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Pharmacology, Biochemistry and Behavior 74 (2002) 187–197

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Involvement of dopamine D2 receptors of the central amygdala on the acquisition and expression of morphine-induced place preference in rat

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Received 14 February 2002; received in revised form 10 June 2002; accepted 9 August 2002

Abstract

In the present study, the effects of intra-central amygdala (CeA) injections of dopamine (DA) D2-like receptor agonist and antagonist on the acquisition and expression of morphine-induced place preference in male Wistar rats have been investigated. Subcutaneous administration of different doses of morphine sulphate (0.5–10 mg/kg) produced a dose-dependent conditioned place preference (CPP). Using a 3-day schedule of conditioning, it was found that the DA D2/D3 receptor agonist, quinpirole (0.3–3 μg/rat), or the DA D2 receptor antagonist, sulpiride (0.04–5 μg/rat), did not produce a significant place preference or place aversion. Intra-CeA administration of quinpirole (0.3 and 1 μg/rat) with an ineffective dose of morphine (0.5 mg/kg) elicited a significant CPP. On the other hand, quinpirole (0.3 μg/rat) injection into the CeA induced CPP in combination with the lower doses of morphine (0.5 and 2.5 mg/kg), but decreased the response of higher dose (7.5 mg/kg) of morphine. This response of quinpirole was attenuated by sulpiride (0.2 μg/rat). Sulpiride by itself (0.04–5 μg/rat) reduced the acquisition of morphine (7.5 mg/kg)-induced place preference. The administration of the higher dose of sulpiride (1 and 5 μg/rat) or the higher dose of quinpirole (3 μg/rat) during acquisition decreased the locomotor activity of the animals on the testing days. The injection of the low dose of quinpirole (0.3 μg/rat) on the test day reduced the expression of morphine-induced CPP, but the high dose of quinpirole (3 μg/rat) potentiated this expression. The administration of sulpiride (5 μg/rat) attenuated the quinpirole response. The injection of sulpiride (1 and 5 μg/rat) abolished the expression of morphine-induced CPP. It is concluded that the CeA DA D2-like receptors may play an active role in morphine reward.

Keywords: Dopamine D2 receptors; Morphine; Quinpirole; Sulpiride; Conditioned place preference; Central amygdala; Rat

1. Introduction

Some conditioned place preference (CPP) studies suggest that the amygdala may play an important role in the reward produced by drugs of abuse (Brown and Fibiger, 1993; O'Dell et al., 1999; Lu et al., 2000). Among the diverse nuclei of the amygdala, it appears that the central amygdala (CeA) is involved in arousal, expression of emotions and forming associations between environmental stimuli and affective states, typically involving autonomic responses (Kaada, 1972; Kapp et al., 1992). The CeA is a part of the extended amygdala that connects anatomically with the nucleus accumbens (Nac) and receives dense dopaminergic afferents from the ventral tegmental area (VTA). It also has more DA terminals relative to other amygdaloid nuclei (Ungerstedt, 1971; Ben-Ari et al., 1975; Kilts and Anderson, 1987; Woodward et al., 1999). Dopamine (DA) exerts its action by binding to specific membrane receptors (Gingrich and Caron, 1993). The DA receptor subtypes are divided into two major subclasses: D1-like (D1 and D5) and D2-like (D2, D3 and D4) (see Vallone et al., 2000; Jaber et al., 1996). The distribution of D1 and D2 DA receptors in the nuclei of the rat amygdaloid complex estimated by quantitative light microscopy has the highest density of [^{125}I]iodosulpiride (DA D2 receptor antagonist) binding sites in the CeA (Scibilia et al., 1992). Considering the anatomy and functions of the CeA, it is likely that it influences the mesocorticolimbic dopaminergic system (originating with cell bodies in the VTA that

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PII: S0091-3057(02)00989-9

project to the Nac) that mediates opiate reward (Di Chiara and North, 1992; Koob et al., 1993; Naranjo et al., 2001). Numerous investigations suggest that VTA and Nac are the key sites mediating the reinforcing actions of opiates (Wise, 1998; McBride et al., 1999). As the CeA also projects back to the VTA and the Nac, it is likely to have an important role in the control of motivation and the effects of drug conditioned cues (Wallace et al., 1992; Woodward et al., 1999). It appears that opiates have more than one site of rewarding action, which may include the VTA (Tsuji et al., 1996; Jaeger and van der Kooy, 1996; Olmstead and Franklin, 1997a,b), the Nac (Laviolette et al., 2002; Tolliver et al., 2000), the hippocampus (Corrigall and Linseman, 1988; Lu et al., 2000), the periaqueductal gray (Motta and Brandao, 1993) and the amygdala (Kelsy and Amold, 1994; Lu et al., 2000).

It has long been known that morphine induces a conditioned preference for the place in which it has been administered in rats (Shippenberg et al., 1993; Popik and Danysz, 1997) and DA receptors appear to play an important role in this response (De Fonseca et al., 1995; Tzschentke, 1998). On the basis of the DA receptor distribution in the CeA, it has been suggested that the functions of the extended amygdala might be regulated by DA afferents at multiple key sites of D2 receptor action (Scibilia et al., 1992). The purpose of the present study is to investigate the role of DA D2 receptors of the CeA on the acquisition and expression of morphine-induced place preference.

2. Materials and methods

2.1. Animals

Male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 240–280 g at the time of the surgery were used. The animals were housed four per cage, in a colony room with a 12/12-h light/dark cycle (7:00–19:00 h lights on) at 22 ± 1 °C. They had free access to food and tap water except during the time of experiments. All animals were allowed to adapt to the laboratory conditions for at least 1 week before surgery and were handled for 5 min/day during this adaptation period. Each animal was used once only. Seven animals were used in each group of experiments. The experiments were carried out during the light phase of the cycle. The experimental protocol was approved by the Research and Ethics Committee of the Faculty of Science, Tehran University (200, 11 July 2000).

2.2. Surgical and infusion procedures

The animals were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg ip) and positioned in a kopf stereotaxic instrument. The skin was incised and the skull was cleaned. Two 23-gauge guide cannulae made of stainless steel tubing were secured by acrylic dental cement and anchored to stainless steel screws fixed to the skull. Stainless steel cannulae were implanted 1 mm above the CeA based on the Atlas of Paxinos and Watson (1986). The final coordinates, determined were as follows: A = − 2.2, L = ± 4.1, V = − 7.8. To prevent clogging, the stainless steel stylets (30 gauge) were placed in the guide cannulae until the animals were given the CeA injection. The animals were allowed 7 days to recover before place conditioning processes.

For drug infusion, the stylets were withdrawn and replaced by the injection units (30-gauge stainless steel tubing), terminating 1 mm below the tip of the guides. Each injection unit has been connected by polyethylene tubing to 1-μl Hamilton syringe. The left and right CeA were infused with a 0.5-μl solution on each side (1 μl/rat) over a 60-s period. The cannulae were left in place an additional 60 s to allow diffusion, then the stylets were reinserted into the guide cannulae. During the infusion procedure, the experimenter loosely held the animals.

2.3. Apparatus

The place conditioning apparatus is based on that used by Carr and White (1983) with a minor modification and consisted of three wooden compartments. Two of the compartments were identical in size (40 × 30 × 30 cm), but one of them was white with a smooth floor and the other was black with vertical white stripes, 3 cm wide and also had a textured floor. The third compartment was a red tunnel (40 × 15 × 30 cm) and it was protruded from the rear of the two large compartments and connected the entrances to them. A guillotine door separated the compartments.

2.4. Behavioral testing

2.4.1. Place conditioning

CPP was conducted using a minor modification of a biased procedure according to the method of De Fonseca et al. (1995). It was consisted of a 5-day schedule with three distinct phases: preconditioning, conditioning and testing.

2.4.1.1. Preconditioning. On Day 1, the animals were placed in the middle of the apparatus and they were allowed to freely explore the three compartments for the next 15 min. The time spent by the animals in each compartment was recorded. All the animals preferred the black compartment with white stripes (i.e., they spent over 80% of the time on that side) and were conditioned to the other side (white compartment).

2.4.1.2. Conditioning. This was conducted during 3 days and included two sessions each day. The animals were conditioned for 45 min in the white compartment immediately after subcutaneous administration of morphine sulfate or intra-CeA injection of DA D2 receptor agonist and antagonist at 9:00–11:00 h. After 6 h, the animals received a single subcutaneous injection of saline and were placed for 45 min in the other (black) compartment. The animals were

confined to one compartment by closing the guillotine doors during these sessions. On the second day of conditioning, the animals received the saline injections in the morning session and the drug administration in the evening session. The third day of conditioning had the same schedule as the first one. This schedule has been chosen for avoiding circadian variability (morning/evening) (De Fonseca et al., 1995).

2.4.1.3. Testing. The testing phase was carried out on day 5. As in the preconditioning phase, the guillotine door was raised and the animals had free choice in the apparatus for 15 min. Then the time spent in the white compartment was recorded for each animal and the change of preference was calculated as the difference (in seconds) between the time spent in the drug-paired compartment (white compartment) on the testing day, and the time spent in this compartment in the preconditioning session. The position of the animal was defined by the position of its forelimbs and head.

2.4.2. Locomotor testing

Locomotor activity in the two main compartments was measured during the testing phase. For this purpose, the ground area of white and black compartments was divided into four equal-sized squares. Locomotion was measured as the number of crossings from one square to another during 15 min.

2.5. Drugs

The drugs used in the present study were morphine sulfate (Temad, Tehran, Iran), quinpirole and sulpiride (Sigma, St. Louis, CA, USA). All drugs were dissolved in sterile 0.9% saline just before the experiment, except for sulpiride that dissolved in one drop of glacial acetic acid and made up to a volume of 5 ml with sterile 0.9% saline and then diluted to the required concentration. Quinpirole and sulpiride were administered intra-CeA and morphine was injected subcutaneously. The control animals received either saline or vehicle.

2.6. Drug treatments

2.6.1. Experiment 1. Dose–response curve for morphine-induced place preference

In this experiment, we established a dose response function for morphine place conditioning. Different doses of morphine (0.5, 2.5, 5, 7.5 and 10 mg/kg sc) were tested for their ability to produce a place conditioning. Five groups of animals were injected with morphine and saline (subcutaneously) on alternate sessions. A separate group of animals was given saline (subcutaneously) only during the conditioning phase in order to confirm that the injections and the conditioning schedule were not affecting the time allotment in the apparatus. This group was used as control. Locomotor activity was also measured in the testing phase.

2.6.2. Experiment 2. Effects of quinpirole with or without morphine on the acquisition of CPP

2.6.2.1. Effect of quinpirole on the acquisition of CPP. Three doses of the DA D2 receptor agonist, quinpirole (0.3, 1 and 3 μg/rat, intra-CeA) were given to three groups of the animals just before the administration of saline (1 ml/kg sc) during the conditioning phase. One additional group received saline (1 μl/rat, intra-CeA), just before saline (1 ml/kg sc) during the conditioning phase and served as a control. All groups were tested 24 h after the conditioning sessions, with no preceding injection. Locomotor activity was also measured in the testing phase.

2.6.2.2. Effect of quinpirole on the acquisition of morphine-induced CPP. Four groups of animals received saline (1 μl/rat, intra-CeA) or quinpirole (0.3, 1 and 3 μg/rat, intra-CeA), immediately before the administration of morphine (0.5 mg/kg sc), during the conditioning sessions. The animals were tested 24 h after the last conditioning session, with no preceding injection. Locomotor activity was also measured during testing.

2.6.3. Experiment 3. Effects of sulpiride with or without morphine on the acquisition of CPP

2.6.3.1. Effect of sulpiride on the acquisition of CPP. The ability of sulpiride (0.04, 0.2, 1 and 5 μg/rat, intra-CeA) on place conditioning under the 3-day schedule was tested in four groups of animals. An additional group received vehicle (1 μl/rat, intra-CeA) plus saline (1 ml/kg sc) during the conditioning phase and was used as control. Locomotor activity was also evaluated during testing.

2.6.3.2. Effect of sulpiride on the acquisition of morphine-induced CPP. Five groups of animals were injected with different doses of sulpiride (0.04, 0.2, 1, and 5 μg/rat, intra-CeA) or vehicle (1 μl/rat, intra-CeA) immediately before morphine administration (7.5 mg/kg sc) during the conditioning sessions. All animals were tested 24 h after the last conditioning session, with no preceding injection. Locomotor activity was also measured during the testing phase.

2.6.4. Experiment 4. Effects of sulpiride on quinpirole response during morphine conditioning

We used different doses of quinpirole (0.3, 1 and 3 μg/rat) with one dose of morphine (0.5 mg/kg) that could not produce a significant CPP alone. Thus, eight groups of seven animals received an intra-CeA injection of vehicle (1 μl/rat) or the DA D2 receptor antagonist, sulpiride (0.2 μg/rat). After 5 min, they were injected by either saline (1 μl/rat, intra-CeA) or quinpirole (0.3, 1 and 3 μg/rat, intra-CeA). Finally, after 5 min, they received morphine (0.5 mg/kg sc) or saline (1 ml/kg sc) during the conditioning phase. All animals were tested 24 h after the last conditioning session,

with no preceding injection. During testing, the locomotor activity of the animals was measured.

2.6.5. Experiment 5. Effects of quinpirole with or without sulpiride on the acquisition of morphine-induced CPP

One dose of quinpirole in combination with different doses of morphine was used in this experiment. Five groups of animals received vehicle (1 μl/rat, intra-CeA) followed by saline (1 μl/rat, intra-CeA) injections with a 5-min interval before either morphine (0.5, 2.5, 5 and 7.5 mg/kg sc) or saline (1 ml/kg sc) during the conditioning phase. Another five groups of the animals received an intra-CeA injection of vehicle (1 μl/rat, intra-CeA) 5 min before an intra-CeA injection of quinpirole (0.3 μg/rat, intra-CeA) and then were injected by either morphine (0.5, 2.5, 5 and 7.5 mg/kg sc) or saline (1 ml/kg sc) during conditioning phase. A further five groups were pretreated with sulpiride (0.2 μg/rat, intra-CeA) and, after 5 min, they received a dose of quinpirole (0.3 μg/rat, intra-CeA) before either morphine (0.5, 2.5, 5 and 7.5 mg/kg sc) or saline (1 ml/kg sc) injection during the conditioning phase. All animals were tested 24 h after the last conditioning session, with no preceding injection. Locomotor activity was evaluated during testing.

2.6.6. Experiment 6. Effects of quinpirole or sulpiride on the expression of morphine-induced CPP

Nine groups of animals underwent the experimental procedure of place conditioning with morphine (7.5 mg/kg sc). On the 5th day, 5 min before testing, seven groups were injected with quinpirole (0.3, 1 and 3 μg/rat, intra-CeA) or sulpiride (0.04, 0.2, 1 and 5 μg/rat, intra-CeA) and two other control groups received either saline (1 μl/rat, intra-CeA) or vehicle (1 μl/rat, intra-CeA). Locomotor activity was also evaluated during testing in this group.

2.6.7. Experiment 7. Effects of quinpirole with or without sulpiride on the expression of morphine-induced CPP

Eight groups of animals underwent the experimental procedure of place conditioning with morphine (7.5 mg/kg sc). On the 5th day, 5 min before testing, they received an intra-CeA injection of vehicle (1 μl/rat) or sulpiride (5 μg/rat) and, after 5 min, they were injected with either saline (1 μl/rat, intra-CeA) or quinpirole (0.3, 1 and 3 μg/rat, intra-CeA). Locomotor activity was measured during testing.

2.7. Histology

After completion of the experimental sessions, each animal was given a lethal dose of sodium pentobarbital and transcardically perfused with a phosphate-buffered saline solution (pH 7.4), followed by 10% formalin. The brains were removed, blocked and cut coronally in 40-μm sections through both cannulae placements. The tissues were stained with cresyl violet to determine the injection locations.

2.8. Statistical analysis

In all experiments, the conditioning scores are expressed as differences in the time spent on the drug-associated side between the preconditioning and the testing phases. Locomotor activities are expressed as crossing of lines in both of the main compartments during the testing phase. Data are expressed as mean ± S.E.M. ($n = 7$). Analysis of data was performed using one-way or two-way ANOVA. Following a significant F-value, post-hoc analyses (Tukey's test) were performed for assessing specific group comparisons. The level of statistical significance was set at $P < .05$.

3. Results

3.1. Histology

Fig. 1 illustrates the approximate point of the drug injections in the CeA. The histological results were plotted

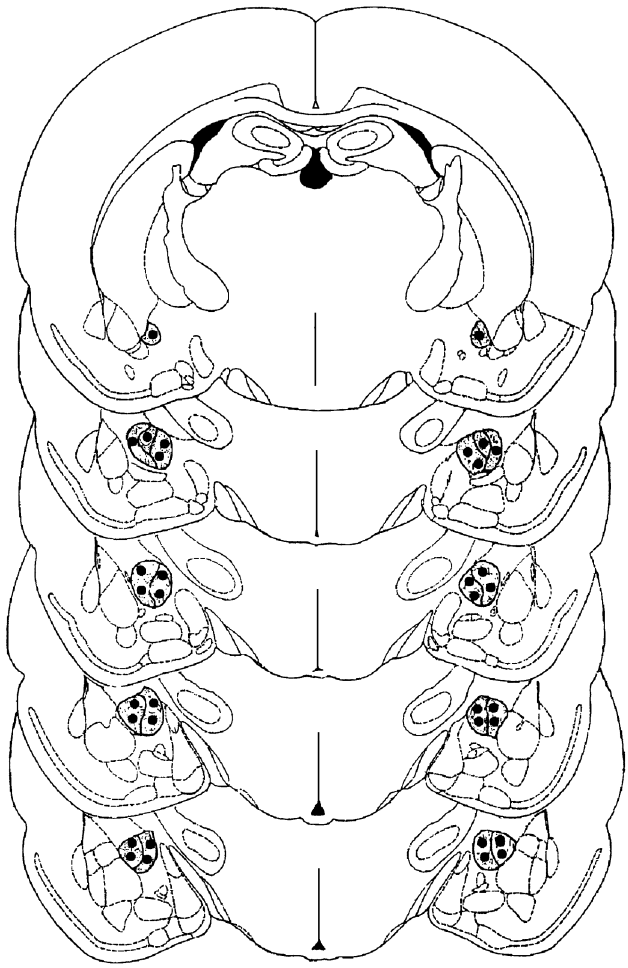

Fig. 1. The approximate placements of injection cannulae within the central amygdala were indicated by the circles. Representative sections of the central amygdala (−1.8, −2.12, −2.3, −2.56 and −2.8 mm from bregma) were taken from the rat brain atlas of Paxinos and Watson (1986).

on representative sections taken from the rat brain atlas of Paxinos and Watson (1986). Data from the animals with injection sites located outside the CeA were not used in the analysis.

3.2. Experiment 1. Dose–response curve for morphine-induced place preference

The conditioning treatments with morphine induced a CPP for the drug-associated place (Fig. 2A). One-way ANOVA revealed that morphine caused a significant dose-related preference [$F(5,36)=18.7$, $P<.0001$]. Significant conditioning was observed at doses of 5, 7.5 and 10 mg/kg. The maximum response was obtained with 7.5 mg/kg of morphine. No significant effect was observed for locomotor activity in the testing phase [$F(5,36)=0.1$, $P>.05$] (Fig. 2B).

3.3. Experiment 2. Effects of quinpirole with or without morphine on the acquisition of CPP

Fig. 3A shows the effects of bilateral intra-CeA injection of quinpirole in the absence or presence of morphine on the acquisition of CPP. Data were analyzed by a two-way ANOVA. The results indicated a significant main effect of treatment [$F(1,48)=134.9$, $P<.0001$], dose [$F(3,48)=15.2$, $P<.0001$] and Treatment × Dose interaction [$F(3,48)=22.1$, $P<.0001$]. In addition, one-way ANOVA revealed that the lower dose of morphine (0.5 mg/kg) and quinpirole (0.3, 1 and 3 μg/rat, intra-CeA) alone did not induce a significant place preference [$F(4,31)=1.9$, $P>.05$]. Furthermore, the lower and middle doses of quinpirole (0.3 and 1 μg/rat, intra-CeA) potentiated the morphine (0.5 mg/kg)-induced place preference [$F(3,24)=29.3$, $P<.0001$].

Fig. 3B illustrates the effect of the drugs on the locomotor activity in the testing phase. Two-way ANOVA indicated no significant effect of dose [$F(3,48)=2.3$, $P>.05$], treatment [$F(1,48)=2.2$, $P>.05$] as well as the Treatment × Dose interaction [$F(3,48)=1.7$, $P>.05$]. Quinpirole (0.3, 1 and 3 μg/rat) alone had no effect on the locomotor activity [one-way ANOVA: $F(3,24)=0.14$, $P>.05$], but in combination with morphine, reduced the locomotor activity [one-way ANOVA: $F(3,24)=4.5$, $P<.01$].

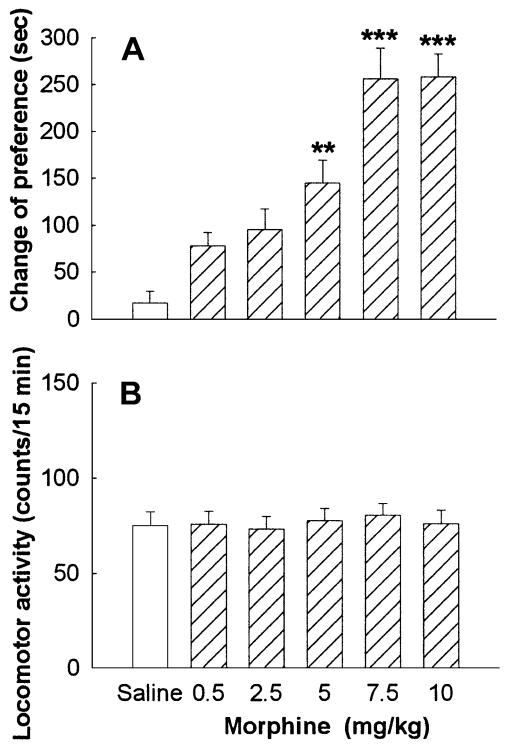

Fig. 2. Place preference produced by morphine. Different doses of morphine (0.5, 2.5, 5, 7.5 and 10 mg/kg) or saline (1 ml/kg) were administered subcutaneously in a 3-day schedule of conditioning. On the testing day, the animals were observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the preconditioning session (Panel A). The locomotor activity was assessed as described in the Materials and methods section (Panel B). Data are expressed as mean ± S.E.M. of seven animals per group. $**P<.01$, $***P<.001$ different from the saline control group.

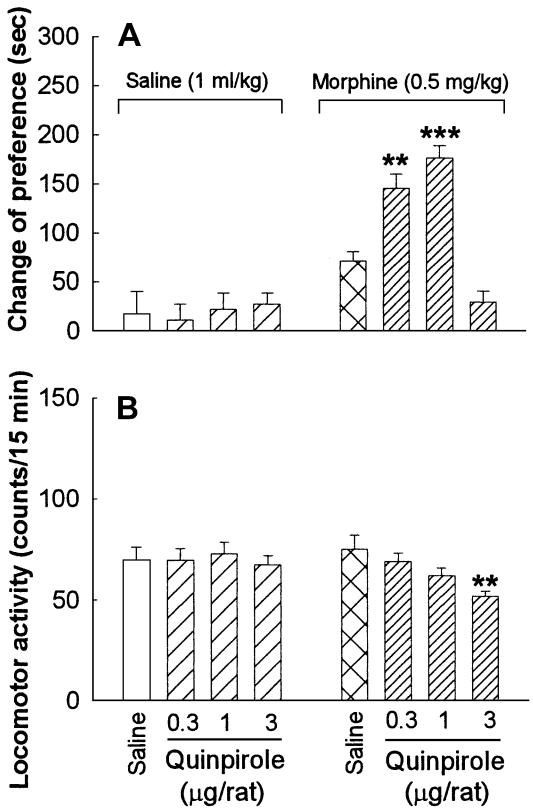

Fig. 3. The effects of bilateral intra-CeA injection of quinpirole, either alone or in combination with morphine, on the acquisition of a conditioned place preference. The animals received quinpirole (0.3, 1 and 3 μg/rat) or saline (1 μl/rat) with or without morphine (0.5 mg/kg sc), in a 3-day schedule of conditioning. On the test day, the animals were observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the preconditioning session (Panel A). The locomotor activity was assessed as described in the Materials and methods section (Panel B). Data are expressed as mean ± S.E.M. of seven animals per group. $**P<.01$, $***P<.001$ different from the saline control group.

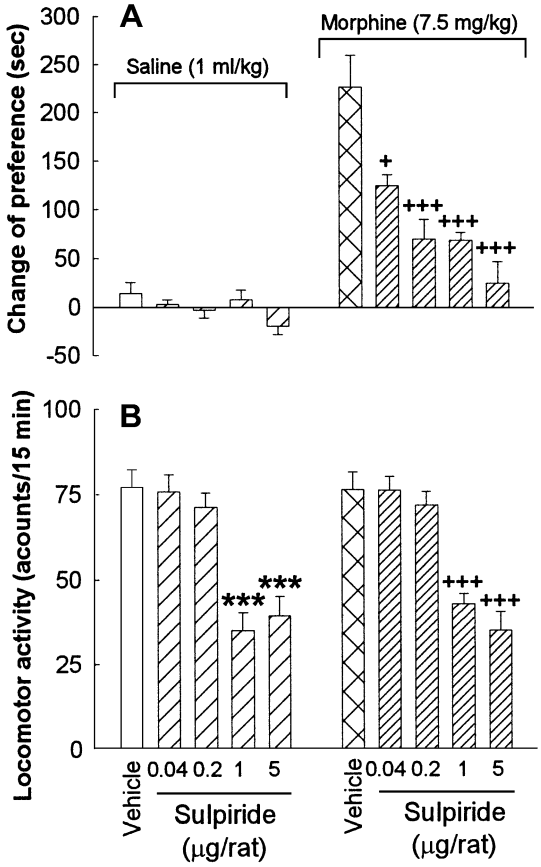

Fig. 4. The effects of bilateral intra-CeA injection of sulpiride, either alone or in combination with morphine, on the acquisition of a conditioned place preference. The animals received sulpiride (0.04, 0.2, 1 and 5 μg/rat) or saline (1 μl/rat) in combination with morphine (7.5 mg/kg sc) or without morphine, in a 3-day schedule of conditioning. On the test day, the animals were observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the preconditioning session (Panel A). The locomotor activity was assessed as described in the Materials and methods section (Panel B). Data are expressed as mean ± S.E.M. of seven animals per group. $^{***}P<.001$ different from the vehicle/saline group. $^{+}P<.05$, $^{+++}P<.001$ compared to the vehicle/morphine group.

3.4. Experiment 3. Effects of sulpiride with or without morphine on the acquisition of CPP

Fig. 4A shows the effects of bilateral intra-CeA injection of sulpiride in the absence or presence of morphine on the acquisition of CPP. Data were analyzed by a two-way ANOVA. The results indicated a significant main effect of treatment [$F(1,60)=102.1$, $P<.0001$], dose [$F(4,60)=15.1$, $P<.0001$] and Treatment × Dose interaction [$F(4,60)=8.9$, $P<.0001$]. In addition, one-way ANOVA indicated that sulpiride (0.04, 0.2, 1 and 5 μg/rat, intra-CeA) alone induced neither a significant place preference nor place aversion [$F(3,24)=2.2$, $P>.05$]. Furthermore, sulpiride dose-dependently inhibited the morphine (7.5 mg/kg)-induced place preference [one-way ANOVA: $F(4,30)=13.7$, $P<.0001$].

Fig. 4B illustrates the effects of the drugs on the locomotor activity in the testing phase. Two-way ANOVA also revealed a significant main effect of dose [$F(4,60)=36.4$, $P<.0001$]. The results indicated no significant effect of treatment [$F(1,60)=0.1$, $P>.05$] nor the Treatment × Dose interaction [$F(4,60)=0.4$, $P>.05$]. One-way ANOVA also revealed that sulpiride (1 and 5 μg/rat, intra-CeA), either alone [$F(4,30)=16.8$, $P<.0001$], or in combination with morphine [$F(4,30)=20.6$, $P<.0001$], decreased the locomotor activity.

3.5. Experiment 4. Effects of sulpiride on quinpirole response during morphine conditioning

Fig. 5A shows the effect of the drugs on morphine-induced CPP. Two-way ANOVA indicated an interaction

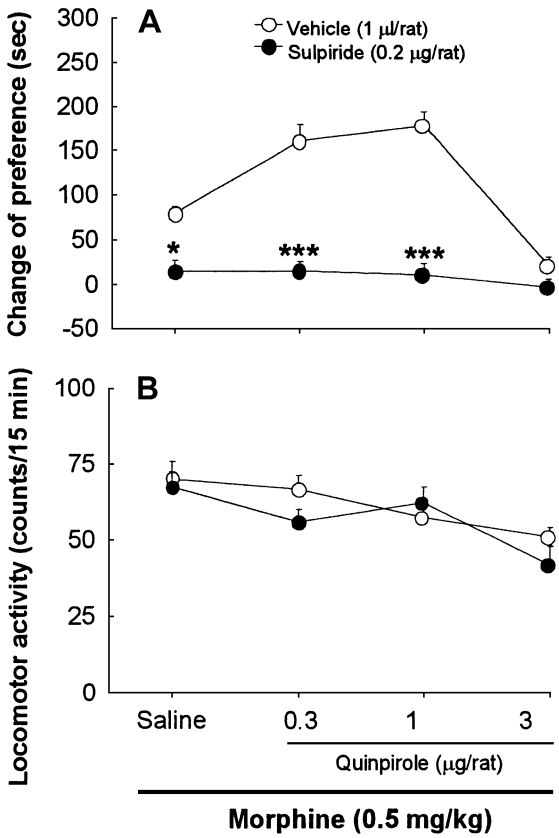

Fig. 5. The effects of bilateral intra-CeA injection of quinpirole alone or combined with sulpiride on the acquisition of morphine-induced place preference. The animals received an intra-CeA injection of either vehicle (1 μl/rat) or sulpiride (0.2 μg/rat) 5 min before intra-CeA injection of either quinpirole (0.3, 1 and 3 μg/rat) or saline (1 μl/rat), and then they were injected with morphine (0.5 mg/kg sc) during conditioning. On the test day, the animals were observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the preconditioning session (Panel A). The locomotor activity was assessed as described in the Materials and methods section (Panel B). Data are expressed as mean ± S.E.M. of seven animals per group. $^{*}P<.05$, $^{***}P<.001$ compared to the vehicle/quinpirole group.

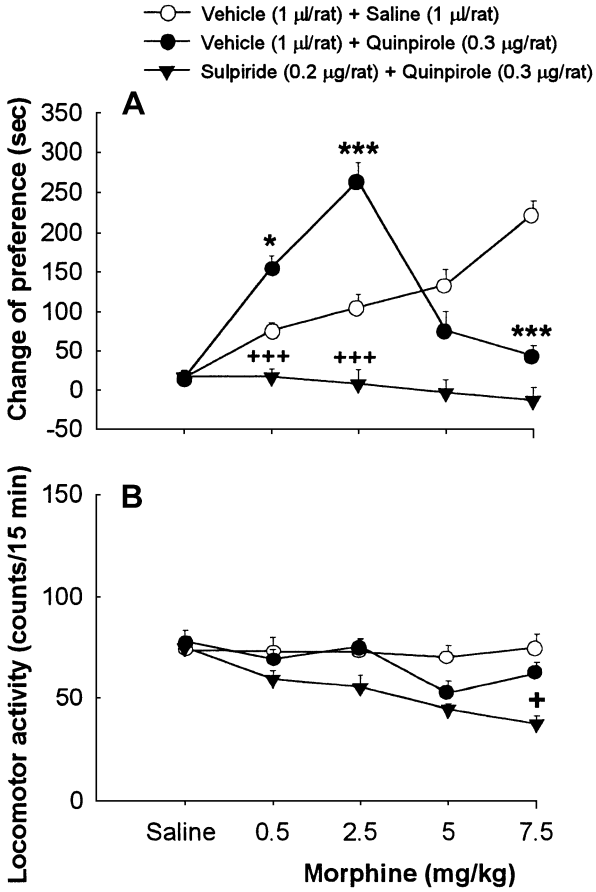

Fig. 6. The effects of bilateral intra-CeA injection of quinpirole, either alone or in combination with sulpiride, on the acquisition of conditioned place preference induced by different doses of morphine. Morphine (0.5, 2.5, 5 and 7.5 mg/kg sc) or saline (1 ml/kg sc) was administered in a 3-day schedule of conditioning. The animals received an intra-CeA injection of either vehicle (1 μl/rat) or sulpiride (0.2 μg/rat) 5 min before the intra-CeA injection of either saline (1 μl/rat) or quinpirole (0.3 μg/rat) immediately before each morphine injection during conditioning. On the test day, the animals were observed for a 15-min. period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the preconditioning session (Panel A). The locomotor activity was assessed as described in the Materials and methods section (Panel B). Data are expressed as mean ± S.E.M. of seven animals per group. $^{*}P<.05$, $^{***}P<.001$ compared to the control (vehicle/saline) group. $^{+}P<.05$, $^{+++}P<.001$ compared to the vehicle/quinpirole group.

[$F(3,48)=13.1$, $P<.0001$] and also a significant difference between the groups of animals [$F(1,48)=117.0$, $P<.0001$], which received quinpirole (0.3, 1 and 3 μg/rat, intra-CeA) immediately before morphine (0.5 mg/kg sc) or sulpiride (0.2 μg/rat, intra-CeA) 5 min before quinpirole (0.3, 1 and 3 μg/rat, intra-CeA) injection plus morphine (0.5 mg/kg sc) in the conditioning sessions. Post-hoc analysis showed that sulpiride decreased the effect of quinpirole on morphine response.

Fig. 5B shows the effect of the drugs on the locomotor activity during testing. Two-way ANOVA revealed a significant effect for dose [$F(3,48)=7.4$, $P<.0001$], but no effect was observed for treatment [$F(1,48)=1.7$, $P>.05$] and the Treatment × Dose interaction [$F(3,48)=1.1$, $P>.05$], on the locomotor activity, by the drugs.

3.6. Experiment 5. Effects of quinpirole with or without sulpiride on the acquisition of morphine-induced CPP

Fig. 6A shows the effect of the drugs on morphine (0, 0.5, 2.5, 5 and 7.5 mg/kg sc)-induced CPP. Two-way ANOVA indicated an interaction [$F(8,90)=21.5$, $P<.0001$] as well as a significant difference [$F(2,90)=69.17$, $P<.0001$] between the groups of animals that received quinpirole (0.3 μg/rat, intra-CeA) immediately before the different doses of morphine or sulpiride (0.2 μg/rat, intra-CeA) just before quinpirole (0.3 μg/rat, intra-CeA) injection plus the different doses of morphine in the conditioning sessions. Post-hoc analysis

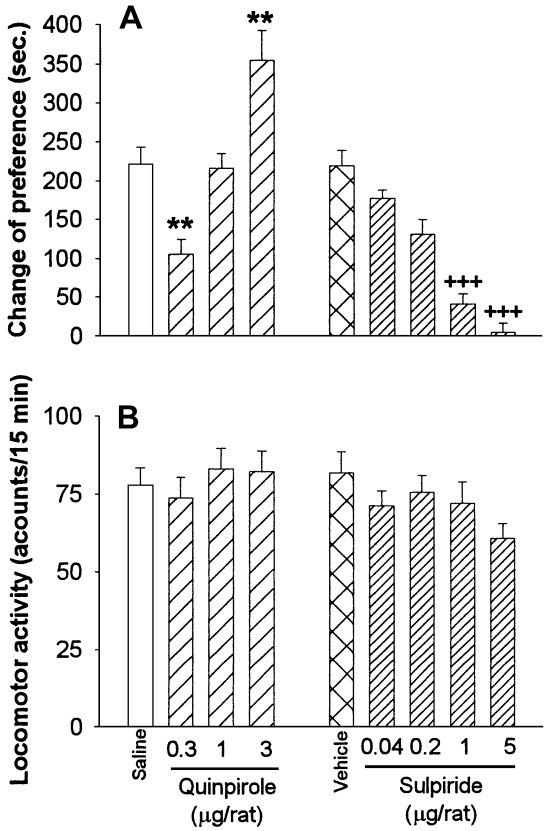

Fig. 7. The effects of bilateral microinjection of quinpirole or sulpiride into the CeA on the expression of morphine-induced place preference. All animals received morphine (7.5 mg/kg sc) or saline (1 mg/kg sc) in a 3-day schedule of conditioning. On the test day, different doses of quinpirole (0.3, 1 and 3 μg/rat), sulpiride (0.04, 0.2, 1 and 5 μg/rat), saline (1 μl/rat) or vehicle (1 μl/rat) were administered into the CeA immediately before testing and each animal was observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the preconditioning session (Panel A). The locomotor activity was assessed as described in the Materials and methods section (Panel B). Data are expressed as mean ± S.E.M. of seven animals per group. $^{**}P<.01$ compared to the saline group. $^{+++}P<.001$ compared to the vehicle group.

confirmed that quinpirole potentiated the acquisition of CPP induced by the lower doses of morphine (0.5 and 2.5 mg/kg), while attenuated the response induced by the higher dose (7.5 mg/kg) of morphine. Sulpiride reversed the quinpirole response and blocked the effect of morphine.

Fig. 6B illustrates the effects of the drugs on the locomotor activity in the testing phase. Two-way ANOVA also revealed a significant main effect of dose [$F(4,90)=6.3$, $P<.0001$] and treatment [$F(2,90=14.4$, $P<.0001$]. No effect was found for the Treatment × Dose interaction [$F(8,90)=2.0$, $P>.05$].

3.7. Experiment 6. Effects of quinpirole or sulpiride on the expression of morphine-induced CPP

Fig. 7A shows the effects of bilateral intra-CeA injection of quinpirole or sulpiride on the expression of morphine-induced CPP. One-way ANOVA indicates that quinpirole (0.3, 1 and 3 μg/rat) had a significant effect on the expression of morphine-induced place preference [$F(3,24)=15.7$, $P<.0001$]. Post-hoc analysis showed that a dose of 0.3-μg/rat of quinpirole inhibited the CPP induced by morphine (7.5 mg/kg), but the middle dose of the drug (1 μg/rat) had no effect, while the higher dose of quinpirole (3 μg/rat) potentiated the expression of morphine-induced CPP. On the other hand, one-way ANOVA revealed that sulpiride (0.04, 0.2 1 and 5 μg/rat, intra-CeA) dose-dependently attenuated the expression of morphine-induced place preference [$F(4,30)=34.8$, $P<.0001$].

As shown in Fig. 7B, quinpirole [$F(3,24)=0.5$, $P>.05$] or sulpiride [$F(4,30)=1.7$, $P>.05$] had no effect on the locomotor activity on the testing phase.

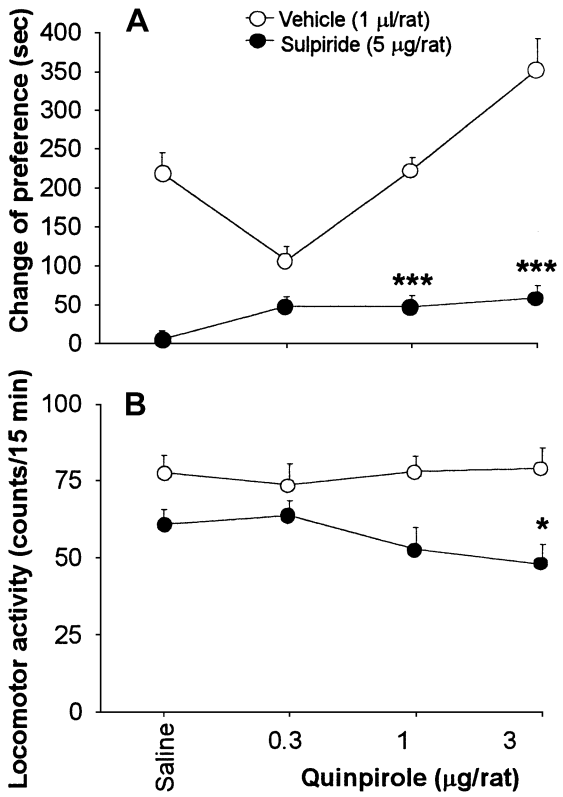

Fig. 8. The effects of bilateral microinjection of sulpiride plus quinpirole into the CeA on the expression of morphine-induced place preference. All animals received morphine (7.5 mg/kg sc) or saline (1 mg/kg sc) in a 3-day schedule of conditioning. On the test day, immediately before testing they received an intra-CeA injection of either vehicle (1 μl/rat) or sulpiride (5 μg/rat) 5 min before intra-CeA injection of either quinpirole (0.3, 1 and 3 μg/rat) or saline (1 μl/rat). Each animal was observed for a 15-min period. The change of preference was assessed as the difference between the time spent on the day of testing and the time spent on the day of the preconditioning session (Panel A). The locomotor activity was assessed as described in the Materials and methods section (Panel B). Data are expressed as mean ± S.E.M. of seven animals per group. * $P<.05$, * * * $P<.001$ different from the control (vehicle/quinpirole) group.

3.8. Experiment 7. Effects of quinpirole with or without sulpiride on the expression of morphine-induced CPP

Fig. 8A shows the effect of the drugs on the expression of morphine-induced CPP. Two-way ANOVA indicated an interaction [$F(3,48)=10.1$, $P<.0001$] and also a significant difference between the groups of animals [$F(1,48)=145.3$, $P<.0001$] that received quinpirole (0.3, 1 and 3 μg/rat, intra-CeA) or sulpiride (5 μg/rat, intra-CeA) 5 min before quinpirole (0.3, 1 and 3 μg/rat, intra-CeA) injection in the testing phase. Post-hoc analysis showed that sulpiride reduced the quinpirole effect.

Fig 8B illustrates that there was a significant effect of group [$F(3,48)=21.5$, $P<.0001$]; no effect was observed for dose [$F(3,48)=0.8$, $P>0.05$] nor the Treatment × Dose interaction [$F(3,48)=1.6$, $P>.05$] on the locomotor activity.

4. Discussion

Intracranial place conditioning studies have been used to show that there are a number of receptors, neuronal pathways and discrete central nervous system (CNS) sites involved in the brain reward mechanisms (see McBride et al., 1999). Although, among the CNS regions examined, there is evidence suggesting that the mesocorticolimbic DA system, which originates in the VTA and projects to the Nac, various limbic and cortical areas is a major neural substrate of the rewarding effects produced by morphine (Olmstead and Franklin, 1997a,b). However, few intracranial place-conditioning studies have been undertaken with the amygdala on the morphine-induced place preference. The present study shows that the DA D2 receptors of the CeA may play a critical role on the acquisition and expression of morphine-induced place preference.

In accordance with previous studies, the subcutaneous administration of morphine produced a CPP in a dose-dependent manner (Shoaib et al., 1995; Tzschentke and Schmidt, 1995).

Some researchers claim that the activation of different subtypes of DA receptors could be essential for the opiate reward (see Tzschentke, 1998). In the present study, we showed that the bilateral microinjections of the DA D2 receptor agonist, quinpirole and the DA D2 receptor antagonist, sulpiride (see Jaber et al., 1996) into the CeA alone did not produce a significant CPP or conditioned place aversion (CPA). In contrast, quinpirole does produce a CPP when injected into the Nac (White et al., 1991; Papp et al., 1993), while sulpiride in the Nac is without effect (Baker et al., 1996). In spite of neuroanatomical studies that have revealed that the CeA bears strong relationships and homologies with the Nac shell (Heimer et al., 1991), and the high distribution of the DA D2 receptors in the CeA (Scibilia et al., 1992), our data show that the injection of quinpirole alone could not initiate rewarding effects as it does in the Nac.

A low dose of morphine (0.5 mg/kg) did not induce a significant CPP. However, injection of lower and middle doses of quinpirole (0.3 and 1 μg/rat) into the CeA plus morphine (0.5 mg/kg) significantly elicited a CPP. The response of quinpirole was attenuated by sulpiride administration. It may be concluded that the potentiation of morphine-induced CPP is mediated through a DA D2 receptor mechanism. Sulpiride (0.04, 0.2, 1 and 5 μg/rat), by itself, dose-dependently decreased morphine conditioning, although other investigators have shown that the systemic or intra-Nac injection of sulpiride has no effect on morphine-induced CPP (Shippenberg and Herz, 1988).

In Experiment 5, quinpirole (0.3 μg/rat, intra-CeA) potentiated the acquisition of CPP induced by the lower doses of morphine (0.5 and 2.5 mg/kg), while it decreased the response induced by the higher dose (7.5 mg/kg) of morphine. Since the administration of the DA D2 receptor antagonist, sulpiride (0.2 μg/rat, intra-CeA), attenuated the potentiation induced by quinpirole, the effect of quinpirole may be produced through a DA D2 receptor mechanism. Hodge et al. (1997) have shown similar biphasic effects by elicited quinpirole. They reported that the microinjection of quinpirole into the Nac produced a biphasic effect on alcohol self-administration. Since the DA D2/D3 receptor agonist, quinpirole, exhibits an affinity for DA D3 receptors at higher doses, it appears likely that the dose-related differential action is due to the various D2-like receptor subtypes. It has further been demonstrated that the activation of DA D2 and D3 receptor subtypes have opposing functional consequences on behaviors (see Richtand et al., 2001). The activation of presynaptic (terminal) DA D2 receptors inhibits both the DA synthesis and release. Some studies also suggest that DA D3 receptors might function as both release- and synthesis-inhibiting autoreceptors in some systems (Meller et al., 1993; Tang et al., 1994). Therefore, the biphasic effect of quinpirole on the acquisition of morphine-induced CPP may be either due to pre- or postsynaptic stimulation of DA D2-like receptors or may be mediated through activation of different DA receptors.

Furthermore, the higher dose of quinpirole (3 μg/rat) with 0.5-mg/kg of morphine or the combination of the low dose of quinpirole (0.3 μg/rat) with 7.5-mg/kg of morphine decreased the locomotor activity, while the other doses of quinpirole had no effect. These results are similar to the data obtained by Gong et al. (1999) who reported that ventral pallidum microinjection of 3-μg/rat of quinpirole, suppressed locomotion, while 0.3–1 μg/rat had no effect. It has also been reported that the D1/D2 activation increases locomotion, while the D3 receptor stimulation inhibits locomotion (Depoortere, 1999). Therefore, the decrease in locomotion by quinpirole may be due to the DA D3 receptor activation. It should be considered that the higher dose of quinpirole plus morphine did not induce a CPP, which may also be a reflection of the decrease in locomotion.

The higher doses of sulpiride (1 and 5 μg/rat, intra CeA) by itself and in combination with morphine, during conditioning, decreased the locomotor activity. Therefore, the effect of the higher dose (but not the lower dose) of sulpiride on morphine CPP may be due to the influence of the drug on locomotion.

Considering that the amygdala is a critical site for the acquisition of emotional association memory (Ono et al., 1995), it seems likely that the inhibition of DA D2 receptors by sulpiride blocks the reward-related incentive learning. Beninger (1983) and Beninger and Miller (1998) suggested that DA receptor agonists support this kind of learning and antagonists block the usual effects of reward on behavior. Furthermore, some studies showed that the amygdala may play a critical role in stimulus-reward learning (Harmer and Phillips, 1999; Harmer et al., 1997) and the lesions in the CeA before conditioning impaired the acquisition of the conditioned responses (see Ono et al., 1995). Therefore, the changes in morphine-induced CPP by activation or inhibition of DA D2 receptors may be influenced by the CeA associated-memory.

De Fonseca et al. (1995) showed that DA has an effective role in the expression of morphine-induced place preference. The expression of morphine-induced CPP may be related to decrease in DA release in the test session. This decrease stimulates the drug-seeking behavior evoked by environmental cues associated with morphine administration. The opposite effects of the lower and higher doses of quinpirole on the expression of the morphine-induced CPP may be related to the affinity of the drug to the DA D2/D3 receptors that may change the DA levels. Intra-CeA administration of sulpiride by itself also decreased the expression of morphine-induced CPP. Sulpiride may block presynaptic DA D2 receptors, and so releases DA, which, in turn, activates postsynaptic DA receptors and thus reduces the expression of morphine-induced CPP. In general, our studies demonstrated for the first time that DA D2 receptors in the CeA have an important role in the acquisition and expression of morphine-induced CPP. Recent studies also support this hypothesis that the DA D2 receptor could be as

important as the DA D1 receptors in morphine reward (Manzanedo et al., 2001).

Acknowledgements

The authors thank Dr. T. Nayer Nouri for his assistance in preparing this manuscript.

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