

Maternal deprivation and handling modify the effect of the dopamine D3 receptor agonist, BP 897 on morphine-conditioned place preference in rats

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Received: 3 August 2006 / Accepted: 3 April 2007 / Published online: 10 May 2007
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Abstract

Rationale Maternal deprivation and handling can lead to a vulnerability to opiate dependence. However, the involvement of the dopamine D3 receptors has not been investigated.

Objectives This study analysed the effects of a selective partial D3 receptor agonist, BP 897, on morphine-conditioned place preference (CPP) in deprived and handled rats.

Materials and methods The effects of BP 897 were studied on the expression and the extinction of morphine CPP. Quantitative autoradiography of D2, D3 receptors and immunohistochemistry of dopamine transporter were performed in some saline- and morphine-treated rats 24 h after the place preference test.

Results Morphine (5 mg/kg) induced a more prolonged morphine CPP in deprived and handled rats than in control animals. BP 897 (0.5 or 2 mg/kg) enhanced the expression of morphine conditioning in control rats. Same doses did not change morphine conditioning in deprived rats. BP 897 (2 mg/kg) suppressed morphine CPP in handled rats. An increase in basal D2 receptor density in the mesencephalon of handled rats, which was suppressed after morphine CPP, was observed. A decrease in D2 receptor levels in

morphine-treated deprived rats occurred in the nucleus accumbens.

Conclusions This study shows that maternal deprivation and handling induced a prolonged morphine CPP, and different changes of D2/D3 receptor functioning revealed after morphine CPP. Early manipulations of infant–mother relationships may have different consequences on the balance of opioidergic and dopaminergic neurotransmission and may be of interest to reveal pharmacological properties of dopamine receptor partial agonists or antagonists potentially useful for therapeutic applications.

Keywords Maternal deprivation · Handling
Place preference paradigm · Morphine
D3 partial agonist BP 897 · DAT · D2 · D3 receptors · Rat

Introduction

Long maternal separation in rodents has been shown to lead to anxiety, stress-induced illness and depression (reviewed in Anisman et al. 1998; Hall 1998; Francis et al. 1999; Matthews and Robbins 2003). A vulnerability to drug dependence as a direct long-term behavioural consequence of disruption of the infant–mother relationships has also been documented. Some data showed that separated rats develop a preference to ethanol (Huot et al. 2001; Ploj et al. 2003; but see also Marmendal et al. 2004; Jaworski et al. 2005) and increase (Kosten et al. 2000, 2005; Zhang et al. 2005) or decrease (Matthews et al. 1999) the acquisition of cocaine self-administration. In addition, a hypersensitivity of separated rats to psychostimulant-induced hyperlocomotor activity and modifications of dopaminergic systems have been reported (Kehoe et al. 1996; Hall et al. 1999; Brake et al. 2004). More recently, we have shown that

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deprivation of infant–mother–litter (maternal deprivation) relationship leads to an enhancement of anxiety, a hypersensitivity to the reinforcing effect of morphine and to morphine dependence (Vazquez et al. 2005a, b). Enduring neurobiological changes have been also described with hyposensitivity of the enkephalinergic system in deprived (D) rats that could explain their sensitivity to opiate dependence (Vazquez et al. 2005b). On top of opioidergic system, dopaminergic systems play a fundamental role in brain reinforcement processes (Di Chiara and Imperato 1988; reviewed in Van Ree et al. 2000; Koob and LeMoal 2001; Wise 2004). It is well established that endogenous opioids and opiate compounds exert reward and reinforcing effects via dopamine-dependent and dopamine-independent mechanisms (Shippenberg et al. 1993; Sellings and Clarke 2003). It is therefore important to evaluate the possible participation of dopaminergic system in the sensitivity of D rats to opiates. Among the dopaminergic receptors involved in reinforcing processes, D3 receptors present a great interest. D3 receptors are highly expressed in brain structures involved in reward and reinforcing processes, such as the nucleus accumbens (N.Acc.) and the ventral tegmental area (VTA; Sokoloff et al. 1990; Bouthenet et al. 1991). D3 receptor agonists have been shown to modify cocaine, nicotine and ethanol reward (reviewed in Le Foll et al. 2000) by diminishing intracranial auto-stimulation (Depoortere et al. 1996), cocaine self-administration (Caine and Koob 1993) and the rewarding value of cocaine (Pilla et al. 1999; Duarte et al. 2003; Garcia-Ladona and Cox 2003). In addition, interactions between D3 receptors, endogenous opioid systems and opiates have been recently documented (Narita et al. 2003; Spangler et al. 2003). However, there are still discrepancies about the potential role of D3 receptors in opiate dependence. 7-OH-DPAT, a rather preferential D3 receptor full agonist, impairs the acquisition and the expression of morphine conditioned place preference (CPP) in rats (Rodriguez De Fonseca et al. 1995), whereas BP 897, an *in vivo* selective partial D3 receptor agonist (Pilla et al. 1999), does not modify the expression of morphine CPP (Duarte et al. 2003) and either decreases or increases the acquisition of morphine CPP (Duarte et al. 2003; Francès et al. 2004a). An increase in morphine-induced reward was observed in D3 knockout mice (Narita et al. 2003; Francès et al. 2004b) or after a decrease in D3 receptors in limbic forebrain of mice (Mizuo et al. 2004a). Conversely, a highly selective D3 receptor antagonist, SB-277011A, administered in rats blocked the acquisition and the expression of opiate CPP (Ashby et al. 2003). D3 receptors may be a component for the modulation of opiate-inducing reward, but the exact direction of their role seems to depend on the doses of the D3 compounds, on the species studied and on the specific experimental conditions.

Another approach to reveal the influence of D3 receptors on opiate reward is to examine the effects of D3 receptor compounds on animals known to present a vulnerability to opiate dependence. In this study, we decided to focus on the model of maternal deprivation. Maternal deprivation consisted of separating pups from their mother and littermates 3 h/day from the ages of 1–14 days. Non-deprived rats, representing the control group, had experienced human intervention for animal care and were therefore named animal facility rearing group (AFR, see Pryce and Feldon 2003). We also studied a group of rats submitted to a brief handling (1 min/day from the ages of 1–14 days), as an increase in oral morphine self-administration has been revealed in handled (H) rats compared to AFR animals (Vazquez et al. 2006). BP 897 acts as a D3 receptor agonist or antagonist at low doses, depending on the level of receptor occupancy and as a weak D2 antagonist at doses above 10 mg/kg (Pilla et al. 1999; Wood et al. 2000; Wicke and Garcia-Ladona 2001). Besides their autoreceptor function (Sokoloff et al. 1990; Diaz et al. 2000), it has been suggested that the activation of D3 receptors can regulate extracellular dopamine by modulating the dopamine transporter (DAT; Zapata and Shippenberg 2002). We therefore studied the effects of BP 897 injected at the doses of 0.5 and 2 mg/kg on the expression and the extinction of morphine CPP in AFR, D and H rats. Some AFR, D and H rats receiving saline or morphine during CPP were killed, and the relative density of D2 and D3 receptors and of DAT in the brain was quantified by autoradiography and by immunautoradiography (respectively) in the striatum [caudate-putamen nucleus (CPu) and N.Acc.] and the mesencephalon [substantia nigra (SN) and ventral tegmental area (VTA)] regions of the brain involved in opiate reward (Shippenberg et al. 1993; Van Ree et al. 2000).

Experimental procedures

Subjects

Five series of 20 pregnant Long–Evans rats on day 14 of gestation (Janvier, Le Genest St Isle, France) were used. The dams gave birth 1 week \pm 12 h after inclusion. Litters were housed in clear plastic cages in a well-ventilated, temperature-controlled ($22 \pm 1^\circ\text{C}$) and humidity-controlled ($50 \pm 5\%$) environment on a 12-h light 12-h dark cycle (lights on at 0800–2000 hours). Dams received rat chow and water *ad libitum*, and the cages and all of the shavings were changed only once weekly to avoid excessive handling.

The experimental procedure and care of the animals were in accordance with local committee guidelines and the

“Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research” (National Research Council 2003).

Maternal deprivation

Maternal deprivation and handling were performed as described in Vazquez et al. (2005a, b, 2006). The day of birth was designated day 0. On postnatal day 1, female pups were removed from the litters. Random redistribution of male pups among dams was done to homogenise possible effects of genetic and prenatal factors and to obtain similar litter size (standardised to six to seven male pups). It cannot be excluded that litters may have suffered from prenatal stress due to the transport of pregnant rats and that cross-fostering may change maternal behaviour. However, the same procedure was applied in all pups from the AFR, H and D groups before deprivation or handling, allowing valid data comparisons. Two investigators collaborated in the determination of each pup's sex, and each pup received similar handling during this procedure. The litters were each assigned to an experimental group. From day 1, mothers were removed from their home cage and put in a new cage for 3 h, the same procedure being applied at each deprivation. Neonates belonging to the maternal deprivation group (D) were individually placed in temperature- (30–34°C) and humidity-controlled cages divided into compartments in a room separated from their mothers. Pups' cages contained 2 cm of fresh shavings covered with absorbing paper. Pups were isolated daily from days 1 to 14 from 1300 to 1600 hours. At the end of the deprivation period, each litter of pups was replaced in the housing cage, and then the dam was transferred back to the housing cage. To obtain minimum handling, pups were transferred to and from their cages quickly and gently. D pups received no other handling except that required to change the bedding in their cages once weekly. A second group of pups, named handled (H), were individually taken and weighed daily from day 1 to day 14 always from 1300 to 1400 hours. Mothers were removed from their home cage and put in a new cage for 6 to 7 min, the same procedure being applied at each handling. At the end of litter weighing, the dam was transferred back to the housing cage. After this period, H pups received no other handling except that required to change the bedding in their cages once weekly. Rat pups not subjected to maternal deprivation remained with their mothers during this period and received no special handling other than that necessary to change the bedding in their cages once weekly (animal facility rearing, AFR). From days 15 to 21, all pups remained with their mothers. On day 21 or 22, pups were weaned from their mothers and housed in groups of three or four until 2.5–3 months of age.

Place preference paradigm

Place preference experiments were performed as described in Vazquez et al. (2005b, 2006).

The place preference apparatus consisted of a Plexiglas box divided into two independent square compartments (45×45×30 cm, width × length × height), 14 cm spaced out from each other, and both accessible from a rectangular exterior area (18×36×30 cm, w × l × h). The box was placed in a soundproof testing room illuminated by two indirect 20-W lights. Two distinctive sensory cues differentiated the compartments: the wall and floor colouring (black or striped) and the floor texture (rough or smooth). The combinations were as follows: (a) black wall, rough floor, (b) striped wall, smooth floor so that naive rats spent approximately the same amount of time in each of the two compartments. The neutral area to access to compartments had grey walls and floor. The position of the rat was recorded by a video camera and time spent in each compartment analysed by a program provided with the Videotrack II, 2.12 version computer (Viewpoint, Lyon, France). The rat was scored as being within a compartment if the head and both forepaws were in that area.

One compartment was randomly chosen to be associated with morphine administration and the other with saline. The drug-assigned compartment could be either the more or the less preferred. Care was taken to ensure that all treatments were equally balanced between compartments. Experiments were conducted between 0900 and 1900 hours.

The place preference conditioning schedule consisted of four phases:

- 1) In the preconditioning phase (day 1), rats were placed in the middle of the neutral area and the time spent in each compartment recorded for the following 20 min. Rats showing strong unconditioned aversion (less than 25% of the session time) or preference (more than 75% of the session time) for any compartment were discarded. Rats were then randomised to treatment or control groups and to one of the two compartments.
- 2) The conditioning phase consisted of six consecutive days of injection (days 2–7). Treated rats received morphine (5 mg/kg i.p.) on days 2, 4 and 6 and saline (1 ml/kg i.p.) on days 3, 5 and 7. Control rats received saline every day. The rats were confined to the compartment by a matching door for the 25 min immediately after the morphine or saline injection. The same procedure was used in another experiment except that D rats received morphine at the dose of 2 mg/kg i.p.
- 3) In the testing phase (day 8), the test was conducted exactly as in the preconditioning phase: 24 h after the final conditioning session, the rats were given free access to each compartment for 20 min.

- 4) The extinction phase was conducted exactly as the testing phase, but 24 h (day 9) and 48 h (day 10) after.

The days 8, 9, 10 rats received BP 897 (0.5 or 2 mg/kg i.p.) or saline (0.1 ml/kg i.p.) 30 min before testing.

A place preference score was calculated as the difference between post-conditioning at days 8, 9 or 10 and pre-conditioning times spent in the compartment associated with drug. Mean \pm SEM was calculated for each group.

Experimental procedure

Experiment 1

Five identical series were carried out with each experimental group represented. In each series, AFR, D and H rats received saline just before the conditioning phase and saline the days 8, 9 and 10 of the testing phase (saline/saline) = group I, saline just before the conditioning phase and BP 897 (0.5 mg/kg) the days 8, 9 and 10 of the testing phase (saline/BP 897 0.5 mg/kg) = group II, saline just before the conditioning phase and BP 897 (2 mg/kg) the days 8, 9 and 10 of the testing phase (saline/BP 897 2 mg/kg) = group III, morphine (5 mg/kg) just before the conditioning phase and saline the days 8, 9 and 10 of the testing phase (morphine/saline) = group IV, morphine (5 mg/kg) just before the conditioning phase and BP 897 (0.5 mg/kg) the days 8, 9 and 10 of the testing phase (morphine/BP 897 0.5 mg/kg) = group V and morphine (5 mg/kg) just before the conditioning phase and BP 897 (2 mg/kg) the days 8, 9 and 10 of the testing phase (morphine/BP 897 2 mg/kg) = group VI (Table 1).

Experiment 2

The dose of 5 mg/kg of morphine was chosen because we previously showed that AFR, D and H animals were conditioned to morphine in the place preference paradigm (Vazquez et al. 2005b, 2006). However, as morphine at the dose of 2 mg/kg was also active, but only in D rats (Vazquez et al. 2005b, 2006), another experiment was done in which the behaviour of D animals were analysed after injection of 2 mg/kg of morphine during the conditioning phase and BP 897 at the dose of 2 mg/kg during the testing phase (saline/saline; morphine/saline; morphine/BP 897 2 mg/kg).

Immunoautoradiographical and autoradiographical experiments

One or two saline- or morphine-treated AFR, D and H rats (groups I saline/saline and IV morphine/saline) from four independent series of experiments showing a mean value of

CPP score and providing from different litter (to avoid any litter effect) were selected for immunoautoradiographical and autoradiographical experiments. They were killed by decapitation 24 h after CPP experiments, and their brains were quickly removed and frozen by immersion in isopentane at -20°C and then stored at -80°C until sectioning. Rostro-caudal series of coronal sections (10- μm thickness) were cut at -20°C in a cryostat (Leica microsystems GmbH, Wetzlar, Germany) according to the frontal plan of the stereotaxis atlas of Paxinos and Watson (1997), thaw-mounted on slides (Superfrost^R Plus slides, Menzel-Glass, Braunschweig, Germany) and frozen at -80°C until use. For the CPU and the N.Acc., two pairs of slices were cut every 160 μm from level +1.6 mm anterior to bregma. For the SN and the VTA, two pairs of slices were cut every 160 μm from level -5.3 mm posterior to bregma.

DAT labelling

Labelling of DAT was performed as described in Martres et al. (1998). After fixation for 5 min at 4°C in 4% paraformaldehyde in phosphate-buffered saline (PBS, 50 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, 150 mM NaCl, pH 7.4) and extensive washes, sections were incubated for 1 h at room temperature in PBS, supplemented with 3% bovine serum albumin (BSA) and 1% goat serum, then incubated overnight at 4°C with anti-DAT antiserum at 1/20,000 dilution. After extensive washes, sections were incubated for 1 h at room temperature in PBS containing 3% BSA, 1% goat serum and 1 mM NaI in the presence of 0.20–0.25 $\mu\text{Ci/ml}$ anti-rabbit [^{125}I]-F(ab')₂ IgG (100 $\mu\text{Ci/ml}$), purified by gel filtration onto a PD10 column (Sephadex G25M, Pharmacia). Sections were abundantly washed in PBS, dried and apposed to Biomax MR films (GE Healthcare Europe GMBH, Orsay, France) for 1–4 days.

D2 receptor labelling

Labelling of D2 receptors was performed as described in Martres et al. (1985). To rule out binding of endogenous dopamine, slides were pre-incubated three times for 5 min at room temperature in 50 mM Tris-HCl buffer pH 7.4 containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 (Tris-ions buffer), supplemented with 0.1% BSA and 0.01% ascorbic acid. Then, slides were incubated for 1 h at room temperature in the same buffer containing 0.3 nM [^{125}I]-iodosulpride. Some adjacent slides were incubated with 10 μM apomorphine to determine non-specific binding. Sections were washed four times for 5 min in ice-cold Tris-ions buffer, rapidly rinsed in ice-cold water and dried. Slides were exposed to Biomax MR films (GE Healthcare Europe GMBH) for 12–24 h.

Table 1 Protocol of morphine-conditioned place preference paradigm in the experiment with 5 mg/kg of morphine

Rats	Types	PreC phase	Conditioning phase							Testing phase		
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	
Saline/saline (I)	AFR (25) D (27) H (26)		S	S	S	S	S	S	S	S	S	S
Saline/BP 897 0.5 mg/kg (II)	AFR (9) D (9) H (10)		S	S	S	S	S	S	BP	BP	BP	
Saline/BP 897 2 mg/kg (III)	AFR (8) D (8) H (8)		S	S	S	S	S	S	BP	BP	BP	
Morphine/saline (IV)	AFR (31) D (29) H (27)		M	S	M	S	M	S	S	S	S	
Morphine/BP 897 0.5 mg/kg (V)	AFR (9) D (11) H (10)		M	S	M	S	M	S	BP	BP	BP	
Morphine/BP 897 2 mg/kg (VI)	AFR (10) D (8) H (9)		M	S	M	S	M	S	BP	BP	BP	

Some rats from I and IV were sacrificed and brains were removed 24 h after the place preference test for autoradiographic and immunohistochemical studies. The number of rats is indicated in parentheses.

AFR Animal facility rearing (control), D deprived rats, H handled rats, S saline, BP BP 897 0.5 or 2 mg/kg i.p., M morphine 5 mg/kg i.p., PreC pre-conditioning

D3 receptor labelling

Labelling of D3 receptors was performed as described in Stanwood et al. (2000) and modified as follows: Slides were pre-incubated at room temperature three times for 5 min in 50 mM HEPES, pH 7.4, supplemented with 1 mM ethylenediamine tetraacetic acid, 0.05% ascorbic acid, 0.1% BSA, 100 μ M GTP (to dissociate D2 receptors from G proteins) and 25 μ M 1,3-di-*o*-tolylguanidine (to dissociate ligand from sigma receptors). Then, slides were incubated for 1 h at room temperature in the same buffer containing 0.25 nM [125 I]-7-OH-PIPAT. Some adjacent slides were incubated with 10 μ M dopamine to determine non-specific binding. Sections were then washed four times for 15 min in 50 mM HEPES buffer at 4°C, briefly dipped in ice-cold water, dried and exposed to Biomax MR film (GE Healthcare Europe GMBH) for 2–3 days.

Quantification of relative density of DAT, D2 and D3 receptors

Standard radioactive microscales (GE Healthcare Europe GMBH) were exposed on each autoradiographic film to ensure that densities of the labelling were in the linear range. After autoradiogram scanning, densities were measured using MCID™ analysis software (Imaging Research, St. Catharines, ON, Canada). Structures were identified

with reference to the rat brain atlas of Paxinos and Watson (1997). The relative densities (nCi/mg) were quantified in both hemispheres after subtraction of non-specific labelling. The values obtained in both hemispheres were then averaged. For each region, the relative density of four sections per slide were meaned to give one value per animal. The mean of relative density \pm SEM was calculated in AFR, D and H rats.

Drugs

Morphine sulphate was purchased from Francopia (Gentilly, France), [125 I]-F(ab')₂ IgG (100 μ Ci/ml) and [125 I]-iodosulpride from GE Healthcare Europe GMBH and [125 I]-7-OH-PIPAT from Perkin Elmer-NEN (Orsay, France). BP 897 was a generous gift from P. Sokoloff.

Statistical analysis

The results of behavioural and biochemical experiments are expressed as means \pm SEM. Behavioural data were analysed by two-way repeated-measures analysis of variance (ANOVA; between-subject for deprivation and treatment factors and within-subject for time), followed by one-way ANOVA and by Newman–Keuls for multiple comparisons. Immunohistochemistry and autoradiography data were analysed by two-way repeated-measures ANOVA (be-

tween-subject for deprivation and treatment factors and within-subject for brain area) followed by one-way ANOVA and by Newman–Keuls for comparisons. All data were analysed with Statview® software (SAS, Cary NC, USA) for Macintosh. The level chosen for statistical significance was $\alpha=5\%$.

Results

Place preference paradigm

There was a significant effect of deprivation, treatment and time factors [$F(2,256)=5.72$, $P<0.003$; $F(5,256)=17.29$, $P<0.0001$; $F(2,512)=26.87$, $P<0.0001$, respectively]. The interaction between deprivation \times treatment factors was significant [$F(10,256)=3.09$, $P<0.001$]. The interactions between time \times deprivation, time \times treatment and time \times deprivation \times treatment factors were not significant [$F(4,512)=0.137$; $F(4,392)=0.24$; $F(10,512)=1.299$; $F(20,512)=0.99$, respectively].

AFR, D and H rats were conditioned to 5 mg/kg of morphine. Post hoc analysis showed significant differences between morphine-treated D ($P<0.001$) and H ($P<0.01$) rats compared to morphine-treated AFR rats. A more prolonged conditioned effect of morphine was observed in D and H animals than in AFR rats.

There was a significant effect of treatment in AFR group [$F(5,270)=22.701$, $P<0.0001$]. Post hoc analysis revealed that there was no difference between saline/BP 897 groups whatever the dose of BP 897 (0.5 and 2 mg/kg). On the other hand, AFR rats injected with morphine at the dose of 5 mg/kg spent significantly more time than saline-treated animals in the morphine-associated compartment. BP 897 injected at the dose of 0.5 or 2 mg/kg in AFR rats significantly increased the effect of morphine in the place preference test. There was a significant effect of treatment in D group [$F(5,273)=18.584$, $P<0.0001$]. Post hoc analysis revealed that there was no difference between saline/BP 897 groups whatever the dose of BP 897 (0.5 and 2 mg/kg). On the other hand, D rats injected with morphine at the dose of 5 mg/kg spent significantly more time than saline-treated animals in the morphine-associated compartment. BP 897 injected at the dose of 0.5 or 2 mg/kg in D rats did not modify the effect of morphine in the place preference test. There was a significant effect of treatment in H group [$F(5,265)=12.254$, $P<0.0001$]. Post hoc analysis revealed that there was no difference between saline/BP 897 groups whatever the dose of BP 897 (0.5 and 2 mg/kg). On the other hand, H rats injected with morphine at the dose of 5 mg/kg spent significantly more time than saline-treated animals in the morphine-associated compartment. The effect of morphine in H rats was not changed

after injection of 0.5 mg/kg of BP 897. The dose of 2 mg/kg of BP 897 completely suppressed morphine-induced conditioning in H rats (Fig. 1).

We previously observed that D rats were more sensitive to morphine than AFR and H animals in the place preference paradigm, as they exhibited morphine place preference at the dose of 2 mg/kg in contrast to AFR and H rats (Vazquez et al. 2005b, 2006). Another experiment was therefore performed in which D animals were tested after injection of 2 mg/kg of morphine just before the conditioning phase and BP 897 at the dose of 2 mg/kg 30 min before the testing phase to evaluate the effect of BP 897 on rats showing similar sensitivity to morphine. In this experiment, morphine induced an increase in the time spent in the morphine-associated compartment in D rats [$F(2,19)=4.79$, $P=0.02$], as expected from previous study (Vazquez et al. 2005b), and BP 897 at the dose of 2 mg/kg did not modify the morphine effect (Fig. 2).

Relative density of DAT

Statistical analysis showed that there was a significant effect of structure [$F(5,190)=69.9$, $P<0.0001$], but that all other effects were not significant.

Higher levels of DAT were present in the SN compared to the core, the cone and the shell of the N.Acc. ($P<0.001$), in the VTA compared to the cone ($P<0.001$) and the shell ($P<0.01$) of the N.Acc., in the CPu compared to the core, the cone and the shell ($P<0.001$) of the N.Acc. and in the core compared to the cone ($P<0.001$) of the N.Acc. (Table 2).

Relative density of D2 receptors

Statistical analysis showed a significant effect of structure [$F(5,185)=306.1$, $P<0.0001$] and the interactions structure \times deprivation [$F(10,185)=2.78$, $P<0.01$] and structure \times deprivation \times treatment: [$F(10,185)=2.7$, $P<0.01$].

In saline AFR rats, higher levels of D2 receptors were present in the CPu compared to the core, the cone and the shell ($P<0.001$) of the N.Acc., to the SN and the VTA ($P<0.001$), in the core compared to the cone of the N.Acc., the VTA ($P<0.001$) and to the SN ($P<0.01$), and in the shell compared to the cone ($P<0.001$) of the N.Acc. and to the VTA ($P<0.01$). In saline D rats, higher levels of D2 receptors were present in the CPu compared to the shell, the core ($P<0.01$) and the cone ($P<0.001$) of the N.Acc., to the SN and the VTA ($P<0.001$), in the core compared to the cone of the N.Acc., the VTA ($P<0.001$) and to the SN ($P<0.01$) and in the shell compared to the cone of the N. Acc. and to the VTA ($P<0.05$). In saline H rats, higher levels of D2 receptors were present in the CPu compared to the core, the cone ($P<0.001$) and the shell ($P<0.01$) of the

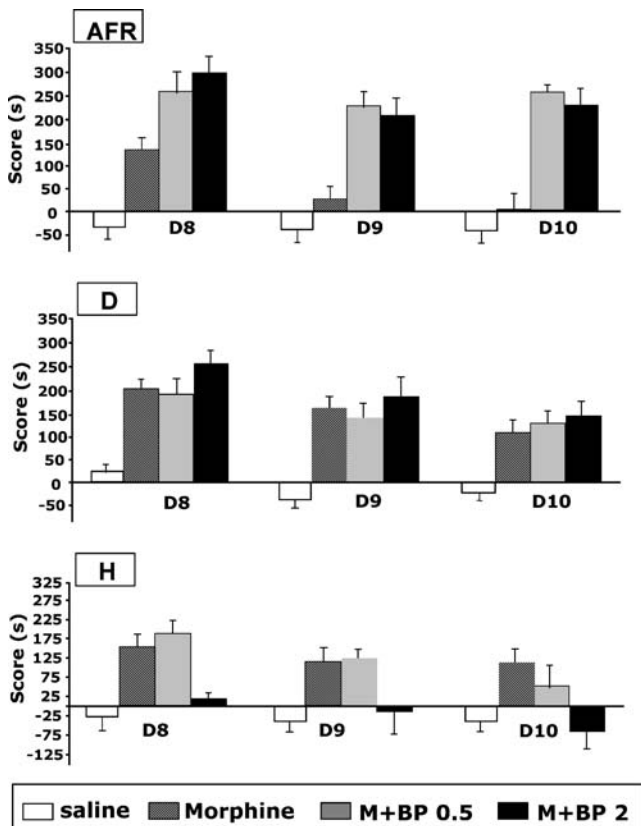


Fig. 1 Effects of BP 897 on morphine-conditioned place preference paradigm in AFR ($n=92$), D ($n=92$) and H ($n=90$) rats. During the place-conditioning period, rats received morphine (5 mg/kg) on days 2, 4 and 6 and saline on days 3, 5 and 7 immediately before confinement in the associated compartment. During the expression [day 8 (D8)] and extinction [day 9 (D9) and day 10 (D10)] periods, rats received BP 897 (0.5 or 2 mg/kg) or saline 30 min before testing in the place preference paradigm (for details see Table 1). Rats were tested at 2.5–3 months of age. Only the results of the groups I, IV, V and VI are shown in the figure. The results are expressed as a score (s) calculated as the difference between the post-conditioning and the pre-conditioning times spent in the compartment associated with the drug. M +BP 0.5=morphine + BP 897 0.5 mg/kg, M+BP 2=morphine + BP 897 2 mg/kg

N.Acc., to the SN and the VTA ($P<0.001$) and in the core compared to the cone ($P<0.05$) of the N.Acc. (Table 3).

There was a significant effect of deprivation and treatment in the SN [deprivation: $F(2,37)=3.41$, $P<0.05$, treatment: $F(1,37)=8.1$, $P<0.01$]. A significant increase in D2 receptor density was observed in the SN [$F(2,19)=5.08$, $P<0.01$] of the saline H group compared to the saline AFR (+38%) and saline D (+38%) rats. The D2 receptor density of H rats treated with morphine was decreased in the SN [$F(1,11)=10.8$, $P<0.01$], (-31%) compared to H rats treated with saline.

There was a significant effect of the interaction deprivation \times treatment in the VTA [$F(2,37)=4.12$, $P<0.05$]. A significant increase in D2 receptor density was observed in the VTA [$F(2,19)=5.55$, $P<0.01$] of the saline H group

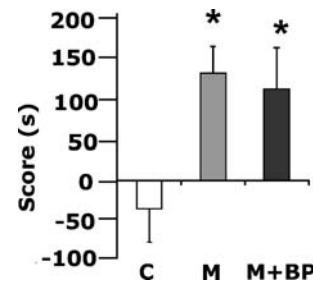


Fig. 2 Effects of BP 897 on the expression (D8) period of conditioned place preference paradigm in D rats for 2 mg/kg of morphine ($n=27$). During the place-conditioning period, rats received morphine (2 mg/kg) on days 2, 4 and 6 and saline on days 3, 5 and 7 immediately before confinement in the associated compartment. During the expression period, rats received BP 897 (2 mg/kg) or saline 30 min before testing in the place preference paradigm. Rats were tested at 2.5–3 months of age. The results are expressed as a score (s) calculated as the difference between the post-conditioning and the pre-conditioning times spent in the compartment associated with the drug. C Saline/saline, M morphine, M+BP morphine + BP 897. * $P<0.05$ vs saline/saline group, Newman–Keuls test

compared to the saline AFR (+27%) and the saline D (+31%) rats. The D2 receptor density of H rats treated with morphine was decreased in the VTA [$F(1,11)=23.9$, $P<0.001$], (-43%) compared to H rats treated with saline.

There was a significant effect of treatment and structure in D rats [treatment: $F(1,13)=5.23$, $P<0.05$; structure: $F(5,65)=142.8$, $P<0.0001$]. The D2 receptor density of D

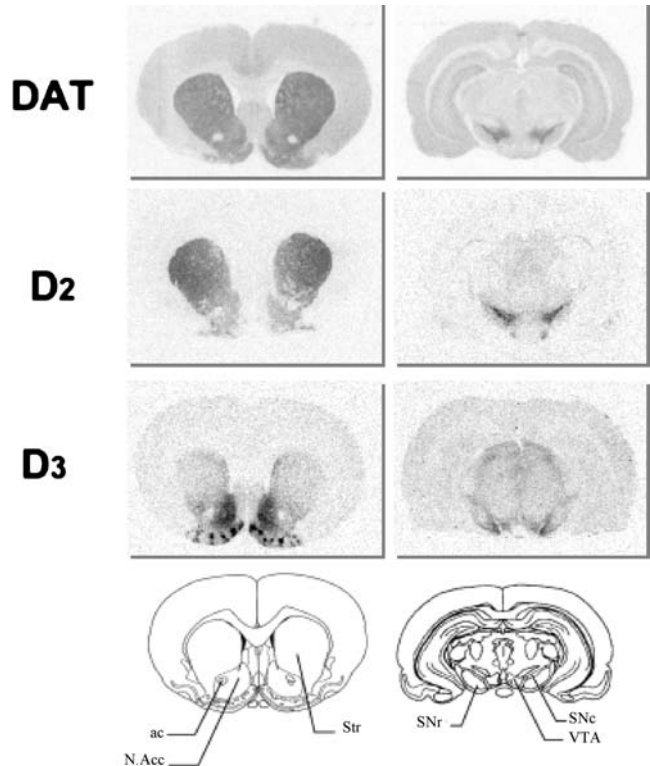


Fig. 3 Representative autoradiograms of the distribution of DAT and D2 and D3 receptors in the striatum (left side) and the mesencephalon (right side) of AFR rats (frontal brain sections)

Table 2 Relative density of DAT in the striatum and mesencephalon of AFR, D and H rats after morphine-conditioned place preference paradigm

	AFR		D		H	
	Saline (7)	Morphine (7)	Saline (8)	Morphine (8)	Saline (7)	Morphine (7)
Striatum						
CPu	231±1	236±6	238±6	237±5	233±3	233±6
N.Acc. core	206±2	206±7	206±5	207±4	207±5	202±6
N.Acc. cone	178±2	155±20	173±5	160±8	166±5	153±14
N.Acc. shell	201±2	207±14	206±9	193±5	208±8	199±9
Mesencephalon						
SN	221±9	238±13	223±9	235±13	230±5	211±6
VTA	225±5	223±8	232±7	220±8	230±6	207±10

Relative densities are the means±SEM of specific immunohistochemical labelling in nCi/mg. The number of rats is indicated in parentheses. *CPu* Caudate putamen nucleus, *N.Acc.* nucleus accumbens, *SN* substantia nigra, *VTA* ventral tegmental area

rats treated with morphine was decreased in the cone [$F(1,13)=10.22$, $P<0.01$], (−34%) and in the shell [$F(1,13)=8.6$, $P<0.01$], (−27%) of the N.Acc. compared to D rats treated with saline (Table 3).

Relative density of D3 receptors

The results of quantitative autoradiography of D3 receptor density showed a significant effect of the structure × treatment interaction [$F(5,190)=2.4$, $P<0.05$].

Higher levels of D3 receptors were present in the cone of the N.Acc. compared to the CPu ($P<0.001$), the core ($P<0.01$) of the N.Acc., the SN and the VTA ($P<0.001$), in the shell of the N.Acc. compared to the CPu, the core, the SN and the VTA ($P<0.001$). Lower levels of D3 receptors were present in CPu compared to the others structures ($P<0.001$).

A significant decrease in D3 receptor density was observed after morphine treatment in the CPu [$F(1,42)=4.74$, $P<0.05$], in the cone [$F(1,42)=6.53$, $P<0.01$] and in the shell [$F(1,42)=5.44$, $P<0.05$] of the N.Acc, but not in the SN and in the VTA (Table 4).

Discussion

This study showed that early manipulations of infant–mother relationships lead to a dysfunction of the dopaminergic system. This is revealed by both changes in the sensitivity to the reward effect of morphine after injection of BP897 and in the D2 receptor levels.

The injection of 5 mg/kg of morphine induced a more prolonged conditioned place preference in D and H rats than in AFR animals. This was expected from our previous

Table 3 Relative density of D2 receptors in the striatum and mesencephalon of AFR, D and H rats after morphine-conditioned place preference paradigm

	AFR		D		H	
	Saline (8)	Morphine (7)	Saline (8)	Morphine (7)	Saline (6)	Morphine (7)
Striatum						
CPu	183±3	179±5	187±2	168±10	173±12	170±10
N.Acc. core	103±2	98±7	104±3	82±9	92±13	90±8
N.Acc. cone	76±4	63±6	74±3	49±7*	57±7	56±8
N.Acc. shell	99±2	87±7	104±4	76±9*	93±13	80±10
Mesencephalon						
SN	85±3	81±8	85±10	78±10	118±6**	82±8*
VTA	79±3	65±6	77±6	69±9	101±6**	58±6*

Relative densities are the means±SEM of specific autoradiographic labelling in nCi/mg. The number of rats is indicated in parentheses.

CPu Caudate putamen nucleus, *N.Acc.* nucleus accumbens, *SN* substantia nigra, *VTA* ventral tegmental area

* $P<0.01$ vs respective saline group

** $P<0.01$ vs AFR saline group, Newman–Keuls test

Table 4 Relative density of the D3 receptors in the striatum and mesencephalon of AFR, D and H rats after morphine-conditioned place preference paradigm

	AFR		D		H	
	Saline (7)	Morphine (7)	Saline (8)	Morphine (8)	Saline (7)	Morphine (7)
Striatum						
CPu	22±2	21±1	25±2	18±2	21±3	18±2
N.Acc. core	53±5	50±4	56±3	40±5	45±6	43±4
N.Acc. cone	104±5	96±7	110±5	81±8	96±6	92±6
N.Acc. shell	92±6	76±10	96±6	69±11	80±8	78±8
Mesencephalon						
SN	43±2	42±3	46±2	41±3	46±2	44±3
VTA	51±2	48±2	52±2	49±3	53±4	54±4

Relative densities are the means±SEM of specific autoradiographic labelling in nCi/mg. The number of rats is indicated in parentheses. *CPu* Caudate putamen nucleus, *N.Acc.* nucleus accumbens, *SN* substantia nigra, *VTA* ventral tegmental area

deprivation studies (Vazquez et al. 2005b). No significant prolonged effect on morphine CPP has been previously found in H rats, probably due to the lower number of animals tested than in the present study (Vazquez et al. 2006).

BP 897 induced different effects in AFR, D and H rats on the expression of morphine CPP. BP 897 increased and decreased morphine-induced conditioning in AFR and H rats, respectively, and had no effect in D animals.

BP 897 injected at the doses of 0.5 or 2 mg/kg did not induce a direct effect on the expression in the place preference paradigm as reported in other studies (Duarte et al. 2003; Francès et al. 2004a). However, this compound enhanced the expression of morphine conditioning in AFR rats. These results are in agreement with data showing that the very selective D3 antagonist, SB-277011A, administered in rats impairs both acquisition and expression of heroin CPP (Ashby et al. 2003). BP 897 has been also reported to enhance the acquisition of morphine CPP in mice (Francès et al. 2004a). D3 receptors are expressed at somatodendritic and terminal levels of both dopaminergic and non-dopaminergic neurons within the nigrostriatal and mesolimbic dopaminergic system (Sokoloff et al. 1990; Diaz et al. 2000; Stanwood et al. 2000). Dopaminergic transmission is under the control of presynaptic modulation of dopamine release through D2 and/or D3 autoreceptors (Gobert et al. 1995; Tepper et al. 1997), postsynaptic modulation of D1 and D2 receptor-mediated transmission via inhibitory or excitatory postsynaptic D3 receptors (Kling-Petersen et al. 1995; Ridray et al. 1998) and/or D3 receptor-mediated modulation of dopamine reuptake (a D3-preferring agonist increases extracellular dopamine clearance and a D3 antagonist induces the opposite; Zapata and Shippenberg 2002). Regarding BP 897-induced increase in the expression of morphine CPP in AFR rats, the mechanism involved could occur via the postsynaptic D3

receptors, as the stimulation of D2/D3 autoreceptors will lead to the inverse effect, i.e. a decrease in morphine CPP (reviewed in Adell and Artigas 2004). A possible mediation by the blockade of D2/D3 autoreceptors was unexpected, owing the low effective dose of 0.5 mg/kg of BP 897. The results obtained with BP 897 in AFR rats showed that stimulation of postsynaptic D3 receptors may enhance the rewarding effect of morphine.

In contrast to AFR rats, we observed no effect of 0.5 or 2 mg/kg of BP 897 on morphine CPP in D animals. We have previously shown that D rats were more sensitive to morphine than AFR animals in the place preference paradigm. Indeed, 5 mg/kg is the first morphine active dose in AFR and H rats, while 2 mg/kg is already effective in D rats (Vazquez et al. 2005b, 2006). We therefore performed another experiment in which D animals were tested after administration of 2 mg/kg of morphine during the conditioning phase and of 2 mg/kg of BP 897 during the testing phase to evaluate the effect of BP 897 on rats showing similar sensitivity to morphine than AFR and H animals. In these conditions, no effect of BP 897 was observed on morphine CPP in D rats, emphasising the lack of sensitivity of D rats to the D3 receptor agonist at the doses tested. No significant change of D3 receptor levels was observed in saline- or morphine-treated D rats. However, this does not exclude possible difference in D3 receptor activity particularly after morphine treatment. Recent data showed that prenatal and neonatal exposure to an endocrine disruptor bisphenol-A induced an enhanced morphine rewarding effect and a decreased functional dopamine D3 receptors (Mizuo et al. 2004a, b). It was suggested that D3 receptors may play a potential role in the negative modulation of the dopamine D1/D2 receptor-dependent rewarding effects induced by μ -opioid receptor agonists (Narita et al. 2003, reviewed in Richtand et al. 2001). It was therefore possible that maternal deprivation

led to maladaptive changes of D3 receptor regulation that were revealed after morphine CPP. The lack of BP 897 effect on morphine CPP may be, at least in part, involved in the sensitivity of D rats to the rewarding effect of morphine or could be a consequence of the hypersensitivity of D rats to the rewarding effects of morphine previously reported (Vazquez et al. 2005b).

No difference of DAT density was observed in the CPu, the N.Acc., the SN and the VTA of D rats compared with AFR animals, contrary to previous data showing a decrease in the DAT relative density observed in a different maternal separation model (Brake et al. 2004). However, the lack of change in the density of DAT does not exclude possible difference in DAT activity between AFR and D rats. No change in D2 receptor levels was observed in saline-treated D animals compared with saline-treated AFR rats as previously reported (Ploj et al. 2003; Brake et al. 2004). However, D2 receptor density was significantly decreased in morphine-injected D rats compared with saline D animals. D2 receptors decreased in the cone (−34%) and in the shell (−27%) of the N.Acc., but not in the mesencephalon. The decrease in D2 receptors after morphine CPP may be caused by adaptative changes. There is direct evidence for the implication of dopamine D2 receptors in the morphine-induced rewarding effect (Maldonado et al. 1997). In addition, a decrease in D2 mRNAs and protein was found in the striatum of rodents after chronic morphine treatment (Staley and Mash 1996; Spangler et al. 2003). One can hypothesise that the semi-chronic morphine treatment used in our CPP procedure was sufficient to induce enhanced dopamine release and consequently decreased postsynaptic D2 receptor density.

Regarding H rats, BP 897 injected at the dose of 0.5 mg/kg slightly decreased morphine CPP, while 2 mg/kg completely suppressed the expression of morphine conditioning, as reported, using both a highly selective D3 antagonist (Ashby et al. 2003) or D3 agonists (Rodriguez De Fonseca et al. 1995). BP 897 acts as a partial D3 selective agonist or antagonist at low doses and as a weak D2 receptor antagonist at doses above 10 mg/kg (Pilla et al. 1999; Wood et al. 2000; Wicke and Garcia-Ladona 2001). In this regard, the BP 897 suppression of the morphine CPP in H rats could be due to its antagonistic properties. No change of D3 receptors was observed in morphine H rats compared to saline H animals. However, this does not exclude that the difference in BP 897 response may be due to modifications of D3 receptor function such as transduction pathways. In agreement, D3 receptor stimulation *in vitro* (Griffon et al. 1997) and *in vivo* (Ridray et al. 1998) produces both opposite and synergistic interaction with the cyclic AMP pathway. Both D3 and D2 receptors are located as autoreceptors on SN and VTA dopaminergic neurons (Sokoloff et al. 1990; Diaz et al. 2000; reviewed in Kalivas

and Nakamura 1999). These autoreceptors inhibit impulse flow, neurotransmitter synthesis and release of dopamine (White and Wang 1984; reviewed in Adell and Artigas 2004). Indeed, it cannot be excluded that 2 mg/kg of BP 897 acts on the D3/D2 autoreceptors. In this case, BP 897 would act to decrease the firing of dopaminergic cells, and thus, would prevent morphine CPP.

No change of D2 receptors was found in saline and morphine H rats compared to saline and morphine AFR animals, respectively, in the striatum, contrasting with the high increase in D2 receptor levels observed in the SN (38%) and the VTA (27%) of saline H rats, in agreement with previous report in handling (15 min) rats (Ploj et al. 2003). A hypothesis could be advanced that handling manipulation would lead to a hypodopaminergia in an area-dependent manner in adult rats, although further study is required. The increase in D2 binding sites in the mesencephalon of H rats could explain the antagonistic effect of 2 mg/kg BP 897 on the expression of morphine CPP. However, even if we cannot exclude this hypothesis, this seems to be unlikely, as the enhancement of D2 receptor levels was completely suppressed after morphine treatment in H animals. This latter effect on D2 receptor levels could be due to the well-known effect of morphine on the dopaminergic system in the mesencephalon to increase firing rate of neurons and enhance the release of dopamine with D2 receptor returning to normal levels as a consequence.

This study showed that maternal deprivation and handling induced different changes of D2/D3 receptor functioning revealed after morphine CPP. Maternal deprivation led to a hyposensitivity of the effect of the D3 agonist, BP 897. On the other hand, handling led to an antagonistic effect of BP 897 in morphine CPP and an increase in D2 autoreceptor density, which was restored after morphine-conditioned CPP. Our previous data showed a hypersensitivity to morphine-induced place preference conditioning and oral morphine dependence associated with a hypoactivity of enkephalinergic system in D rats, while an increase in oral morphine dependence was observed in H animals (Vazquez et al. 2005b, 2006). Deprivation of the mother and littermates constitutes a more severe postnatal manipulation than human brief handling. However, although more drastic changes on opioidergic system were found in maternally D rats than in H animals, the present results emphasise that human brief handling constitutes in itself an experimental treatment.

Altogether, our results showed that early manipulations of infant–mother relationships may have different consequences on the balance of opioidergic and dopaminergic neurotransmission and could explain the different pharmacological effects of the partial D3 agonist in morphine CPP paradigm. These results reinforced the idea that the D3

receptors played an important role in homeostatic control of dopaminergic system activity in relation with opiate systems. However, beneficial use of agonists or antagonists, in a therapeutically point of view, may differ according to imbalance status of the systems, as we point it out using deprivation versus handling models.

Acknowledgement This study was supported by the Institut National de la Santé et de la Recherche Médicale. S. Weiss was a recipient of a grant from the “Société de Tabacologie” (France).

References

- Adell A, Artigas F (2004) The somatodendritic release of dopamine in the ventral tegmental area and its regulation by afferent transmitter systems. *Neurosci Biobehav Rev* 28:415–431
- Anisman H, Zaharia MD, Meaney MJ, Meralis Z (1998) Do early-life events permanently alter behavioural and hormonal responses to stressors. *Int J Dev Neurosci* 16:149–164
- Ashby CR Jr, Paul M, Gardner EL, Heidbreder CA, Hagan JJ (2003) Acute administration of the selective D3 receptor antagonist SB-277011A blocks the acquisition and expression of the conditioned place preference response to heroin in male rats. *Synapse* 48:154–156
- Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B, Schwartz JC (1991) Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with D2 receptor mRNA. *Brain Res* 564:203–219
- Brake WG, Zhang TY, Diorio J, Meaney MJ, Gratton A (2004) Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *Eur J Neurosci* 19:1863–1874
- Caine SB, Koob GF (1993) Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. *Science* 260:1814–1816
- Depoortere R, Perrault G, Sanger DJ (1996) Behavioural effects in the rat of the putative dopamine D3 receptor agonist 7-OH-DPAT: comparison with quinpirole and apomorphine. *Psychopharmacology (Berl)* 124:231–240
- Diaz J, Pilon C, Le Foll B, Gros C, Triller A, Schwartz JC, Sokoloff P (2000) Dopamine D3 receptors expressed by all mesencephalic dopamine neurons. *J Neurosci* 20:8677–8684
- Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* 85:5274–5278
- Duarte C, Lefebvre C, Chaperon F, Hamon M, Thiebot MH (2003) Effects of a dopamine D3 receptor ligand, BP 897, on acquisition and expression of food-, morphine-, and cocaine-induced conditioned place preference, and food-seeking behavior in rats. *Neuropsychopharmacology* 28:1903–1915
- Francès H, Smirnova M, Leriche L, Sokoloff P (2004a) Dopamine D3 receptor ligands modulate the acquisition of morphine-conditioned place preference. *Psychopharmacology (Berl)* 175:127–133
- Francès H, Le Foll B, Diaz J, Smirnova M, Sokoloff P (2004b) Role of DRD3 in morphine-induced conditioned place preference using DRD3-knockout mice. *Neuroreport* 15:2245–2249
- Francis DD, Caldji C, Champagne FN, Plotsky PM, Meaney MJ (1999) The role of corticotropin-releasing factor-norepinephrine systems in mediating the effects of early experience on the development of behavioral and endocrine responses to stress. *Biol Psychiatry* 46:1153–1166
- Garcia-Ladona FJ, Cox BF (2003) BP897, a selective dopamine D3 receptor ligand with therapeutic potential for the treatment of cocaine-addiction. *CNS Drug Rev* 9:141–158
- Gobert A, Rivet JM, Audinot V, Cistarelli L, Spedding M, Vian J (1995) Functional correlates of dopamine D3 receptor activation in the rat in vivo and their modulation by the selective agonist (+)-S 14297:II. Both D2 and “silent” D3 autoreceptors control synthesis and release in mesolimbic, mesocortical and nigrostriatal pathways. *J Pharmacol Exp Ther* 275:899–913
- Griffon N, Pilon C, Sautel F, Schwartz JC, Sokoloff P (1997) Two intracellular signalling pathways for the dopamine D3 receptor: opposite and synergistic interactions with cyclic AMP. *J Neurochem* 68:1–9
- Hall FS (1998) Social deprivation of neonatal, adolescent, and adult rats distinct neurochemical and behavioral consequences. *Crit Rev Neurobiology* 12:129–162
- Hall FS, Wilkinson LS, Humby T, Robbins TW (1999) Maternal deprivation of neonatal rats produces enduring changes in dopamine function. *Synapse* 32:37–43
- Huot RL, Thivikraman KV, Meaney MJ, Plotsky PM (2001) Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. *Psychopharmacology (Berl)* 158:366–373
- Jaworski JN, Francis DD, Brommer CL, Morgan ET, Kuhar MJ (2005) Effects of early maternal separation on ethanol intake, GABA receptors and metabolizing enzymes in adult rats. *Psychopharmacology (Berl)* 181:8–15
- Kalivas PW, Nakamura M (1999) Neural systems for behavioral activation and reward. *Curr Opin Neurobiol* 9:223–227
- Kehoe P, Shoemaker WJ, Triano L, Hoffman J, Arons C (1996) Repeated isolation in the neonatal rats produces alterations in behavior and ventral striatal dopamine release in the juvenile after amphetamine challenge. *Behav Neurosci* 110:1435–1444
- Kling-Petersen T, Ljung E, Wollter L, Svensson K (1995) Effects of dopamine D3 preferring compounds on conditioned place preference and intracranial self-stimulation in the rat. *J Neural Transm Gen Sect* 101:27–39
- Koob GF, LeMoal M (2001) Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* 24:97–129
- Kosten TA, Miserendino MJ, Kehoe P (2000) Enhanced acquisition of cocaine self-administration in adult rats with neonatal isolation stress experience. *Brain Res* 875:44–50
- Kosten TA, Zhang XY, Kehoe P (2005) Neurochemical and behavioral responses to cocaine in adult male rats with neonatal isolation experience. *J Pharmacol Exp Ther* 314:661–667
- Le Foll B, Schwartz JC, Sokoloff P (2000) Dopamine D3 receptor agents as potential new medications for drug addiction. *Eur Psychiatry* 15:140–146
- Maldonado R, Saiardi A, Valverde O, Samad TA, Roques BP, Borrelli E (1997) Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. *Nature* 388:586–589
- Marmendal M, Roman E, Eriksson CJ, Nylander I, Fahlke C (2004) Maternal separation alters maternal care, but has minor effects on behavior and brain peptides in adult offspring. *Dev Psychobiol* 45:140–152
- Martres MP, Bouthenet ML, Salés N, Sokoloff P, Schwartz JC (1985) Widespread distribution of brain dopamine receptors evidenced with [¹²⁵I]-iodosulpride, a highly selective ligand. *Science* 228:752–755
- Martres MP, Demeneix B, Hanoun N, Hamon M, Giros B (1998) Up- and down-expression of the dopamine transporter by plasmid DNA transfer in the brain. *Eur J Neurosci* 10:3607–3616
- Matthews K, Robbins TW (2003) Early experience as a determinant of adult behavioural responses to reward: the effects of repeated maternal separation in the rat. *Neurosci Biobehav Rev* 27:45–55

- Matthews K, Robbins TW, Everitt BJ, Caine SB (1999) Repeated neonatal maternal separation alters intravenous cocaine self-administration in adult rats. *Psychopharmacology (Berl)* 141:123–134
- Mizuo K, Narita M, Yoshida T, Narita M, Suzuki T (2004a) Functional changes in dopamine D3 receptors by prenatal and neonatal exposure to an endocrine disruptor bisphenol-A in mice. *Addict Biol* 9:19–25
- Mizuo K, Narita M, Miyagawa K, Narita M, Okuno E, Suzuki T (2004b) Prenatal and neonatal exposure to bisphenol-A affects the morphine-induced rewarding effect and hyperlocomotion in mice. *Neurosci Lett* 356:95–98
- Narita M, Mizuo K, Mizoguchi H, Sakata M, Narita M, Tseng LF, Suzuki T (2003) Molecular evidence for the functional role of dopamine D3 receptor in the morphine-induced rewarding effect and hyperlocomotion. *J Neurosci* 23:1006–1012
- Paxinos G, Watson C (1997) *The rat brain in stereotaxic coordinates*. Academic, New York
- Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz JC, Everitt BJ, Sokoloff P (1999) Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature* 400:371–375
- Ploj K, Roman E, Nylander I (2003) Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats. *Neuroscience* 121:787–799
- Pryce CR, Feldon J (2003) Long-term neurobehavioural impact of the postnatal environment in rats: manipulations, effects and mediating mechanisms. *Neurosci Biobehav Rev* 27:57–71
- Richtand NM, Woods SC, Berger SP, Strakowski SM (2001) D3 dopamine receptor, behavioral sensitization, and psychosis. *Neurosci Biobehav Rev* 25:427–443
- Ridray A, Griffon N, Mignon V, Souil E, Carboni S, Diaz J, Schwartz JC, Sokoloff P (1998) Coexpression of dopamine D1 and D3 receptors in islands of Calleja and shell of nucleus accumbens of the rat: opposite and synergistic functional interactions. *Eur J Neurosci* 10:1676–1686
- Rodriguez De Fonseca F, Rubio P, Matin-Calderon JL, Caine SB, Koob GF, Navarro M (1995) The dopamine receptor agonist 7-OH-DPAT modulates the acquisition and expression of morphine-induced place preference. *Eur J Pharmacol* 274:47–55
- Sellings L, Clarke P (2003) Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. *J Neurosci* 23:6295–6303
- Shippenberg TS, Bals-Kubik R, Herz A (1993) Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. *J Pharmacol Exp Ther* 265:53–59
- Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature* 347:146–151
- Spangler R, Wittkowski KM, Goddard NL, Avena NM, Hoebel BG, Leibowitz SF (2003) Elevated D3 dopamine receptor mRNA in dopaminergic and dopaminoreceptive regions of the rat brain in response to morphine. *Mol Brain Res* 111:74–83
- Staley JK, Mash DC (1996) Adaptive increase in D3 dopamine receptors in the brain reward circuits of human cocaine facilities. *J Neurosci* 16:6100–6106
- Stanwood GD, Artymyshyn RP, Kung HF, Lucki I, McGonigle P (2000) Quantitative autoradiographic mapping of rat brain dopamine D3 binding with [¹²⁵I]7-OH-PIPAT: evidence for the presence of D3 receptors on dopaminergic and nondopaminergic cell bodies and terminals. *J Pharmacol Exp Ther* 295:1223–1231
- Tepper JM, Sun BC, Martin LP, Creese I (1997) Functional roles of dopamine D2 and D3 autoreceptors on nigrostriatal neurons analyzed by antisense knockdown in vivo. *J Neurosci* 17:2519–2530
- Van Ree JM, Niesink RJ, Van Wolfswinkel L, Ramsey NF, Kornet MM, Van Furth WR, Vanderschuren LJ et al (2000) Endogenous opioids and reward. *Eur J Pharmacol* 405:89–101
- Vazquez V, Farley S, Giros B, Daugé V (2005a) Maternal deprivation increases behavioural reactivity to stressful situations in adulthood: suppression by the CCK2 antagonist L365,260. *Psychopharmacology (Berl)* 181:706–713
- Vazquez V, Penit-Soria J, Durand C, Besson MJ, Giros B, Daugé V (2005b) Maternal deprivation increases vulnerability to morphine dependence and disturbs the enkephalinergic system in adulthood. *J Neurosci* 25:4453–4462
- Vazquez V, Penit-Soria J, Durand C, Besson MJ, Giros B, Daugé V (2006) Brief early handling increases morphine dependence in adult rats. *Behav Brain Res* 170:211–218
- White FJ, Wang RY (1984) A10 dopamine neurons: role of autoreceptors in determining firing rate and sensitivity to dopamine agonists. *Life Sci* 34:1161–1170
- Wicke K, Garcia-Ladona J (2001) The dopamine D3 receptor partial agonist, BP 897, is an antagonist at human dopamine D3 receptors and at rat somatodendritic dopamine D3 receptors. *Eur J Pharmacol* 424:85–90
- Wise RA (2004) Dopamine, learning and motivation. *Nat Rev Neurosci* 5:483–494
- Wood MD, Boyfield I, Nash DJ, Jewitt FR, Avenell KY, Riley GJ (2000) Evidence for antagonist activity of the dopamine D3 receptor partial agonist, BP 897, at human dopamine D3 receptor. *Eur J Pharmacol* 407:47–51
- Zapata A, Shippenberg TS (2002) D(3) receptor ligands modulate extracellular dopamine clearance in the nucleus accumbens. *J Neurochem* 81:1035–1042
- Zhang XY, Sanchez H, Kehoe P, Kosten TA (2005) Neonatal isolation enhances maintenance but not reinstatement of cocaine self-administration in adult male rats. *Psychopharmacology (Berl)* 177:391–399