_{2A} and 5-HT_{2C} receptors play a role in the expression of cocaine CPP during a retention test 30 days after the last conditioning trial. Our data showed that *recall* of cocaine-induced CPP was not affected by inhibition of 5-HT_{2A} or 5-HT_{2C} receptors in either HR or LR animals. Why did blockade of the 5-HT_{2C} receptors affect *recall* of the cocaine-associated memory at 1, but not 30, days of post-conditioning in the LR phenotype? A growing body of evidence shows that cocaine exposure produces adaptive changes in brain that occur after the drug is discontinued. Some of these brain alterations return to basal levels shortly after the drug is discontinued, while others seem to still be present or start taking place after a long period of forced abstinence (Freeman et al. 2008, 2010). Perhaps, the molecular changes that are responsible for the memory of the association of cocaine-environment and/or the cocaine-induced motivational effects during post-abstinence are different at T1 and T30, and 5-HT_{2C} receptors are critical during early abstinence, but not later. Consistently with this hypothesis, data from our lab

have shown different profiles of gene expression in the nucleus accumbens at different durations of abstinence from cocaine self-administration (Capriles et. al. unpublished data).

The higher susceptibility to the pharmacological manipulations of the 5-HT_{2C} receptor in LR animals that result in an enhanced conditioned response to cocaine could also be secondary to different pharmacokinetic and pharmacodynamic profiles in the HR and LR phenotypes. In the present study, we have used a single dose of the 5-HT_{2A} and 5-HT_{2C} receptor antagonists. The doses were chosen from the literature and have been proven to be effective in affecting various cocaine-associated behaviors (Burmeister et al. 2004; Fletcher et al. 2002, 2006, 2008; Nic Dhonnchadha et al. 2009). However, we cannot dismiss shifts in dose-effects curves, and it is possible that administration of cocaine and/or serotonergic antagonists at different doses than the ones used in the study could have uncovered effects on the HR phenotype, or shown significant effects after administration of the 5-HT_{2A} antagonist. It is important to point out though, that even if this were the case, our results would still support the finding that LR animals are more vulnerable to pharmacological manipulations of the 5-HT_{2C} receptors.

To summarize, we have investigated the contribution of 5-HT_{2A} and 5-HT_{2C} receptors on *development*, *expression* and *recall* of cocaine-induced CPP in the HR-LR model of individual vulnerability to drug abuse. The main finding from these set of experiments is that SB242084 differentially modulates development and expression of conditioned place preference in HR vs. LR animals, as demonstrated by the increased vulnerability to 5-HT_{2C} receptor antagonist properties in the LR phenotype. Therefore, our results suggest that the 5-HT_{2C} receptors play a key role in individual differences on cocaine reward-related learning/memory processes.

Despite our growing knowledge of the cocaine addiction, a high rate of relapse to drug seeking behavior is often reported as a consequence of unsuccessful pharmacotherapies. The present data emphasize that a model of individual differences is indeed necessary to characterize the response to potentially useful compounds, such as those affecting the 5-HT_{2C} receptors, in the treatment of cocaine addicts.

Acknowledgments

This work was supported by NIDA PPG: 5P01DA021633-02; Office Naval Research (ONR) N00014-02-1-0879 to Dr. Huda Akil. Authors would like to thank to Dr. Marco Cecchi for his valuable comments on the manuscript.

References

Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev. 1995; 19:39–51. [PubMed: 7770196]

Bradberry CW, Gruen RJ, Berridge CW, Roth RH. Individual differences in behavioral measures: correlations with nucleus accumbens dopamine measured by microdialysis. Pharmacol Biochem Behav. 1991; 39:877–882. [PubMed: 1763106]

Bubar MJ, Cunningham KA. Serotonin 5-HT2A and 5-HT2C receptors as potential targets for modulation of psychostimulant use and dependence. Curr Top Med Chem. 2006; 6:1971–1985. [PubMed: 17017968]

Bubar MJ, Cunningham KA. Prospects for serotonin 5-HT2R pharmacotherapy in psychostimulant abuse. Prog Brain Res. 2008; 172:319–346. [PubMed: 18772040]

Burbassi S, Cervo L. Stimulation of serotonin2C receptors influences cocaine-seeking behavior in response to drug-associated stimuli in rats. Psychopharmacology (Berl). 2008; 196:15–27. [PubMed: 17899022]

Burmeister JJ, Lungren EM, Kirschner KF, Neisewander JL. Differential roles of 5-HT receptor subtypes in cue and cocaine reinstatement of cocaine-seeking behavior in rats. Neuropsychopharmacology. 2004; 29:660–668. [PubMed: 14627998]

Calcagnetti DJ, Schechter MD. Extinction of cocaine-induced place approach in rats: a validation of the "biased" conditioning procedure. Brain Res Bull. 1993; 30:695–700. [PubMed: 8457916]

Clemett DA, Punhani T, Duxon MS, Blackburn TP, Fone KC. Immunohistochemical localisation of the 5-HT2C receptor protein in the rat CNS. Neuropharmacology. 2000; 39:123–132. [PubMed: 10665825]

Cornea-Hebert V, Riad M, Wu C, Singh SK, Descarries L. Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. J Comp Neurol. 1999; 409:187–209. [PubMed: 10379914]

dela Cruz AM, Herin DV, Grady JJ, Cunningham KA. Novel approach to data analysis in cocaine-conditioned place preference. Behav Pharmacol. 2009; 20:720–730. [PubMed: 19901823]

Di Matteo V, Di Giovanni G, Di Mascio M, Esposito E. SB 242[punctuation space]084, a selective serotonin2C receptor antagonist, increases dopaminergic transmission in the mesolimbic system. Neuropharmacology. 1999; 38:1195–1205. [PubMed: 10462132]

Eberle-Wang K, Mikeladze Z, Uryu K, Chesselet MF. Pattern of expression of the serotonin2C receptor messenger RNA in the basal ganglia of adult rats. J Comp Neurol. 1997; 384:233–247. [PubMed: 9215720]

Erb SM, Parker LA. Individual differences in novelty-induced activity do not predict strength of amphetamine-induced place conditioning. Pharmacol Biochem Behav. 1994; 48:581–586. [PubMed: 7938109]

Filip M, Bubar MJ, Cunningham KA. Contribution of serotonin (5-hydroxytryptamine; 5-HT) 5-HT2 receptor subtypes to the hyperlocomotor effects of cocaine: acute and chronic pharmacological analyses. J Pharmacol Exp Ther. 2004; 310:1246–1254. [PubMed: 15131246]

Filip M, Bubar MJ, Cunningham KA. Contribution of serotonin (5-HT) 5-HT2 receptor subtypes to the discriminative stimulus effects of cocaine in rats. Psychopharmacology (Berl). 2006; 183:482–489. [PubMed: 16261316]

Fletcher PJ, Grottick AJ, Higgins GA. Differential effects of the 5-HT(2A) receptor antagonist M100907 and the 5-HT(2C) receptor antagonist SB242084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. Neuropsychopharmacology. 2002; 27:576–586. [PubMed: 12377394]

Fletcher PJ, Rizos Z, Sinyard J, Tampakeras M, Higgins GA. The 5-HT2C receptor agonist Ro60-0175 reduces cocaine self-administration and reinstatement induced by the stressor yohimbine, and contextual cues. Neuropsychopharmacology. 2008; 33:1402–1412. [PubMed: 17653111]

Fletcher PJ, Sinyard J, Higgins GA. The effects of the 5-HT(2C) receptor antagonist SB242084 on locomotor activity induced by selective, or mixed, indirect serotonergic and dopaminergic agonists. Psychopharmacology (Berl). 2006; 187:515–525. [PubMed: 16832658]

Fletcher PJ, Tampakeras M, Sinyard J, Slassi A, Isaac M, Higgins GA. Characterizing the effects of 5-HT(2C) receptor ligands on motor activity and feeding behaviour in 5-HT(2C) receptor knockout mice. Neuropharmacology. 2009; 57:259–267. [PubMed: 19501602]

Freeman WM, Lull ME, Patel KM, Brucklacher RM, Morgan D, Roberts DC, Vrana KE. Gene expression changes in the medial prefrontal cortex and nucleus accumbens following abstinence from cocaine self-administration. BMC Neurosci. 2010; 11:29. [PubMed: 20187946]

Freeman WM, Patel KM, Brucklacher RM, Lull ME, Erwin M, Morgan D, Roberts DC, Vrana KE. Persistent alterations in mesolimbic gene expression with abstinence from cocaine self-administration. Neuropsychopharmacology. 2008; 33:1807–1817. [PubMed: 17851536]

Gong W, Neill DB, Justice JB Jr. Locomotor response to novelty does not predict cocaine place preference conditioning in rats. Pharmacol Biochem Behav. 1996; 53:191–196. [PubMed: 8848450]

Grottick AJ, Fletcher PJ, Higgins GA. Studies to investigate the role of 5-HT(2C) receptors on cocaine- and food-maintained behavior. J Pharmacol Exp Ther. 2000; 295:1183–1191. [PubMed: 11082456]

Hayashi A, Suzuki M, Sasamata M, Miyata K. Agonist diversity in 5-HT(2C) receptor-mediated weight control in rats. Psychopharmacology (Berl). 2005; 178:241–249. [PubMed: 15719229]

Hooks MS, Colvin AC, Juncos JL, Justice JB Jr. Individual differences in basal and cocaine-stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. Brain Res. 1992; 587:306–312. [PubMed: 1525663]

Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB Jr. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse. 1991; 9:121–128. [PubMed: 1821483]

Hyman SE, Malenka RC. Addiction and the brain: the neurobiology of compulsion and its persistence. Nat Rev Neurosci. 2001; 2:695–703. [PubMed: 11584307]

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, Avenell KY, Stean T, Upton N, Bromidge S, Forbes IT, Brown AM, Middlemiss DN, Blackburn TP. SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. Neuropharmacology. 1997; 36:609–620. [PubMed: 9225286]

Liu S, Cunningham KA. Serotonin2C receptors (5-HT2C R) control expression of cocaine-induced conditioned hyperactivity. Drug Alcohol Depend. 2006; 81:275–282. [PubMed: 16146672]

Maurel S, De Vry J, De Beun R, Schreiber R. 5-HT2A and 5-HT2C/5-HT1B receptors are differentially involved in alcohol preference and consummatory behavior in cAA rats. Pharmacol Biochem Behav. 1999; 62:89–96. [PubMed: 9972850]

Meneses A, Hong E. Role of 5-HT1B-5-HT2A and 5-HT2C receptors in learning. Behav Brain Res. 1997; 87:105–110. [PubMed: 9331478]

Misane I, Ogren SO. Multiple 5-HT receptors in passive avoidance: comparative studies of p-chloroamphetamine and 8-OH-DPAT. Neuropsychopharmacology. 2000; 22:168–190. [PubMed: 10649830]

Mueller D, Stewart J. Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. Behav Brain Res. 2000; 115:39–47. [PubMed: 10996406]

Navailles S, Moison D, Cunningham KA, Spampinato U. Differential regulation of the mesoaccumbens dopamine circuit by serotonin2C receptors in the ventral tegmental area and the nucleus accumbens: an in vivo microdialysis study with cocaine. Neuropsychopharmacology. 2008; 33:237–246. [PubMed: 17429406]

Neisewander JL, Acosta JI. Stimulation of 5-HT2C receptors attenuates cue and cocaine-primed reinstatement of cocaine-seeking behavior in rats. Behav Pharmacol. 2007; 18:791–800. [PubMed: 17989517]

Nic Dhonnchadha BÁ, Cunningham KA. Serotonergic mechanisms in addiction-related memories. Behavioural Brain Research. 2008; 195:39–53. [PubMed: 18639587]

Nic Dhonnchadha BA, Fox RG, Stutz SJ, Rice KC, Cunningham KA. Blockade of the serotonin 5-HT2A receptor suppresses cue-evoked reinstatement of cocaine-seeking behavior in a rat self-administration model. Behav Neurosci. 2009; 123:382–396. [PubMed: 19331461]

Piazza PV, Deroche-Gamonent V, Rouge-Pont F, Le Moal M. Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. J Neurosci. 2000; 20:4226–4232. [PubMed: 10818158]

Piazza PV, Rouge-Pont F, Deminiere JM, Kharoubi M, Le Moal M, Simon H. Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. Brain Res. 1991; 567:169–174. [PubMed: 1726140]

Pockros LA, Pentkowski NS, Swinford SE, Neisewander JL. Blockade of 5-HT2A receptors in the medial prefrontal cortex attenuates reinstatement of cue-elicited cocaine-seeking behavior in rats. Psychopharmacology (Berl). 2011; 213:307–320. [PubMed: 21079923]

Prus AJ, James JR, Rosecrans JA. Conditioned Place Preference. 2009

Rouge-Pont F, Deroche V, Le Moal M, Piazza PV. Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. Eur J Neurosci. 1998; 10:3903–3907. [PubMed: 9875367]

Rouge-Pont F, Piazza PV, Kharouby M, Le Moal M, Simon H. Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals predisposed to

amphetamine self-administration. A microdialysis study. Brain Res. 1993; 602:169–174. [PubMed: 8448654]

Schultz W. Predictive reward signal of dopamine neurons. J Neurophysiol. 1998; 80:1–27. [PubMed: 9658025]

Thomsen WJ, Grottick AJ, Menzaghi F, Reyes-Saldana H, Espitia S, Yuskin D, Whelan K, Martin M, Morgan M, Chen W, Al-Shamma H, Smith B, Chalmers D, Behan D. Lorcaserin, a novel selective human 5-hydroxytryptamine2C agonist: in vitro and in vivo pharmacological characterization. J Pharmacol Exp Ther. 2008; 325:577–587. [PubMed: 18252809]

Tzschentke TM, Schmidt WJ. Blockade of morphine- and amphetamine-induced conditioned place preference in the rat by riluzole. Neurosci Lett. 1998; 242:114–116. [PubMed: 9533407]

Vickers SP, Dourish CT, Kennett GA. Evidence that hypophagia induced by d-fenfluramine and d-norfenfluramine in the rat is mediated by 5-HT2C receptors. Neuropharmacology. 2001; 41:200–209. [PubMed: 11489456]

White NM. Reward or reinforcement: what's the difference? Neurosci Biobehav Rev. 1989; 13:181–186. [PubMed: 2682404]

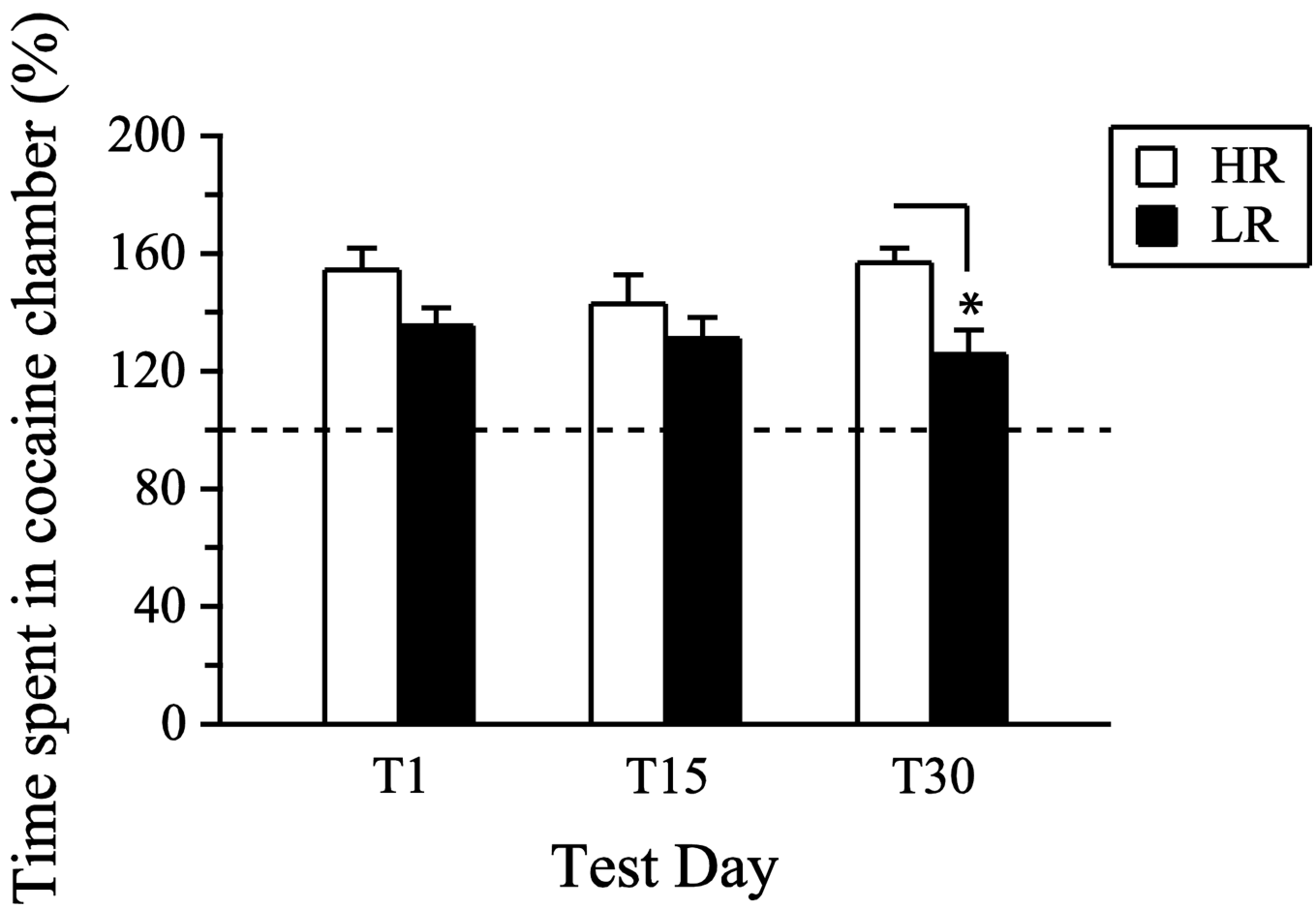

Fig. 1.

Cocaine-induced CPP in vehicle-treated HR and LR rats. The bars represent the percent change in the time spent in the cocaine-paired side on day 1, 15 and 30 relative to the Pre-conditioning scores (Mean ± SEM) in HR and LR rats (n=31–34 per group). *significantly different from HR group, $p<0.05$ (Post-hoc Bonferroni test)

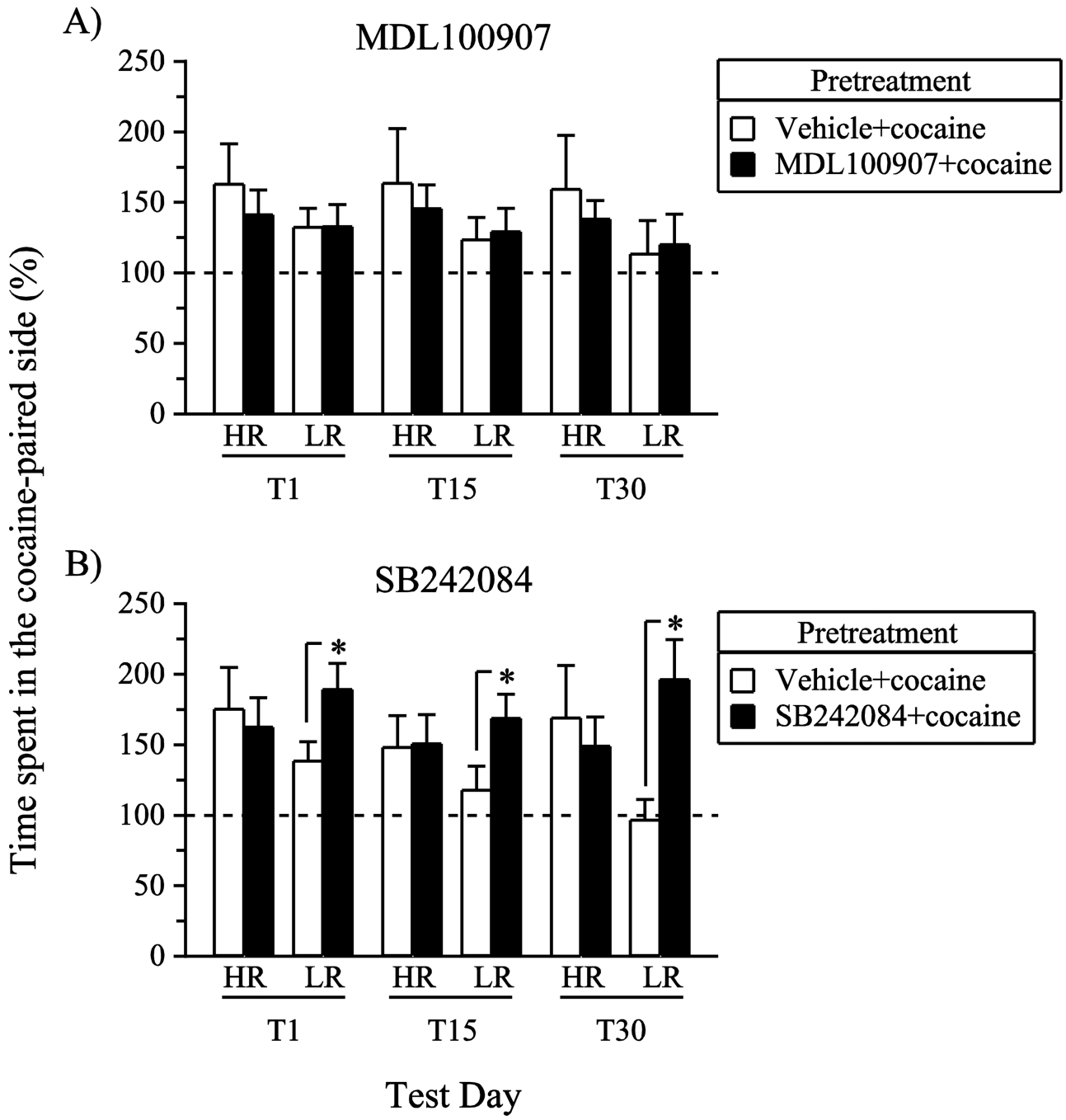

Fig. 2.

Influence of repeated administration of MDL100907 and SB242084 on *development* of cocaine-induced CPP in HR and LR rats. The bars represent the percent change in time spent in the cocaine-paired side 1, 15 and 30 days after the last conditioning trial relative to Pre-conditioning scores (Mean ± SEM) in HR and LR rats treated with: (A) MDL100907 (0.3 mg/kg, i.p.)+cocaine or (B) SB242084 (0.5 mg/kg, i.p.)+cocaine during the conditioning phase (n=6–9 per group). *significantly different from vehicle+cocaine, $p<0.05$ (Post-hoc Bonferroni test)

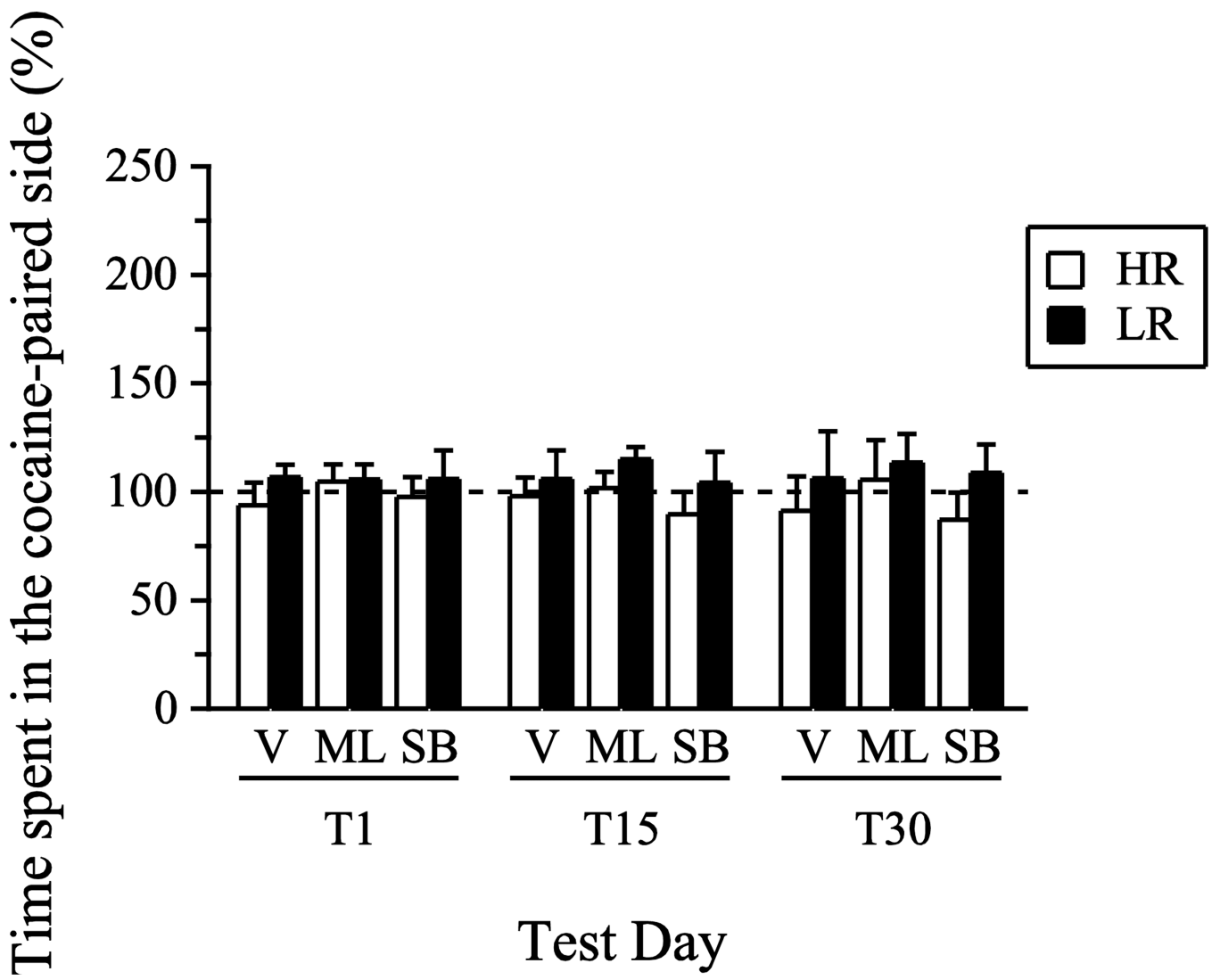

Fig. 3.

Effects of MDL100907 and SB242084 on CPP acquisition in absence of cocaine, in HR and LR animals. The bars represent the percent change in time spent in the saline-paired side 1, 15 and 30 days after the last conditioning trial relative to Pre-conditioning scores (Mean ± SEM) in HR and LR rats pretreated with: vehicle (V, saline containing 8.0% hydroxypropyl-β-cyclodextrin, 1ml/kg, i.p.), MDL100907 (ML, 0.3 mg/kg, i.p.) or SB242084 (SB, 0.5 mg/kg, i.p.) during the conditioning phase (n=5 per group)

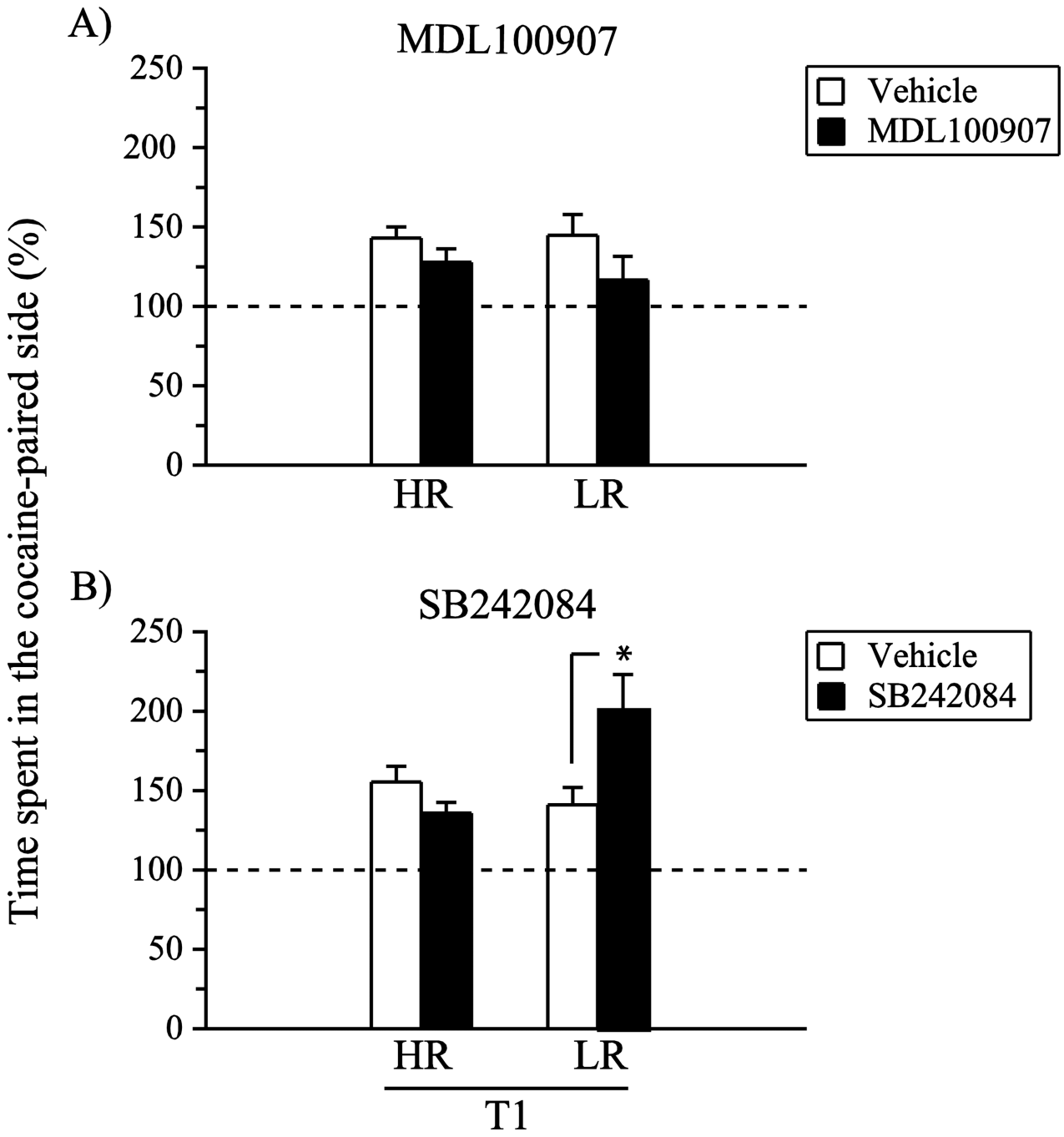

Fig. 4.

Influence of MDL100907 and SB242084 on *expression* of cocaine-induced CPP in HR and LR rats 24h after the last conditioning trial. The bars represent the percent change in time spent in the cocaine-paired side on Post-conditioning day 1 (T1) relative to Pre-conditioning scores (Mean ± SEM) in HR and LR rats after acute administration of (A) MDL100907 (0.3 mg/kg, i.p.) or (B) SB242084 (0.5 mg/kg, i.p.) compared to vehicle controls (n=6–11 per group). *significantly different from vehicle, $p<0.05$ (Post-hoc Bonferroni test)

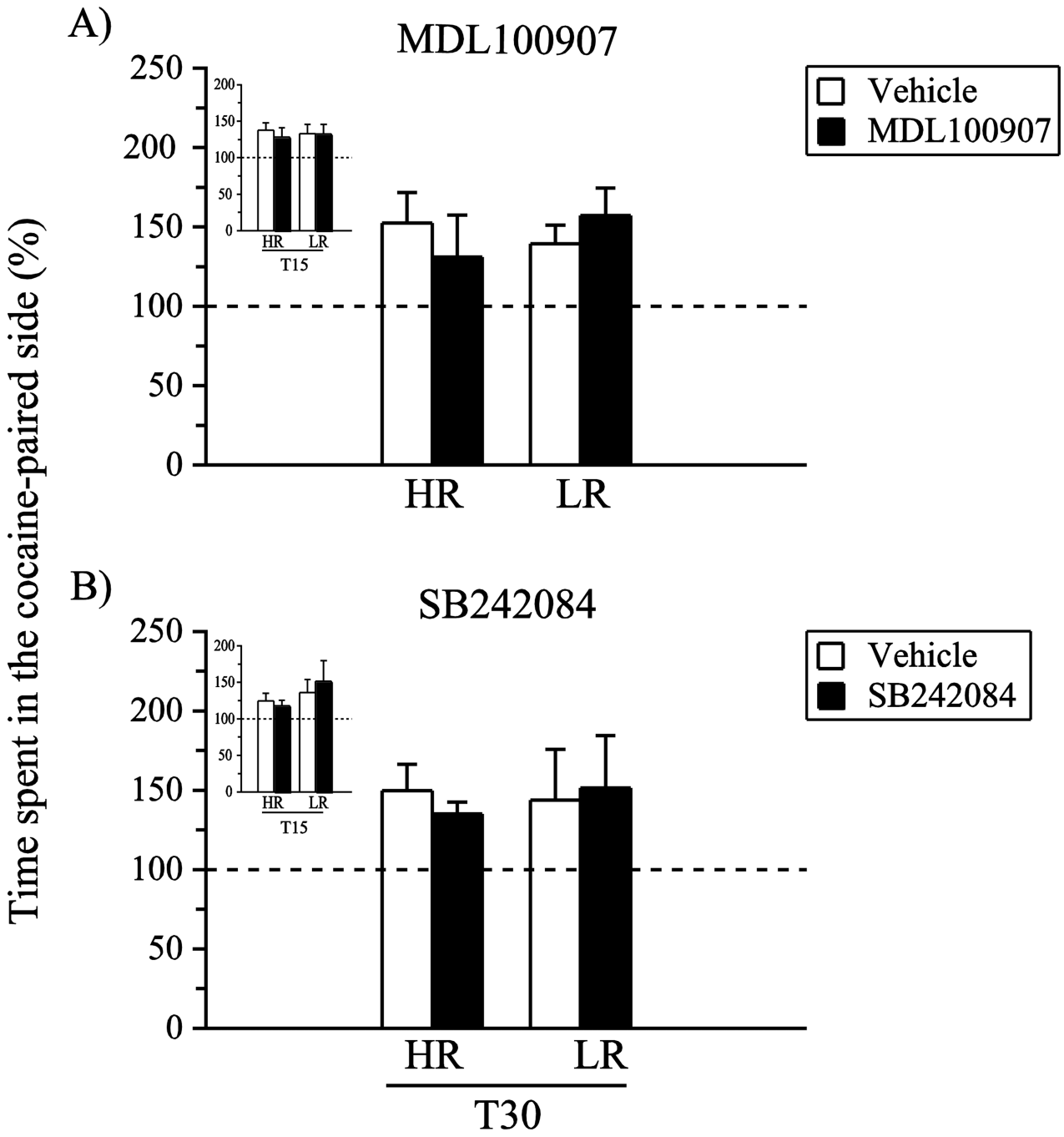

Fig. 5.

Lack of acute effects of MDL100907 and SB242084 on *recall* of cocaine-induced CPP in HR and LR rats 30 days after the last conditioning trial. The bars represent the percent change in time spent in the cocaine-paired side on Post-conditioning day 15 (T15) (upper left) and day 30 (T30) relative to Pre-conditioning (Mean ± SEM) after acute administration of (A) MDL100907 (0.3mg/kg, i.p.) or (B) SB242084 (0.5 mg/kg, i.p.) compared to vehicle controls (n=4–6 per group)