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Effect of morphine on conditioned place preference in rhesus monkeys

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ABSTRACT

In rodents, a conditioned place preference (CPP) can be induced by morphine. In the current study, we designed a biased place conditioning paradigm to test the rewarding effects of morphine in freely moving rhesus monkeys. Five monkeys were first placed in three serial rooms with the doors open between them for three days. After this habituation period, during which baseline preference for each of the two end rooms was measured, CPP conditioning occurred when the monkeys were injected intramuscularly with morphine at an increasing dose (1.5, 3, 4.5 mg/kg) before they entered the non-preferred room and on alternate days, with saline before they entered the preferred room. Morphine and saline treatment lasted for six days, respectively. CPP was tested 24 hours after the end of CPP training. The result showed that in all five monkeys, CPP was induced by the morphine treatment. The preference lasted for at least 15.3 ± 1.7 months.

Keywords Addiction, conditioning, memory, morphine, place preference, rhesus monkey.

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INTRODUCTION

Two main types of animal models have been employed to study addiction and relapse to drug abuse. One is based on the operant self-administration procedure and the other on the classical conditioned place preference (CPP) procedure (Aguilar, Rodriguez-Arias & Minarro 2009). CPP has commonly been studied in drug addiction by employing animal experiments in which it is easy to control the dose of the drug and estimate the animal's behaviour. There is evidence that rodents can associate drugs with a certain cue and later form a CPP (Tan *et al.* 2007; Tzschentke 2007).

As a close species of humans, rhesus monkeys might also exhibit a CPP induced by addictive drugs. Using a two-chamber procedure, Foltin and Evans showed that monkeys developed a location preference for the chamber where heroin was self-administered by smoking (Foltin & Evans 2001). In this study, the monkeys could not make a decision in the space beyond the drug-paired and non-drug-paired environment. In our current study, we developed a three-room CPP paradigm to test the effect of morphine, an opiate μ-receptor agonist, on the development of CPP (morphine-associated memory) in freely moving rhesus monkeys. The paradigm employed was similar to the CPP commonly used for rodents (Rice, Gordon & Gifford 2002; Hnasko, Sotak & Palmiter 2005; Vindenes *et al.* 2006; Marquez *et al.* 2009). The middle room was the start room in which the monkey could make a choice before freely moving into one of the other two rooms. We injected the monkeys intramuscularly (i.m.) with increasing doses of morphine during the CPP conditioning and tested the monkeys for CPP 24 hours after the conclusion of the conditioning. About 15 months after the conditioning, CPP was tested again to measure if there was extinction of the morphine-associated memory in the monkeys.

MATERIALS AND METHODS

Subjects

A total of five rhesus monkeys (*Macaca mulatta*) (four males and one female, 10.2 ± 3.5 years old,

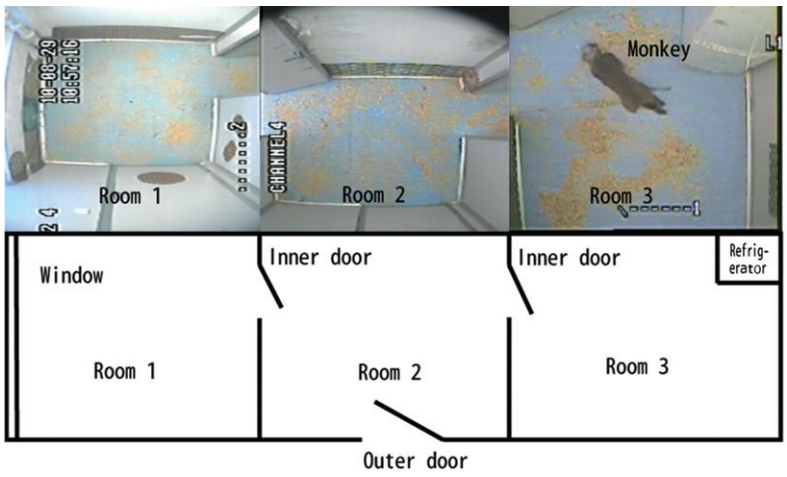

Figure 1 Structure of the conditioned preference place paradigm. There was a window and a refrigerator in room 1 and room 3, respectively. Room 2 was the start room with two inner doors. The monkeys could move freely into the other two rooms through these doors. The upper part of the figure shows the three rooms that were monitored by a camera. Room 3 shows a monkey walking in the room. The scheme of the rooms is shown in the lower part of the figure

7.8 ± 0.4 kg) from the breeding colonies at the Kunming Institute of Zoology (KIZ) were used in the experiments. The monkeys were housed singly under standard conditions (a 12-hour light/dark cycle with light on from 07:00 to 19:00 hours; humidity at 60%, temperature at 21 ± 2°C) in the animal house.

Monkeys received a primate collar under ketamine hydrochloride (5 mg/kg) anaesthesia at least one week before the start of the experiment. Thus, they were habituated to the injection procedure in their home cages before the onset of the CPP conditioning.

The experiments were conducted in accordance with the guidelines for the National Care and Use of Animals approved by the National Animal Research Authority.

CPP

Apparatus

The apparatus was composed of three square rooms connected in a row (Fig. 1). The size of room 1 and 3 was 190 × 190 × 265 cm (l × w × h), room 2 was 210 × 210 × 265 cm. There was a covered window in room 1. Room 2, the middle room (start room), had three doors. One door (90 × 210 cm, w × h) opened to the outside. The other two inner doors were of the same size (65 × 190, w × h) and opened to room 1 and room 3, respectively. There was a refrigerator (50 × 50 × 155 cm, l × w × h) in the corner of room 3. The light was supplied by lamps on the ceiling of each room, thus the brightness was similar in the three rooms. A camera was installed on the top of each room and the monkey's behaviour was recorded by a computer outside the room.

A specifically coloured piece of paper was glued on the wall of each room to provide different visual cues to the monkeys. There was always sawdust on the floors of the rooms. The sawdust was removed if it became soiled by the monkey's excrement and urine, mixed each day after the experiment and totally changed once per week.

Drug

The drug used in the experiments was morphine hydrochloride ($C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$) from Sheng Yang the 1st Medical Company, 10 mg/1 ml per ampule.

Training procedure

Habituation (pre-CPP)

A total of five monkeys were used. The monkeys were individually guided into room 2 (start room) where they were released so that they could freely move in the three rooms for 50 minutes on three consecutive days. Fruit and vegetables were spread across the floors during the habituation but not during CPP conditioning and CPP test. The monkeys usually searched for food in each room and were thus allowed to become completely familiar with the three rooms.

CPP conditioning

As shown in Fig. 2, after a three-day habituation period, the monkeys were trained to form CPP in room 1 or room 3 with the door closed. Since each monkey had a preferred room during the habituation, we chose the room the monkey did not prefer as its drug-paired room during the conditioning in which it stayed for 50 minutes after the morphine injection, while the other room that they preferred during the habituation served as the saline-paired room.

On the first experimental day (D1) of CPP conditioning, the monkeys were individually injected i.m. with 1.5 mg/kg morphine (Liu *et al.* 2005). The dose was then increased to 3 and 4.5 mg/kg on the following alternate days (D3, D5, D7, D9, D11). The highest dose of 4.5 mg/kg was maintained for the last four days. After the injection, the monkeys were guided to stay in the drug-paired room for 50 minutes.

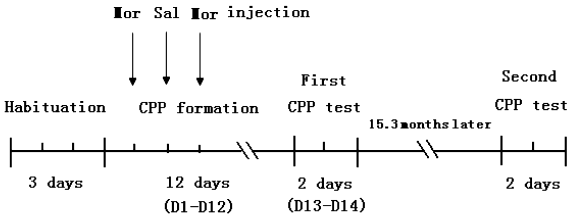

Figure 2 CPP training schedule. Habituation was conducted three days before CPP conditioning. CPP conditioning lasted 12 days (D1–12). The first CPP test was conducted on D13 and D14. After 15.3 ± 1.7 months, the second CPP test was conducted

Starting on day two (D2), one day after the morphine administration, the monkeys received an i.m. saline injection of the same volume as the morphine injection. The monkeys were administered saline on six days that alternated with the morphine days (D2, D4, D6, D8, D10, D12). The monkeys were allowed to stay in the saline-paired room for 50 minutes.

CPP test (post-CPP)

As shown in Fig. 2, after the three days of habituation and 12 days of conditioning, the monkeys were tested for CPP. The testing was conducted 24 hours after the CPP conditioning (post-CPP-0 months) for 30–40 minutes on days 13 (D13) and 14 (D14). The monkeys were tested by releasing them after they had entered the middle room (start room) with the two inner doors opened. Then 15.3 ± 1.7 months after the morphine conditioning was stopped, three monkeys were again tested for CPP (post-CPP-15 months) in order to estimate possible extinction of the morphine-associated memory.

The video tracking was analyzed using a KIZ Smart-lab software and a stopwatch where appropriate. The first room choice, duration of visits and walking distance were recorded for each monkey entering the rooms during 0–5 minutes, 5–20 minutes and 20–30 minutes.

Data analysis

The statistical package SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. The preference scores in duration of visits were expressed by (duration of visits in drug-paired room) / (duration of visits in drug-paired room + duration in saline-paired room) (Portillo & Paredes 2009). The preference scores in the number of visits and walking distance were calculated in the same way. The first room choice and preference scores for moving in each room measured the place preference of each monkey. The number of visits and walking distance, on the other hand, were recorded as an index of locomotor activity.

The difference in the preference scores and locomotor activity between the drug-paired and saline-paired rooms before and after CPP conditioning was assessed for each monkey by a paired *t*-test and analysis of variance analysis. Differences were considered significant when $P \leq 0.05$.

RESULTS

Habituation (pre-CPP)

The monkeys always entered equally the three rooms to find food at the beginning of adaptation. After the food was eaten, they preferred to stay a longer time in one room than in any of the other rooms. Thus, finally, each monkey had his favourite room. Two monkeys preferred to stay in room 1, the other three monkeys preferred room 3.

The room the monkeys first entered from the start room was usually their preferred room. The percentage of the first choice being the preferred room was significantly higher than that for the opposite room. The statistical result is shown in Table 1.

Similarly, the monkeys had higher preference scores with respect to the duration of visits, number of visits, and walking distance during 0–5 minutes, 5–20 minutes, total 20 and 30 minutes for the preferred room than the non-preferred rooms (Table 1). We chose their non-preferred room as the drug-paired room and the preferred room as the saline-paired room.

CPP test (post-CPP)

The result showed that 24 hours after the conditioning, all of the monkeys preferred the drug-paired room, which was not their favourite room during the adaptation period. The percentage of the drug-paired environment as the first choice increased. In addition, after 15.3 ± 1.7 months, three monkeys still preferred the drug-paired room as their first choice (Fig. 3, Table 1).

When CPP was tested 24 hour (post-CPP-0 months) or 15.3 ± 1.7 months (post-CPP-15 months) after CPP conditioning, the preference scores for duration of visits (Fig. 4a,d), number of visits (Fig. 4b,e) and walking distance (Fig. 4c,f) in the drug-paired room during post-CPP were higher than during pre-CPP (Table 1).

The monkeys would also stay in the middle room (start room) during the CPP test. As shown in Fig. 5a,b, the duration the monkey stayed in the drug-paired room was longer than that spent in the saline-paired room within the recorded durations (Table 2). Similarly, when CPP was tested 24 hours after the CPP conditioning, the monkey stayed longer in the drug-paired room than in the middle room. However, 15 months after the CPP conditioning, there was no difference between the times spent in the drug-paired room and in the middle room.

Table 1 Statistical results in pre-CPP and post-CPP.

	0–5 minutes	*5–20 minutes*	*20–30 minutes*	*0–20 minutes*	*0–30 minutes*
Pre-CPP (scores in preferred versus non-preferred room, *P* values)					
% first choice	0.005				
% duration of visits	0.002	0.029		0.013	0.034
% number of visits	0.031	0.053		n.s.	n.s.
% walking distance	0.002	0.027		0.026	n.s.
Post-CPP-0 months (scores during post-CPP versus pre-CPP, *P* values)					
% first choice	0.0002				
% duration of visits	< 0.001	0.001	0.002	< 0.001	0.001
% number of visits	< 0.001	< 0.001	0.004	0.001	0.003
% walking distance	< 0.001	0.001	0.008	0.001	0.003
Post-CPP-15 months (Scores during post-CPP versus pre-CPP, *P* values)					
% first choice	0.036				
% duration of visits	< 0.001	0.012	0.028	0.002	0.006
% number of visits	0.002	0.038	n.s.	0.023	n.s.
% walking distance	< 0.001	0.017	n.s.	0.007	0.035

n.s. = not significant.

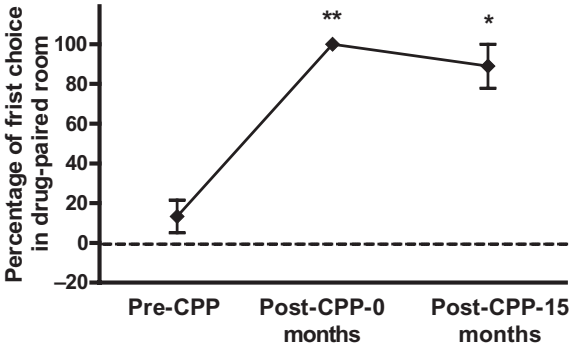

Figure 3 The percentage of the first choice visits of the monkeys to the drug-paired room before (pre-CPP) and after CPP conditioning (post-CPP). Data are expressed as an average of the first entries over three days during pre-CPP and over two days during post-CPP. $^{*}P < 0.05$, $^{**}P < 0.01$ percentage of the first choice visits to the drug-paired room during post-CPP versus pre-CPP. Post-CPP-0 months and post-CPP-15 months represent 24 hours and 15.3 ± 1.7 months after CPP conditioning, respectively

Locomotor activity

Number of visits

When CPP was tested 24 hours after the CPP conditioning, the monkeys visited the drug-paired room more often than the saline-paired room but the difference was not significant partly because the total number of visits decreased after the CPP conditioning. However, 15.3 ± 1.7 months after the CPP conditioning, the monkeys entered the drug-paired room more often than the saline-paired room during 0–20 minutes and 0–30 minutes (Fig. 6a, Table 3).

When the total number of the monkeys' visits to the three rooms was analyzed, there was no difference between the total number of visits during pre-CPP and post-CPP-0 months or post-CPP-15 months, although the monkeys paid fewer visits after the morphine injections because they preferred to stay in the drug-paired room without moving around.

Walking distance

When CPP was tested 24 hours or 15.3 ± 1.7 months after the CPP conditioning, the monkeys walked a longer distance in the drug-paired room than in the saline-paired room during 0–20 minutes and 0–30 minutes. They moved less in the start room than in the drug-paired room during the post-CPP-0 months test within 0–20 minutes and 0–30 minutes. However, the monkeys tended to walk a longer distance in the start room 15.3 ± 1.7 months after the CPP conditioning, but the difference was not significant when compared with the distance in the drug-paired room (Fig. 6b, Table 3).

When analyzing the total distance of the monkeys walking in the three rooms, there was no difference between pre-CPP and post-CPP-0 months or post-CPP-15 months.

DISCUSSION

Our data show that monkeys can establish a CPP associated with morphine at an increasing dose. During the habituation (pre-CPP), the rhesus monkeys usually stayed in their preferred room. After the monkeys had been injected with morphine (1.5, 3, 4.5 mg/kg) in six days (every other day), they changed their preferred environment to that associated with the morphine treatment. This suggested that the monkeys had formed a morphine

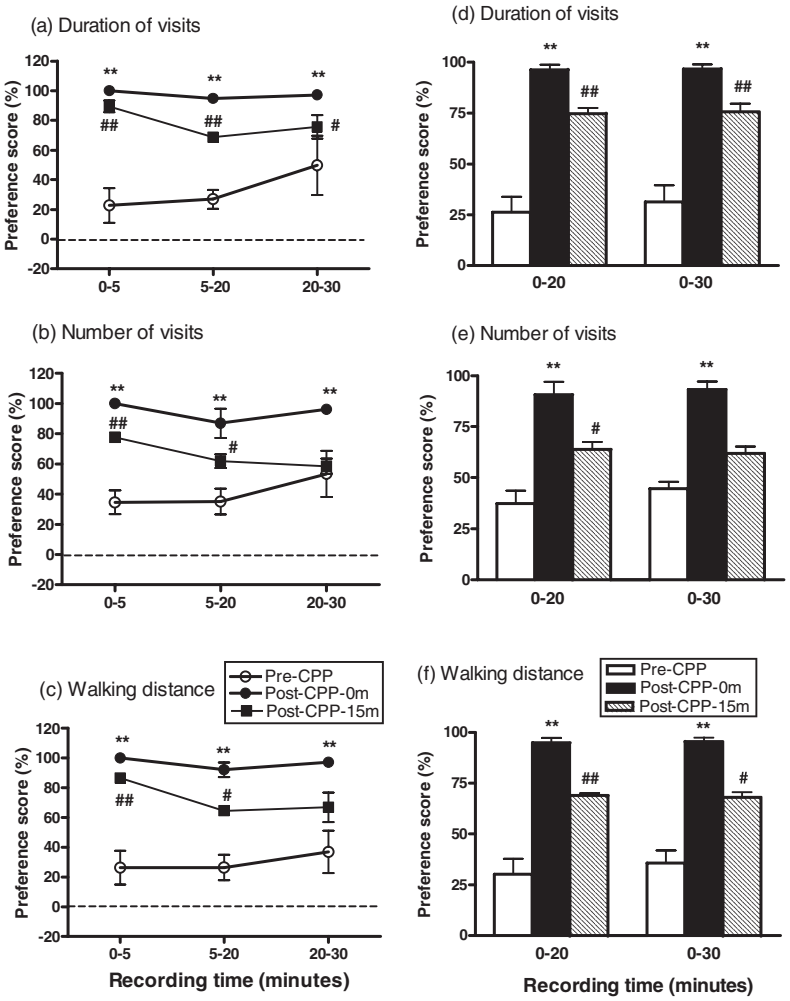

Figure 4 The preference scores for the monkeys displayed by the duration of visits (a, d), number of visits (b, e) and walking distance (c, f) in the drug-paired room within 0–5, 5–20, 20–30, 0–20 and 0–30 minutes before (pre-CPP) and after CPP conditioning (post-CPP). $^{**}P < 0.01$ when comparing the scores before CPP (pre-CPP) with the scores 24 hours after CPP (post-CPP-0 months), $\#P < 0.05$, $\#\#P < 0.01$: scores in pre-CPP versus post-CPP-15 months (15.3 ± 1.7 months after CPP formation)

associated memory. This memory lasted at least 15.3 ± 1.7 months after the conditioning. This finding was indicated by the higher preference scores for the drug-paired than saline-paired room when CPP was tested both 24 hours and 15.3 ± 1.7 months after the CPP conditioning.

The monkeys also stayed in the middle room, which was the start room during the habituation and CPP test. However, when they were tested for CPP 24 hours after its formation, they were found to spend less time and walk less in the start room as compared with the drug-paired room. It was interesting to find that when CPP was tested 15.3 ± 1.7 months after its formation, the monkeys spent about as much time and walked about the same distance in the start room as in the drug-paired room. We assumed that the monkeys were eager to search for the drug in the drug-paired room shortly after the CPP formation. However, after a long period of time (about 15 months), they might have tried to avoid the negative feeling of not being able to get the drug by staying in the middle room.

Since the monkeys had to cross the middle room to get either to the drug-paired or saline-paired room, the number of visits to the start room was high if the monkeys kept going to all three rooms. This was obvious during the habituation and the CPP test 15.3 ± 1.7 months after the CPP conditioning. The number of visits to the rooms tended to be low during post-CPP-0 months, while the walking distance did not decrease, suggesting that the monkeys preferred to stay in the morphine-associated room after the CPP conditioning without a change in locomotor activity.

Dependence to morphine can be produced by injection of increasing doses of morphine. We chose to escalate the morphine doses according to a previous study in our laboratory (Liu *et al.* 2005) and other references (Listos *et al.* 2008). Our previous study showed that morphine injection with increasing doses (0.5, 1.6, 5.0 and 8.0 mg/kg, for 10 days) changed the electroencephalogram recorded in the orbitofrontal cortex and dorsolateral prefrontal cortex (DLPFC) in rhesus monkeys (Liu *et al.* 2005). In the current experiment, we used lower

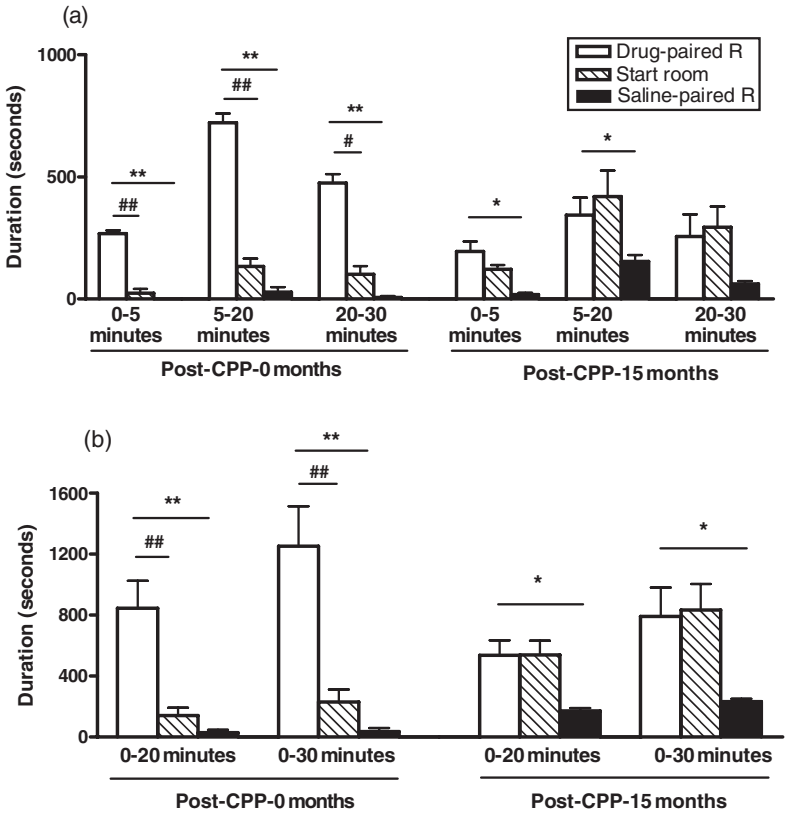

Figure 5 The duration of the visits of the monkeys in the drug-paired room (Drug-paired R), start room and saline-paired room (Saline-paired R) during the CPP test 24 hours (post-CPP-0 months) and 15.3 ± 1.7 months (post-CPP-15 months) after conditioning (a, b). $*P < 0.05$, $**P < 0.01$ when comparing duration in the drug-paired room with duration in the saline-paired room. $\#P < 0.05$, $\#\#P < 0.01$, when comparing duration in the drug-paired room with duration in the start room

Table 2 Statistical results of the duration (or time spent) in the three rooms.

	0–5 minutes	*5–20 minutes*	*20–30 minutes*	*0–20 minutes*	*0–30 minutes*
Duration in drug-paired versus saline-paired room (*P* values)					
Post-CPP-0 months	< 0.001	0.001	0.002	0.001	0.002
Post-CPP-15 months	0.029	0.026	n.s.	0.024	0.046
Duration in drug-paired versus middle room (*P* values)					
Post-CPP-0 months	< 0.001	0.009	0.02	0.008	0.01
Post-CPP-15 months	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. = not significant.

doses of morphine (1.5, 3.0 and 4.5 mg/kg) in order to prevent the monkeys from long-lasting physiological effects. No significant withdrawal behaviour was observed in the monkeys after the morphine treatment was stopped except for a slight increase in aggressiveness for a few weeks. During CPP conditioning, on the other hand, the monkeys always displayed sedative symptoms after the morphine injection, reflected by lying down in a relaxed way in the centre of the drug-paired room. After the saline injection, the monkeys sat peacefully on the floor or walked leisurely around the saline-paired room.

In conclusion, we found that with an increasing dose of morphine treatment, rhesus monkeys could establish CPP that lasted at least 15.3 ± 1.7 months. We interpret this finding to indicate that rhesus monkeys can form long-term morphine-associated memory.

The demonstration of along-lasting morphine-induced CPP in a primate species is a good basis for further investigations on the neurobiological and pharmacological mechanisms involved in the acquisition, maintenance and potential decay of such conditioned rewarding effects and their relevance to human drug addiction and relapse.

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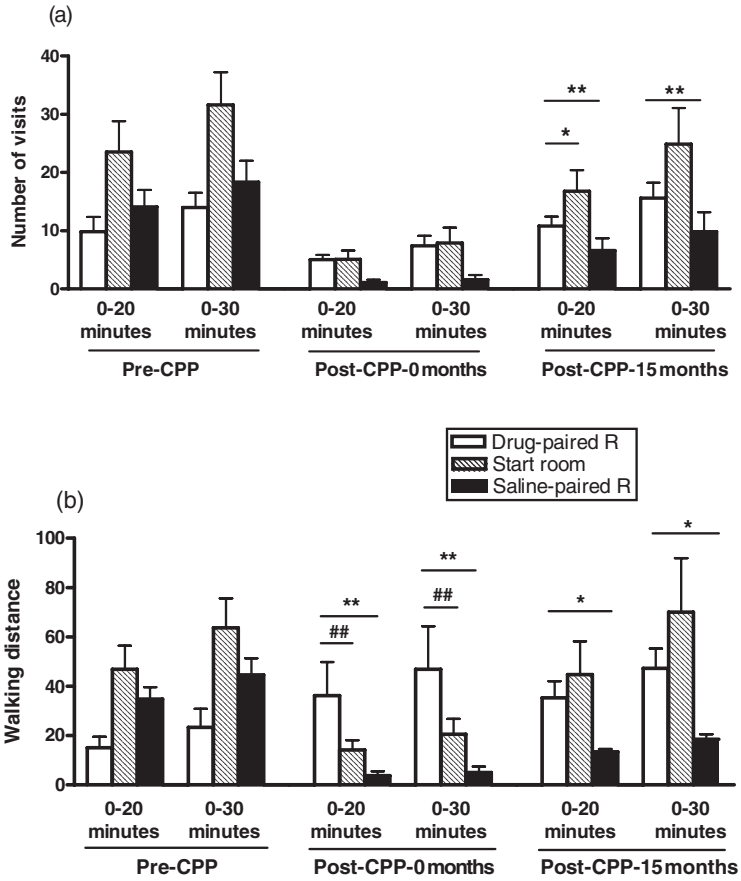

Figure 6 The number of visits (a) to and walking distance (b) of monkeys in the drug-paired room (Drug-paired R), start room and saline-paired room (Saline-paired R) during the CPP test 24 hours (post-CPP-0 months) and 15.3 ± 1.7 months (post-CPP-15 months) after conditioning. $*P < 0.05$, $**P < 0.01$ when comparing duration in the drug-paired room with duration in the saline-paired room. $\#P < 0.05$, $\#\#P < 0.01$ when comparing duration in the drug-paired room with duration in the start room

Table 3 Statistical results of locomotor activity.

	0–20 minutes	*0–30 minutes*
Number of visits in drug-paired versus saline-paired room (*P* values)		
Pre-CPP	n.s.	n.s.
Post-CPP-0 m	n.s.	n.s.
Post-CPP-15 months	0.0065	0.007
Walking distance in drug-paired versus saline-paired room (*P* values)		
Pre-CPP	n.s.	n.s.
Post-CPP-0 months	0.009	0.01
Post-CPP-15 months	0.03	0.02
Walking distance in drug-paired versus middle room (*P* values)		
Pre-CPP	n.s.	n.s.
Post-CPP-0 months	0.01	0.008
Post-CPP-15 months	n.s.	n.s.

n.s. = not significant.

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Authors Contribution

Wang JH wrote the manuscript, designed the paradigm with Ma YY and Hu XT and performed the experiment with other contributors. Wu XJ, Li CY, Wei JK, Jiang HH, Liu CR, Yu CY, Ma H and Duan W collected the data. SC contributed to the manuscript by collaboration and also provided helpful suggestions and revisions on the manuscript.

References

Aguilar MA, Rodriguez-Arias M, Minarro J (2009) Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. Brain Res Rev 59:253–277.

Foltin RW, Evans SM (2001) Location preference related to smoked heroin self-administration by rhesus monkeys. Psychopharmacology 155:419–425.

Hnasko TS, Sotak BN, Palmiter RD (2005) Morphine reward in dopamine-deficient mice. Nature 438:854–857.

Listos J, Talarek S, Fidecka S (2008) Involvement of adenosine receptor agonists on the development of hypersensitivity to acute dose of morphine during morphine withdrawal period. Pharmacol Rep 60:679–685.

Liu N, Liu Y, Fan Y, Yu H, Wilson FA, Ma Y, Hu X (2005) EEG activities in the orbitofrontal cortex and dorsolateral

prefrontal cortex during the development of morphine dependence, tolerance and withdrawal in rhesus monkeys. Brain Res 1053:137–145.

Marquez P, Bebawy D, Lelievre V, Coute AC, Evans CJ, Waschek JA, Lutfy K (2009) The role of endogenous PACAP in motor stimulation and conditioned place preference induced by morphine in mice. Psychopharmacology 204:457–463.

Portillo W, Paredes RG (2009) Conditioned place preference induced by morphine in non-copulating male rats. Behav Brain Res 203:308–311.

Rice OV, Gordon N, Gifford AN (2002) Conditioned place preference to morphine in cannabinoid CB1 receptor knockout mice. Brain Res 945:135–138.

Tan H, Liu N, Wilson FA, Ma Y (2007) Effects of scopolamine on morphine-induced conditioned place preference in mice. Addict Biol 12:463–469.

Tzschentke TM (2007) Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 12:227–462.

Vindenes V, Handal M, Ripel A, Boix F, Morland J (2006) Conditioned place preference induced by morphine and morphine-6-glucuronide in mice. Pharmacol Biochem Behav 85:292–297.