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ORIGINAL INVESTIGATION

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Influence of the dose and the number of drug-context pairings on the magnitude and the long-lasting retention of cocaine-induced conditioned place preference in C57BL/6J mice

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Abstract *Rationale:* The place conditioning procedure is increasingly used to study relapse in drug seeking in mice. However, the retention course of drug-induced place preference has not been systematically characterized. *Methods:* The effects of cocaine doses and number of conditioning trials on both the magnitude and the persistence of cocaine-induced conditioned place preference (CPP) were investigated in C57BL/6J mice. Twelve groups of animals were injected with saline, 4, 8 or 12 mg/kg cocaine (i.p.) and submitted to an unbiased counterbalanced place conditioning protocol including one, two or four drug-pairing sessions. Subsequently, the animals were tested at various time intervals after the last conditioning session. *Results:* One cocaine-pairing session was insufficient to induce a CPP. Two and four pairing sessions resulted in significant place preferences of similar magnitude for all tested doses of cocaine, the place preference induced by the greatest number of pairing sessions being the strongest. In the two-pairing groups, place preference lasted less than 14 days for any tested dose of cocaine. In contrast, all four-pairing groups still showed significant place preference 28 days after the last conditioning session. However, the magnitude of cocaine place preference slowly declined at a rate that was dependent upon cocaine dose. On the 35-day post-conditioning interval, only the 12-mg/kg cocaine group still displayed a significant place preference, whereas place preference was undetectable at 42 and 56 days post-conditioning for all groups. *Conclusions:* The number of cocaine-pairing sessions, but not cocaine dose, affected the magnitude of cocaine place preference in mice when tested 1 day after the last conditioning session. In contrast, both cocaine doses and the number of pairing sessions affected the persistence of cocaine place preference. Overall, these results demonstrate that cocaine-induced place preference is a long lasting phenomenon that is strongly affected by the number of drug-pairing trials.

Keywords Cocaine · Conditioned place preference · Doses · Number of pairings · Persistence · Retention · C57BL/6J mice

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Introduction

The ability of drug-associated cues to induce craving and relapse into drug-seeking behaviors is one of the potential mechanisms by which addiction endures. The conditioned place preference (CPP) technique is an animal model of such cue-eliciting conditioning that can be used to study drug-seeking behaviors (Bardo et al. 1995; Tzschentke 1998). In the CPP technique, animals are given a reinforcing drug in one distinct environment and are administered with vehicle in an alternative environment. After conditioning, the animals are submitted to a preference test during which they have free access to both environments simultaneously. The time spent in each environment is recorded and a drug is said to induce a conditioned place preference when experimental animals spend significantly more time in the drug-associated environment relative to vehicle-injected control animals. Drugs with abuse liability in humans, such as cocaine, amphetamine, heroin, or diazepam reliably produce CPPs in both rats and mice (Tzschentke 1998).

As the CPP test is generally administered the day after the last conditioning session in drug-free animals, the expression of a CPP is dependent upon memory processes, including acquisition, consolidation and retrieval mechanisms (White 1996; Sara 2000). A number of empirical results have confirmed the role of memory processes in drug-induced CPP. The administration of amnesic drugs during the conditioning procedure has been shown to impair the acquisition of drug-induced CPP. For example, MK-801, a non-competitive NMDA antagonist, blocks the

acquisition of morphine- and cocaine-induced place preference in rats (Cervo and Samanin, 1995; Tzschentke and Schmidt 1995). Scopolamine, another drug that impairs learning and memory, also prevents the development of a cocaine-induced CPP in mice (Itzhak and Martin 2000). Specific inhibitors of protein kinases A and C that are involved in long-term potentiation and memory consolidation (Zigmond et al. 1999), also impair the development of cocaine-induced CPP when injected immediately after the conditioning trials (Cervo et al. 1997).

In recent years, the place conditioning procedure has been increasingly used to study the relapse into drug seeking behaviors (e.g., Mueller and Stewart 2000; Parker and McDonald 2000; Mueller et al. 2002; Szumlinski et al. 2002; Sanchez et al. 2003). After the establishment of a reliable conditioned place preference for the drug-paired compartment, this preference is gradually extinguished by repeated expositions of the animals to the drug-paired compartment in a drug-free state. At the end of the extinction procedure, different factors, such as priming drug injections or acute stress may be tested to investigate whether they are able to reinstate a significant conditioned place preference. However, in spite of the growing use of the place conditioning procedure to study drug relapse, the retention course of drug-induced place preferences has not been thoroughly investigated and the factors affecting the maintenance and decline of such CPP have not been systematically characterized. Several studies have shown that drug-induced CPP is maintained over time, up to 12 weeks for morphine (Mueller et al. 2002). Amphetamine-induced CPP has been shown to persist 1 week after the last conditioning trial in rats (Schroeder and Packard 2003). Nicotine-induced stimulus preference is still present 2 weeks after the last conditioning session (Fudala and Iwamoto 1986), while a significant preference for a place that had been associated with delta-9-tetrahydrocannabinol was observed 5 days after conditioning (Lepore et al. 1995). Conditioned place aversions have also been shown to persist over long periods of time. For example, naloxone-induced conditioned place aversion was still observed 1 month after the last conditioning session (Mucha and Iversen 1984). Regarding cocaine, significant CPPs were observed after 4 and 6 weeks in rats (Nomikos and Spyraki 1988; Mueller and Stewart 2000) and 4 weeks in mice (Zhang et al. 2002). Although it has been shown that the CPP for various drugs is a long lasting phenomenon, very few studies have investigated the duration of its persistence until complete disappearance. Despite their potential relevance for the understanding of addiction maintenance, the factors that control CPP persistence are also mostly undefined. Zhang and co-workers (2002) reported the influence of mice strains with C57BL/6J mice showing a greater persistence of cocaine-induced CPP than 129/J mice. According to learning theories, procedural factors, such as the intensity of an unconditioned stimulus and the number of learning trials, have a major impact on learning retention (Spear 1978; Anderson 2000). For instance, in a passive avoidance test, the magnitude of the foot shock used in learning trials determines the retention of an avoidance behavior (Bucherelli and Tassoni 1992). In the same way, the retention of a cocaine-induced CPP should be directly related to the number of conditioning trials and to the dose of cocaine that is administered.

The aim of the present study is to characterize the retention course of a cocaine-induced place preference in mice. Furthermore, the number of conditioning trials and the dose of cocaine were systematically manipulated to assess their impact on CPP persistence overtime. Mice were injected with various doses of cocaine (saline, 4, 8 and 12 mg/kg, i.p.) and were subjected to an unbiased counterbalanced place conditioning procedure including one, two or four conditioning trials. After conditioning, animals were tested in a drug-free state at different time intervals until CPP disappearance.

Materials and methods

Subjects

A total of 192 male C57BL/6J mice, aged 7–8 weeks and experimentally naïve at the beginning of the experiments (Charles River Laboratories, Brussels, Belgium), were housed individually in transparent polycarbonate cages [26 (*L*)× 40.5 (*W*)×20 (*H*) cm] with pine sawdust bedding. Food (standard pellets, Carfil Quality Bvda, Oud-Turnhout, Belgium) and water were available ad libitum. The animal room was maintained on a 12:12 h light–dark cycle (lights on at 8.00 h) and an ambient temperature of 19–22°C. All experimental procedures were carried out in accordance with the standards of care and use of laboratory animals laid down by the European Communities Council (Directive No. 86/609/EEC, 24 November 1986). Protocols were reviewed and approved by the Animal Care Committee of the University of Liège on the basis of these standards.

Pharmacological treatments

Every other day during the experiments, (−)-cocaine hydrochloride (Belgopia, Louvain-La-Neuve, Belgium) was dissolved in an isotonic saline solution (0.9% NaCl), before being administered at 4, 8 or 12 mg/kg at a volume of 0.01 ml/g body weight, the control animals receiving similar volumes of saline solution. All injections were conducted via the peritoneal route.

Behavioural device

We utilized a battery of eight place-preference devices purchased from Technical & Scientific Equipment, Bad Homburg, Germany (TSE Place Preference System, model 257000-MAU) suitable for the application of an "unbiased" place-conditioning design. Each chamber featured three distinct compartments with distinct visual and tactile cues: two large equally sized outer compartments (16.5× 15×20 cm) separated by a smaller central compartment

(6.5×15×20 cm). During place-preference sessions, the separating walls used during conditioning (during which the mouse was confined in one of the outer compartment) were exchanged for walls containing an arched gateway (3.5×4 cm), allowing free movements through the whole apparatus (and thereby preference for one of the outer compartments). The central compartment served as a start-point for place preference tests. All compartments were made of removable opaque PVC tablets. One of the outer compartments was colored white throughout, and the other was colored with alternate black and white 2.5-cm vertical strips, the inside of the central compartment being gray. To provide tactile cues, removable clear acrylic resin tablets whose upper surface was markedly textured were placed on the floor of each outer (choice) compartment. The tablet placed in the left compartment (striped walls) presented a relatively thinly embossed texture with 2-mm^2 punches, whereas that inserted in the right compartment (white walls) was textured with larger 4-mm^2 punches. The central area (gray walls) was provided with a smooth floor. Entrance into and movements within the compartments were automatically recorded via an array of infrared detectors mounted every 28 mm along the entire length of the compartments walls. The infrared detectors allowed the calculation of the time spent (sec) in each compartment, the monitoring of the ambulatory activity that was measured in terms of the total distance traveled (cm) in each compartment and the location of the mouse in the apparatus during the session. One computer operated the eight devices simultaneously. To insure some degree of visual and acoustical isolation, the CPP devices were individually enclosed in white melamine cubicles (60×40×40 cm).

General procedure

All experimental procedures were carried out during the light period of the light–dark cycle, between 9:00 and 13:00 h. Mice were divided into four groups, each group being assigned to one of the four pharmacological treatments (saline, 4, 8 and 12 mg/kg cocaine). The experimental procedure included three main phases: a single-day habituation session, two to eight daily conditioning sessions and several repeated test sessions. On the first day, during the habituation session, all mice were pre-exposed to the apparatus with free access to all three compartments for 20 min and the time spent in each compartment was recorded. During the second phase, beginning the day after the pre-exposure session, animals underwent an unbiased counterbalanced conditioned place preference design. They were further subdivided into three groups that were subjected to two, four or eight conditioning sessions, such that the drug-paired compartment was associated with cocaine on one, two or four occasions. On odd days, mice were injected with their assigned dose of cocaine (0, 4, 8 or 12 mg/kg). Immediately after the injection, they are placed directly into the drug-paired compartment and confined there for 20 min. Within each group, the drug-paired compartment was counterbalanced, such that half of the mice were confined in the striped compartment, while the other half was confined in the white compartment. On even days, mice were injected with saline and confined for 20 min in the opposite compartment (saline-paired compartment). On the third phase, repeated test sessions were carried out in order to assess the persistence of cocaine-induced place preference. On these test sessions, mice were injected with saline and immediately placed into the central area of the apparatus with free access to all compartments for 20 min, the time spent in each compartment being recorded. The first CPP test, which was carried out the day after the last conditioning session, allowed to assess the effects of the cocaine dose (0, 4, 8 or 12 mg/kg) and the number of pairing sessions (one, two or four cocaine-pairing sessions) on the magnitude of cocaine-induced CPP. In order to study the retention of cocaine-induced CPP, mice were subsequently retested at various time intervals until disappearance of the CPP. The time intervals between successive test sessions were defined according to previously published results and in order to minimize the extinction of the CPP. Indeed, multiple tests in drug-free animals are likely to produce an extinction of the CPP and it was therefore necessary to minimize the number of repeated test sessions. Zhang and co-workers (2002) showed in C57BL/6J mice that a cocaine-induced CPP after four pairing sessions is still apparent 4 weeks after the last conditioning session. Therefore, in the group that was subjected to four drug-pairing sessions, the mice were retested every 4 weeks after the last conditioning session. To date, the persistence of a cocaine-induced CPP in mice after two drug-pairing sessions has never been tested. Therefore, we have chosen a shorter time interval (2 weeks) between successive test sessions. Finally, in the group that was submitted to a single drug-pairing session, no significant CPP was observed on the first test. Therefore these mice were not retested.

Data analysis

To ensure that the present place conditioning design was actually unbiased, a mixed-model two-way analysis of variance (ANOVA) was performed on the time spent in each compartment during the pre-conditioning session, the group (saline, 4, 8 or 12 mg/kg cocaine for one, two and four drug-pairing sessions, 12 levels) and the compartment (striped versus white, two levels) being incorporated as between-subject and within-subject factors, respectively. A score of place preference was calculated for each animal by subtracting the time spent in the drug-paired compartment during the pre-conditioning habituation session from the time spent in that compartment on the test session. A score of zero would indicate an absence of change in place preference, whereas positive and negative scores would reflect preferences or aversions for the drug-paired side. To test the effects of cocaine doses and number of pairing sessions, a two-way ANOVA was calculated on the results of the first CPP test with cocaine dose and number of drug-pairing sessions defined as between-subject factors. To

study the effects of cocaine doses and number of pairing sessions on the persistence of cocaine-induced CPP, the results of the groups that were submitted to two and four drug-pairing sessions were analyzed separately using two-way ANOVAs with repeated measures. Experimental group and test session were defined as between-subject and within-subject factors, respectively. After every ANOVA, relevant between-mean differences were assessed via the Fisher's Protected Least Significant Difference test (PLSD). Statistical significance was conventionally set at $p<0.05$.

Results

Effects of cocaine doses and pairing sessions on the magnitude of the CPP

As shown in Table 1, all groups of animals spent equal amounts of time in the white and in the striped compartments. This profile of effects was supported by a two-way ANOVA indicating no effect of both the experimental group ($F_{11,132}=1.36$, $p=0.200$) and the compartment ($F_{1,132}=0.80$, $p=0.372$) and no interaction between these factors ($F_{11,132}=0.40$, $p=0.953$).

The effects of cocaine dose and number of drug pairings on the magnitude of cocaine-induced CPP were assessed on the first test session (Fig. 1). A two-way ANOVA (experimental group × drug-pairing sessions) indicated a significant effect of cocaine doses ($F_{3,132}=16.033$, $p<0.001$), a significant effect of the number of drug-pairing sessions ($F_{2,132}=8.816$, $p<0.001$) and a significant interaction between these factors ($F_{6,132}=3.125$, $p<0.01$). Mice subjected to a single cocaine-pairing session showed no significant place preference for the compartment that was associated with cocaine administration. In contrast, after two or four cocaine pairing sessions, mice showed a significant preference for the drug-paired compartment relative to their respective saline control groups, although this effect was similar for all cocaine doses. In contrast, the magnitude of cocaine-induced CPP was affected by the number of drug-pairing sessions. After four cocaine-pairing sessions, mice showed a stronger CPP relative to the groups that were submitted to two pairing sessions. This is supported by significant Fisher PLSD tests for both 4 and 12 mg/kg cocaine, whereas the 8 mg/kg cocaine group failed to achieve statistical significance.

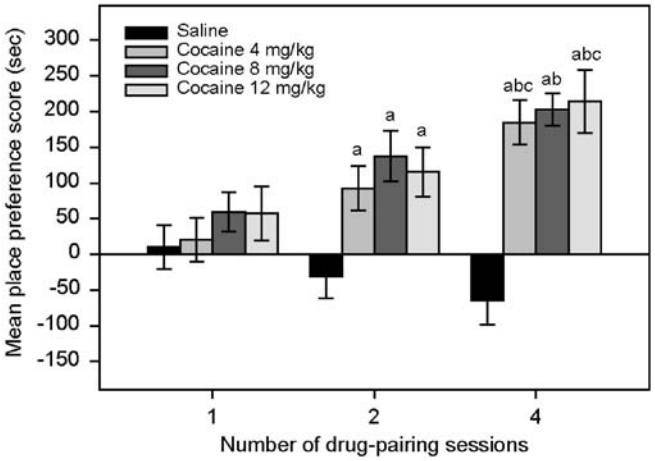

Fig. 1 Conditioned place preference obtained 24 h after conditioning with saline, 4, 8 or 12 mg/kg cocaine and following one, two or four drug-pairing sessions. Place preference was scored in terms of the difference between the time spent in the drug-paired compartment before and after conditioning. **a** Value significantly greater than that of the corresponding saline control group for a given number of drug-pairing sessions, **b** value significantly greater than that obtained for the mice having underwent a single drug-pairing session for a given cocaine dose, **c** value significantly greater than that of the mice having underwent two drug-pairing sessions at a given cocaine dose, as yielded by Fisher PLSD tests taken at least at $p<0.01$. *Vertical brackets* represent SEMs × 2

Table 1 Mean±SEM time (sec) spent in the two large compartments of the place conditioning apparatus (striped compartment, white compartment) by the different groups of mice on the habituation session

Experimental groups	Striped compartment	White compartment
One-drug pairing groups		
Saline	453±14	427±25
4 mg/kg	435±14	435±23
8 mg/kg	459±22	436±34
12 mg/kg	479±19	430±24
Two-drug pairings groups		
Saline	465±23	431±33
4 mg/kg	442±22	480±24
8 mg/kg	439±23	471±27
12 mg/kg	471±23	439±26
Four-drug pairings groups		
Saline	459±22	423±19
4 mg/kg	407±27	405±30
8 mg/kg	424±28	438±26
12 mg/kg	444±24	427±31

All groups of mice spent a comparable amount of time in the striped and white compartments

Table 2 shows the actual time spent in the drug-paired compartment for all testing sessions. A two-way ANOVA performed on these data for the first test session confirms the results obtained above with the scores of place preference. There were significant main effects for both cocaine doses ($F_{3,132}=19.604$, $p<0.001$) and number of pairing sessions ($F_{2,132}=10.845$, $p<0.001$) and a significant interaction between these factors ($F_{6,132}=3.251$, $p<0.01$).

Effects of cocaine doses and pairing sessions on the persistence of CPP

Figure 2 shows the decay of cocaine-induced CPP in mice that had been submitted to two cocaine-pairing sessions. The two-way ANOVA indicated no effect of the experimental group ($F_{3,44}=1.802$, $p=0.160$) but a significant effect of the test session ($F_{1,44}=9.685$, $p<0.01$) and a significant interaction between these factors ($F_{3,44}=4.863$, $p<0.01$). One day after conditioning, animals treated with 8

Table 2 Actual time spent (mean±SEM, sec) in the drug-paired compartment for all test sessions

Groups	Test 1	Test 2	Test 3
One drug-pairing session, test session on day 1			
Saline	446±31		
4 mg/kg	451±20		
8 mg/kg	516±25		
12 mg/kg	479±33		
Two drug-pairing sessions, test sessions on days 1 and 14			
Saline	389±33	441±41	
4 mg/kg	532±24*	472±30	
8 mg/kg	570±28**	483±34	
12 mg/kg	555±23**	454±39	
Four drug-pairing sessions, test sessions on days 1, 28 and 56			
Saline	395±24	396±30	437±36
4 mg/kg	613±21**	520±28	428±40
8 mg/kg	614±30**	551±35*	427±43
12 mg/kg	644±38**	601±75**	443±36
Four drug-pairing sessions, test sessions on days 1, 35 and 42			
Saline	407±36	394±26	431±37
4 mg/kg	550±19*	478±36	393±32
8 mg/kg	625±38**	552±26*	327±19
12 mg/kg	573±36**	525±28*	364±22

During the conditioning procedure, the drug-paired compartment was associated with saline, 4, 8 or 12 mg/kg cocaine on one, two or four occasions

*$p<0.05$

**$p<0.01$ relative to their respective saline control groups

and 12 mg/kg showed significant place preferences for the cocaine-paired compartment relative to the saline control group (LSD, $p<0.05$). Mice of the 4-mg/kg cocaine group displayed a non-significant tendency for place preference (Fisher PLSD, $p=0.054$). Cocaine-induced CPP then declined and was undetectable 14 days after the last conditioning session in all groups.

Figure 3 shows the decay of cocaine-induced CPP in mice that had been submitted to four cocaine-pairing sessions. The two-way ANOVA indicated a significant effect of the experimental group ($F_{3,44}=6.946$, $p<0.001$), a significant effect of the test session ($F_{2,88}=32.301$, $p<0.0001$) and a significant interaction between these factors ($F_{6,88}=6.829$, $p<0.0001$). One day after the last conditioning session, all cocaine-conditioned mice showed a significant preference for the cocaine-paired compartment relative to the saline control group (Fisher PLSD, $p<0.01$). However, the magnitude of this CPP was not dependent upon cocaine doses as all cocaine groups showed similar levels of place preference. Four weeks later (on day 28), cocaine-induced CPP was still significant in all cocaine groups. However, a slight decrease in the magnitude of the CPP was apparent, although it was significant only for the 4-mg/kg cocaine group that spent less time in the cocaine-paired compartment relative to the first test on day 1 (Fisher PLSD, $p<0.01$). At 8 weeks (56 days), cocaine-induced CPP had become undetectable in all groups.

As shown in Fig. 3, cocaine-induced CPP after four drug-pairing sessions was still apparent at 4 weeks, but had disappeared at 8 weeks. Therefore, to determine more precisely the retention of cocaine-induced CPP in the interval between 4 and 8 weeks, an independent experiment was carried out. This experiment was identical to the protocol used for the four cocaine-pairing experiment described above, except that successive test trials were conducted 1, 35 (i.e., 5 weeks) and 42 (i.e., 6 weeks) days after the last

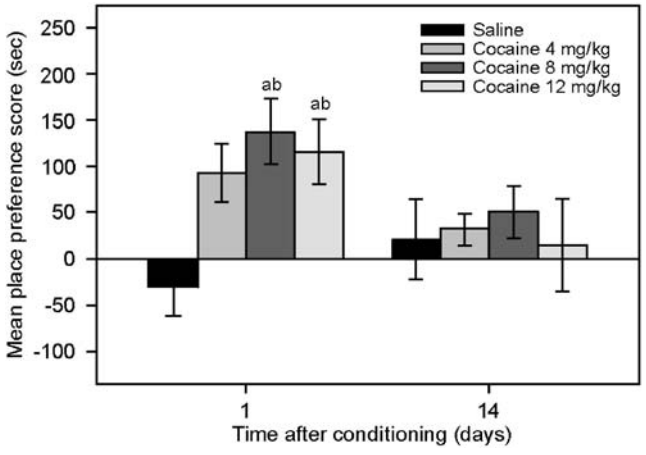

Fig. 2 Conditioned place preference in mice after two drug-pairing sessions with saline, 4, 8 or 12 mg/kg cocaine. Repeated place preference tests were carried out 1 and 14 days following the last conditioning session. **a** Value significantly greater than that of the corresponding saline control group at a given post-conditioning test, **b** value significantly greater than the corresponding one (same dose) obtained on the 14-day retention interval, as yielded by Fisher PLSD tests taken at least at $p<0.05$. The other details are the same as in Fig. 1 legend

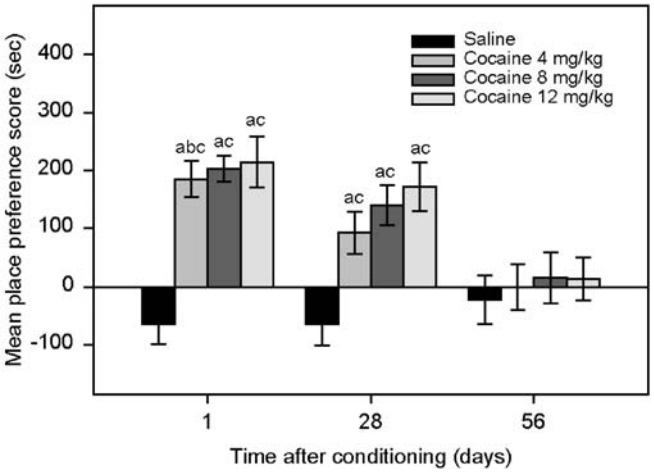

Fig. 3 Conditioned place preference in mice after four drug-pairing sessions with saline, 4, 8 or 12 mg/kg cocaine. Repeated place preference tests were carried out 1, 28 and 56 days after the last conditioning session. **a** Value significantly higher than that of the saline control group within each post-conditioning test session, **b** value significantly higher than that obtained 28 days after the last conditioning session at a given cocaine dose, **c** value significantly higher than that obtained 56 days after the last conditioning session at a given cocaine dose, as yielded by Fisher PLSD tests taken at least at $p<0.05$. The other details are the same as in Fig. 1 legend

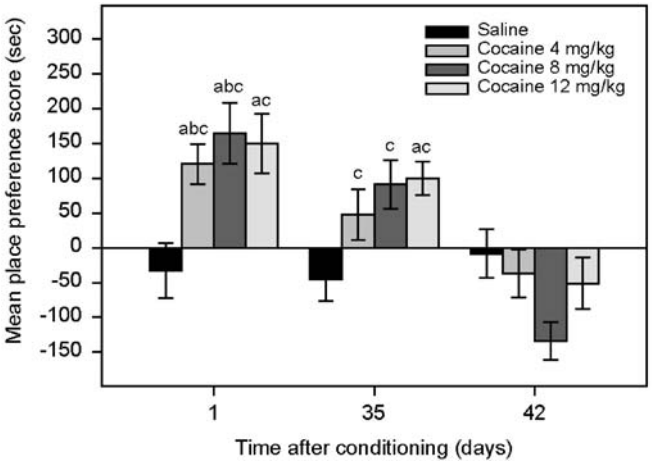

Fig. 4 Conditioned place preference in mice after four pairing sessions with saline, 4, 8 and 12 mg/kg cocaine. Repeated place preference tests were carried out 1, 35 and 42 days after the last conditioning session. Place preference was scored in terms of the difference between the time spent in the drug-paired compartment before and after conditioning. **a** Value significantly higher than its saline control group, **b** value significantly higher than that obtained 35 days after the last conditioning session, **c** value significantly higher than that obtained 42 days after the last conditioning session, as yielded by Fisher PLSD tests taken at least at $P<0.05$. The other details are the same as in Fig. 1 legend

conditioning session. Figure 4 shows the decay of cocaine-induced CPP in this independent experiment. The two-way ANOVA revealed no significant effect of the experimental group ($F_{3,44}=1.997$, $p=0.128$), but a significant effect of the test session ($F_{2,88}=46.016$, $p<0.0001$) and a significant interaction between these factors ($F_{6,88}= 9.252$, $p<0.0001$). One day after the last conditioning session, all cocaine-conditioned mice showed a significant preference for the cocaine-paired compartment relative to the saline control group (Fisher PLSD, $p<0.05$), replicating the results obtained with the previous four cocaine-pairing experiment. Five weeks later (on day 35), a significant place preference for the cocaine-paired compartment was found only for the 12-mg/kg cocaine group. Although the time these mice spent in the cocaine-paired compartment was reduced relative to day 1, this reduction failed to achieve statistical significance (Fisher PLSD, $p=0.139$). In contrast, the time spent by both other cocaine groups in the drug-paired compartment was significantly lower relative to day 1 and these groups failed to achieve a significant place preference for the cocaine-paired compartment. One week later (on day 42), cocaine-induced CPP had become undetectable in all groups.

Discussion

In the present study, we have shown that the magnitude of a cocaine-induced CPP is affected by the number of drug-pairing sessions, whereas it is not dose-dependent across doses between 4 and 12 mg/kg. In contrast, the retention of cocaine-induced CPP is dependent upon both cocaine dose and the number of drug-pairing sessions. This later result is in agreement with current learning theories according to which the magnitude of an unconditioned stimulus and the number of learning trials strongly determine the memory retention of learned tasks.

In place conditioning studies, cocaine-induced CPP is most often tested after four drug-pairing sessions. With such a protocol, C57BL/6J mice show a significant cocaine-induced CPP, whose magnitude is not dependent upon cocaine doses across the 4–12 mg/kg range (Cunningham et al. 1999; Seale and Carney 1991; Zhang et al. 2002). In a meta-analysis, Bardo and collaborators (1995) reported that the impact of cocaine doses is very difficult to demonstrate with the place conditioning technique. It has been suggested that the ascending graded portion of the dose–response curve for cocaine in the place conditioning technique lies between 1 and 5 mg/kg (Nomikos and Spyraki 1988; Spyraki et al. 1982), such that a ceiling effect would be attained with higher doses. A consistent result was obtained in the present study, in which 4–12 mg/kg cocaine doses induced significant place preferences, but of equivalent magnitude.

Amongst the few studies having systematically investigated the relation between the number of drug-pairing sessions and the magnitude of cocaine-induced place preference, Nomikos and Spyraki (1988) have tested cocaine-induced CPP in rats after one, two, three or four cocaine-pairing sessions. In their study, only four pairing sessions resulted in a significant CPP for cocaine, whereas one, two and three pairing sessions only induced a non-significant tendency toward CPP. In contrast, Bardo and co-workers (1986) were able to show a significant CPP after a single cocaine-pairing session in rats and several other studies demonstrated cocaine-induced CPP after two pairing sessions in rats (Gong et al. 1995; Le Pen et al. 1996; Russo et al. 2003). The reasons for such discrepancies are unknown, but could be related to a number of uncontrolled technical and procedural differences. In mice, previous studies obtained cocaine-induced CPP after two pairing-sessions (Miner 1997; Sora et al. 1998; Becker et al. 2002; Hall et al. 2003). In particular, Miner (1997) showed that C57BL/6J mice are able to develop a significant CPP after only two cocaine-pairing sessions. However, to our knowledge, no published study in mice tried to induce a significant CPP for cocaine after a single drug-pairing session. The present study confirms that C57BL/6J mice display a cocaine-induced CPP after two pairing sessions and further shows that a single cocaine-pairing session is insufficient to induce a significant CPP in spite of a relatively large number of animals per group ($n=12$). Therefore, there is a significant discrepancy between rats that are able to develop a cocaine-induced CPP after a single drug-pairing session and mice that fail to do so. Such a difference may be due to the poorer general learning abilities of mice relative to rats (McNamara et al. 1996). Finally, the present study also demonstrates that the number of conditioning trials affects the magnitude of cocaine-induced CPP with four pairing sessions producing a significantly stronger preference than two sessions.

The results of the present study clearly show that the retention of cocaine-induced CPP is particularly sensitive to the effects of procedural variables such as the dose of cocaine and the number of drug-pairing sessions. Whereas the number of drug-pairing sessions had only a moderate impact on the magnitude of cocaine-induced place preference on the first test session (Fig. 1), its effects on the retention of the place preference was dramatic. After two drug-pairing sessions, cocaine-induced CPP lasted less than 2 weeks since it was undetectable 14 days after the last conditioning trial (Fig. 2). In contrast, after four drug-pairing sessions, cocaine-induced CPP was still significant 5 weeks after the last conditioning session for the 12-mg/kg dose and 4 weeks after conditioning for the other tested doses (4 and 8 mg/kg). These later results also show that cocaine doses affect the retention of the place preference, with higher doses of cocaine leading to a longer persistence over time.

Previous studies had already shown that cocaine-induced place preference after four drug-pairing sessions lasts 4 weeks in both rats (Mueller and Stewart 2000; Nomikos and Spyraki 1988) and mice (Zhang et al. 2002). In agreement with the present results, these studies observed that the CPP induced by cocaine doses of 10 and 20 mg/kg was unaltered 4 weeks after the last conditioning trial. The present study further indicates that the CPP induced by cocaine doses in the range between 8 and 12 mg/kg starts to decline at the fifth week after the last conditioning trial.

Although the present study shows a decline in the magnitude of cocaine-induced CPP over time, it cannot ascertain if this effect is only due to forgetting or whether extinction processes contributed to the overall decline. The procedure used in the present study tried to minimize the number of repeated test sessions. In the four drug-pairing groups, mice were submitted to a maximum of three consecutive CPP tests in a drug free state, although it is still possible that an extinction process contributed to the decline in the CPP magnitude on the third test session. However, it is very unlikely that the decline in cocaine-induced CPP is only attributable to extinction processes. Indeed, Mueller and Stewart (2000) showed that the multiplication of successive test sessions under extinction conditions did not affect the decline of cocaine-induced CPP over 6 weeks. At 6 weeks, there were no significant differences in the magnitude of the CPP between rats that were subjected to either one, two or four extinction sessions.

Finally, it is noteworthy that the retention of cocaine-induced CPP appears to be more sensitive to experimental manipulations than the common test of place preference on the first day after the last conditioning trial. In the present study, cocaine doses between 4 and 12 mg/kg did not influence the magnitude of the CPP on the first test, whereas cocaine doses had a significant impact on the retention of the CPP. Similarly, the number of conditioning trials had only a moderate impact on the magnitude of the CPP on the first test, while the impact on the retention of CPP was dramatic. A similar difference was obtained in a previous study that compared cocaine-induced CPP in two strains of mice (Zhang et al. 2002). On the first test session, both C57BL/6J and 129/J mice displayed similar levels of CPP with 5, 10 and 20 mg/kg cocaine, although at the fourth week, C57BL/6J mice showed a better retention of the CPP than 129/J mice.

Recent self-administration studies showed that cocaine-seeking behaviors are long lasting and may persist as long as 9 months after a single cocaine self-administration experience (Grimm et al. 2001; Ciccocioppo et al. 2004). Relative to these later studies, the maximum persistence of cocaine-induced CPP found in the present study (7 weeks after four pairing sessions with 12 mg/kg cocaine) may look surprisingly short. However, the self-administration and the place conditioning procedures differ in many respects. Differences in the persistence of cocaine-induced behaviors may be linked to the active versus passive drug administration that are used in the self-administration and place conditioning procedure, respectively. Indeed, passive versus active drug administrations have been shown to induce differential neuroadaptive effects (Jacobs et al. 2003). Another notable difference between these experimental procedures lies in the number of drug associations. In drug self-administration studies, even a session defined as a single cocaine experience involves 40–50 reinforcements of the lever-pressing behavior with cocaine infusions (Ciccocioppo et al. 2004). In contrast, the place conditioning procedure requires only very few, generally four, associations of the drug-paired compartment with cocaine. For example, the present study shows that C57BL/6J mice develop a significant place preference after only two cocaine-pairing sessions. As a consequence, these two experimental techniques may be useful to investigate different aspects of the persistence of drug-induced behaviors. For example, the place conditioning procedure would be better suited to compare the occurrence of drug relapse after either systematic extinction or simple forgetting, as the later is less likely to develop after many drug associations.

In conclusion, the magnitude of cocaine-induced place preference on the first test session was not dependent upon cocaine doses, but was significantly affected by the number of drug-pairing sessions. In contrast, there was a significant impact of both factors on the retention of this CPP. Overall, the present results indicate that cocaine-induced place preference is a long lasting phenomenon that is highly dependent upon the number of drug-pairing sessions and can last 5 weeks in C57BL/6J mice after four pairing sessions with 12 mg/kg cocaine.

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References

Anderson JR (2000) Learning and memory, 2nd edn. Wiley, New York

Bardo MT, Neisewander JL, Miller JS (1986) Repeated testing attenuates conditioned place preference with cocaine. Psychopharmacology 89:239–243

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

Becker A, Grecksch G, Kraus J, Loh HH, Schroeder H, Hollt V (2002) Rewarding effects of ethanol and cocaine in mu opioid receptor-deficient mice. Naunyn-Schmiedeberg's Arch Pharmacol 365:296–302

Bucherelli C, Tassoni G (1992) Duration of retrograde amnesia induced by tetrodotoxin inactivation of the parabrachial nuclei is inversely related to the intensity of footshock in rat's passive avoidance response. Behav Brain Res 49:175–180

Cervo L, Samanin R (1995) Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. Brain Res 673: 242–250

Cervo L, Mukherjee S, Bertaglia A, Samanin R (1997) Protein kinases A and C are involved in the mechanisms underlying consolidation of cocaine place conditioning. Brain Res 775:30–36

Ciccocioppo R, Martin-Fardon R, Weiss F (2004) Stimuli associated with a single cocaine experience elicit long-lasting cocaine-seeking. Nat Neurosci 7:495–496

Cunningham CL, Dickinson SD, Grahame NJ, Okorn DM, McMullin CS (1999) Genetic differences in cocaine-induced conditioned place preference in mice depend on conditioning trial duration. Psychopharmacology 146:73–80

Fudala PJ, Iwamoto ET (1986) Further studies on nicotine-induced conditioned place preference in the rat. Pharmacol Biochem Behav 25:1041–1049

Gong W, Neill DB, Justice JB Jr (1995) Increased sensitivity to cocaine place-preference conditioning by septal lesions in rats. Brain Res 683:221–227

Grimm JW, Hope BT, Wise RA, Shaham Y (2001) Incubation of cocaine craving after with withdrawal. Nature 412:141–142

Hall FS, Drgonova J, Goeb M, Uhl GR (2003) Reduced behavioral effects of cocaine in heterozygous brain-derived neurotrophic factor (BDNF) knockout mice. Neuropsychopharmacology 28:1485–1490

Itzhak Y, Martin JL (2000) Scopolamine inhibits cocaine-conditioned but not unconditioned stimulant effects in mice. Psychopharmacology 152:216–223

Jacobs EH, Smit AB, de Vries TJ, Schoffelmeer AN (2003) Neuroadaptative effects of active versus passive drug administration in addiction research. Trends Pharmacol Sci 24:566–573

Le Pen G, Duterte-Boucher D, Costentin J (1996) Place conditioning with cocaine and the dopamine uptake inhibitor GBR12783. NeuroReport 7:2839–2842

Lepore M, Vorel SR, Lowinson J, Gardner EL (1995) Conditioned place preference induced by delta 9-tetrahydrocannabinol: comparison with cocaine, morphine, and food reward. Life Sci 56:2073–2080

McNamara RK, Namgung U, Routtenberg A (1996) Distinctions between hippocampus of mouse and rat: protein F1/GAP-43 gene expression, promoter activity, and spatial memory. Brain Res Mol Brain Res 40:177–187

Miner LL (1997) Cocaine reward and locomotor activity in C57BL/6J and 129/SvJ inbred mice and their F1 cross. Pharmacol Biochem Behav 58:25–30

Mucha RF, Iversen SD (1984) Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: a procedural examination. Psychopharmacology 82:241–247

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

Mueller D, Perdikaris D, Stewart J (2002) Persistence and drug-induced reinstatement of a morphine-induced conditioned place preference. Behav Brain Res 136:389–397

Nomikos GG, Spyraki C (1988) Cocaine-induced place conditioning: importance of route of administration and other procedural variables. Psychopharmacology (Berl) 94:119–125

Parker LA, McDonald RV (2000) Reinstatement of both a conditioned place preference and a conditioned place aversion with drug primes. Pharmacol Biochem Behav 66(3):559–561

Russo SJ, Jenab S, Fabian SJ, Festa ED, Kemen LM, Quinones-Jenab V (2003) Sex differences in the conditioned rewarding effects of cocaine. Brain Res 970:214–220

Sanchez CJ, Bailie TM, Wu WR, Li N, Sorg BA (2003) Manipulation of dopamine d1-like receptor activation in the rat medial prefrontal cortex alters stress- and cocaine-induced reinstatement of conditioned place preference behavior. Neuroscience 119:497–505

Sara SJ (2000) Retrieval and reconsolidation: toward a neurobiology of remembering. Learn Mem 7:73–84

Schroeder JP, Packard MG (2003) Systemic or intra-amygdala injections of glucose facilitate memory consolidation for extinction of drug-induced conditioned reward. Eur J Neurosci 17:1482–1488

Seale TW, Carney JM (1991) Genetic determinants of susceptibility to the rewarding and other behavioral actions of cocaine. J Addict Dis 10:141–162

Sora I, Wichems C, Takahashi N, Li XF, Zeng Z, Revay R, Lesch KP, Murphy DL, Uhl GR (1998) Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. Proc Natl Acad Sci U S A 95:7699–7704

Spear NE (1978) The processing of memories: forgetting and retention. Erlbaum, Hillsdale, NJ

Spyraki C, Fibiger HC, Phillips AG (1982) Cocaine-induced place preference conditioning: lack of effects of neuroleptics and 6-hydroxydopamine lesions. Brain Res 253:195–203

Szumlinski KK, Price KL, Frys KA, Middaugh LD (2002) Unconditioned and conditioned factors contribute to the 'reinstatement' of cocaine place conditioning following extinction in C57BL/6 mice. Behav Brain Res 136:151–160

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 56: 613–672

Tzschentke TM, Schmidt WJ (1995) *N*-Methyl-D-aspartic acid-receptor antagonists block morphine-induced conditioned place preference in rats. Neurosci Lett 193:37–40

White NM (1996) Addictive drugs as reinforcers: multiple partial actions on memory systems. Addictions 91:921–949

Zhang Y, Mantsch JR, Schlussman SD, Ho A, Kreek MJ (2002) Conditioned place preference after single doses or "binge" cocaine in C57BL/6J and 129/J mice. Pharmacol Biochem Behav 73 :655–662

Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR (1999) Fundamental neuroscience. Academic Press, San Diego