

# Behavioral Expression of Opiate Withdrawal is Altered after Prefrontocortical Dopamine Depletion in Rats: Monoaminergic Correlates

Emilio Fdez. Espejo, Ph.D., M.D., María I. Serrano, Ph.D., M.D., Stéphanie Caillé, Ph.D., and Luis Stinus, Ph.D.

*The objective of this study was to establish the effects of prefrontocortical dopamine depletion on opiate withdrawal and prefrontocortical neurochemical changes elicited by morphine dependence and withdrawal. The dopaminergic content was also measured in the nucleus accumbens during withdrawal, in order to detect reactive changes induced by prefrontocortical lesion. Withdrawal was induced by naloxone in morphine-dependent rats. Monoamine levels were analyzed post-mortem by high performance liquid chromatography. The results showed that chronic morphine dependence did not modify basal levels of monoamines in sham rats, revealing neuroadaptation of prefrontocortical dopamine, noradrenaline and serotonin systems to chronic morphine. The neuroadaptive phenomenon remained after prefrontocortical lesion (> 79% dopamine depletion). On the other hand, a strong increase of dopamine, noradrenaline, and serotonin contents in the medial prefrontal cortex of sham rats was detected during opiate withdrawal. However, in lesioned rats, the increase of prefrontocortical dopamine and serotonin*

*content, but not that of noradrenaline, was much lower. In the nucleus accumbens, prefrontocortical lesion reactively enhanced the dopaminergic tone and, although opiate withdrawal reduced dopaminergic activity in both sham and lesioned rats, this reduction was less intense in the latter group. At a behavioral level, some symptoms of physical opiate withdrawal were exacerbated in lesioned rats (writhing, mastication, teeth-chattering, global score) and exploration was reduced. The findings hence indicate that: (i) prefrontocortical monoaminergic changes play a role in the behavioral expression of opiate withdrawal; (ii) the severity of some withdrawal signs are related to the dopaminergic and serotonergic tone of the medial prefrontal cortex rather than to the noradrenergic one, and (iii) an inverse relationship between mesocortical and mesolimbic dopaminergic systems exists.*

**[Neuropsychopharmacology 25:204–212, 2001]**

© 2001 American College of Neuropsychopharmacology.  
Published by Elsevier Science Inc.

**KEY WORDS:** *Medial prefrontal cortex; Nucleus accumbens; Opiate withdrawal; Dopamine; Noradrenaline; Serotonin; 6-hydroxydopamine*

From the Departamento de Fisiología Médica (EFE) and Departamento de Farmacología (MIS), Facultad de Medicina, Universidad de Sevilla, Sevilla, Spain; and Laboratoire de Neurobiologie des désadaptations, Université de Bordeaux II, Bordeaux, France (SC, LS).

Address correspondence to: Emilio F. Espejo, Departamento de Fisiología Médica y Biofísica, Facultad de Medicina, Universidad de Sevilla, Av. Sanchez Pizjuan 4, E-41009 Sevilla, Spain.

Received June 7, 2000; revised October 6, 2000; accepted December 21, 2000.

When morphine is chronically perfused into the prefrontal cortex, the systemic administration of naloxone does not precipitate a withdrawal syndrome (Bozarth 1994). However, during naloxone-precipitated withdrawal, a significant enhancement of c-fos expression is detected in the prefrontal cortex (Hayward et al. 1990), along with a strong increase of dopamine (DA) and noradrenaline (NA) release (Redmond 1987; Rossetti et al. 1993; Bassareo et al. 1995). These facts point towards a possible role of prefrontocortical monoamines on opiate withdrawal. Furthermore, it has been postulated that

the severity of withdrawal parallels changes in NA cortical output, and that some of the behavioral signs of withdrawal may be mediated by an increased activity of the frontocortical noradrenergic system (Gunne 1959, 1963; Rossetti et al. 1993; Redmond 1987). Prefrontal serotonin (5-HT) appears not to be involved in physical dependence to morphine (Blasig et al. 1976; Bhargava 1979; Silverstone et al. 1993), except for a possible role on jumping behavior during opiate withdrawal (Cervo et al. 1983). However it is known that a complex interdependence between biogenic amines exists within the prefrontal cortex, suggesting that changes in dopamine and noradrenaline could also affect serotonin neurotransmission after morphine (Gobert et al. 1998).

Within the prefrontal cortex, the medial part (mPFC) is a hub area which plays an important role in the DA system network, since it receives input from the mesocortical DA system, and sends efferents to the nucleus accumbens, dorsal striatum and midbrain DA cell groups. In this context, an inverse relationship between mesocortical and mesolimbic DA systems appears to exist, since the prefrontal cortex possesses corticofugal excitatory neurons that innervate striatal and DA midbrain cell regions, and that prefrontocortical dopaminergic activity inhibits these corticofugal neurons (Glowinski et al. 1984; Grace 1991). Furthermore, the medial prefrontal cortex receives important noradrenergic and serotonergic inputs from locus coeruleus and raphe nuclei, respectively.

The objectives of this study were to establish the effects of selective dopamine depletion in the medial prefrontal cortex on somatic opiate withdrawal and prefrontocortical neurochemical changes elicited by morphine dependence and withdrawal. Dopamine, noradrenaline and serotonin neurotransmission in the mPFC were evaluated. Dopamine content was also measured in the nucleus accumbens during withdrawal in order to detect if an inverse relationship between mesocortical and mesolimbic dopamine systems exists. Rats were rendered dependent by subcutaneous implantation of morphine pellets, and withdrawal was precipitated by the opiate antagonist naloxone.

## MATERIALS AND METHODS

### Subjects

Male Wistar rats (275–325 g) from the breeding colony of the Faculty of Medicine of the University of Seville, Spain, were housed in groups of seven. Laboratory temperature was kept at  $22 \pm 1^\circ\text{C}$ , and a 12-h light-dark cycle (lights on at 08:00 hours) was maintained throughout the experiment. Food (lab chow) and water were available *ad libitum*.

### Surgery and 6-OHDA Lesion

Anaesthesia was induced by an intraperitoneal injection of chloral hydrate (425 mg/kg). Thirty minutes before 6-OHDA lesion, rats were injected with desipramine (15 mg/kg, i.p.) in order to protect noradrenergic terminals from 6-hydroxydopamine toxicity. Rats were then placed into a Kopf stereotaxic apparatus with the incisor bar set 3.3 mm below the interaural line. After scalp incision, burr holes were drilled over the injection sites and a blunted 30-Gauge cannula, connected to a 10  $\mu\text{l}$  Hamilton syringe, was lowered to the injection site.

The following coordinates were used: AP = +3.5 with respect to bregma, L =  $\pm 0.8$ , V = -3.3 (-4.3 below skull surface). At each injection site, 1  $\mu\text{l}$  of a solution containing 6-OHDA (2.5  $\mu\text{g}/\text{l}$ ), 0.9% saline, and 0.2% ascorbic acid was injected over 6 min. The cannula was left in place for 1 min after the injection, and then slowly withdrawn at the end of injections. Immediately after surgery, rats were injected with penicillin (100,000 I.U., i.m.). Sham-operated rats followed the same protocol except for the fact that a 6-OHDA-free solution containing 0.9% NaCl and 0.2% ascorbic acid was injected.

### Drug Procedure and Groups

Two weeks after 6-OHDA-induced or sham lesion, rats were subcutaneously implanted in the back with one placebo or morphine pellet (75 mg, National Institute of Drug Abuse, Baltimore, MD, USA), under deep anaesthesia. The incision was sealed with surgical clips and antiseptic applied to the wound area. One 75 mg morphine pellet maintains a constant level of morphine dependence during at least 14 days (Gold et al. 1994). Placebo rats (sham,  $n = 9$ ; lesioned or 6-OHDA group,  $n = 10$ ) served for HPLC measurements of non-treated naive animals. Morphine dependent rats (sham morphine dependence,  $n = 10$ , 6-OHDA morphine dependence group,  $n = 9$ ) were used for studies of chronic morphine dependence. Finally, the remainder morphine-treated animals served for studies of naloxone-induced opiate withdrawal (sham withdrawal,  $n = 10$ ; 6-OHDA withdrawal,  $n = 9$ ).

Naloxone hydrochloride was dissolved in saline (0.9% NaCl) and used to precipitate morphine withdrawal, being supplied by Research Biochemicals International (Natick, MA, USA). Every rat received saline 0, 100, 500, and 1000  $\mu\text{g}/\text{kg}$  naloxone, at 1, 3, 5, and 7 days after pellet implantation, following a within-subject design and progressively changing the initial dose, in order to minimize learning effects of repeated tests (Maldonado et al. 1992; Gold et al. 1994). Naloxone was injected immediately before experimental tests, at a volume of 1 ml/kg body weight.

## Behavioral Procedure

Animals were tested in a cage with transparent cover (20 × 40 × 25 cm) to allow videotaping. Each test lasted 10 min, a duration of exposure which allows the animal to display most of its behavioral responses during the withdrawal syndrome. Every test was carried out during the light period (13:00 to 18:00 hours). Behavior was videotaped under white light illumination, and video tapes were later played and behavior analysed automatically after direct keyboard entry to a computer programmed to perform statistical and ethological analyses. Tape speed could be modified (×1, ×1/5) for scoring fast behavioral patterns more accurately. Behavior was analysed by two highly-trained observer blind for treatment (inter-rater reliability ≥ 0.9).

Behavior was recorded by using the complete sampling method (Slater 1978). An ethogram comprising seven behavioral patterns was employed. Table 1 shows pattern denominations, and brief descriptions, which were taken partially from several authors (Grant and Mackintosh 1963; Barnett 1975; Gmerek 1988; Espejo et al. 1994, 1995). Several vegetative signs were also quantified: weight loss, intensity of chromodacryorrhea, and rhinorrhea, as well as number of animals suffering from diarrhea. Chromodacryorrhea and rhinorrhea were scored as 0 (absent), 1 (low), 2 (moderate), 3 (intense), and 4 (very intense). Weight loss was evaluated before (matching group) and 1 hour after each test. Each 1% loss above the matching weight was quantified as 1. Morphine withdrawal syndrome was also rated according to the "etho-score" (Espejo et al. 1995), whose formula is as follows:

$$\text{Etho-score} = (\text{MF}/10) + \text{WL}$$

where MF = mastication frequency and WL = weight loss.

## Neurochemical Analysis

Monoamine levels were measured in the medial PFC in every group and in the nucleus accumbens in controls and abstinent rats. Six days after placebo or morphine pellet implantation, placebo rats and morphine-treated rats without naloxone were killed by decapitation followed by immediate head freezing in liquid nitrogen for 10s. This procedure allowed the determination of monoamine levels in rats with placebo pellets and those with chronic morphine dependence as explained. On the other hand, monoamine levels were also determined in tissue taken of morphine dependent rats during precipitated withdrawal. Thus, on day 7 after pellet implantation, three rats per group were killed by decapitation 80 min after receiving the last and highest naloxone dose (1000 µg/kg), followed by immediate head freezing in liquid nitrogen for 10s.

The frozen heads were later stored at -4°C to allow them to reach a more manageable temperature before brain removal. Bilateral medial prefrontal cortex and nucleus accumbens were localized, sectioned, and immediately stored at -80°C. Later, tissue samples were homogenized in 0.5 ml of an ice-cold solution containing 0.4 M HClO<sub>4</sub>, 0.5M sodium acetate, and 0.5 M acetic acid, and centrifuged at 27,000 rpm for 60 min at 4°C. The supernatants were decanted and filtered through a 0.45 µm filter (Sartorius), and frozen at -80°C until high liquid chromatography (HPLC) assay. The electrochemical performance was based essentially on the method described by Saito et al (1992).

Aliquots (10 µl) of each sample were injected directly into the HPLC system (System Gold, Beckman), consisting of a solvent delivery pump with a pulse-dampener, an automatic sample injector (Carnegie Medicine) and an analytical C18 reverse-phase column (Ultrasphere 3 µm particle size, 75 × 4.6 mm ID; Beckman). The ESA

**Table 1.** Ethogram of the Rat's Behavior during Morphine Withdrawal

| Pattern          | Description  |
|------------------|--|
| Exploration      | The rat explores the environment, including quiet or mobile exploration, such as walking or rearing.   |
| Grooming         | The rat licks or scratches its fur or hands, licks its genitalia, or cleans the face with forelegs   |
| Wet dog shake    | The rat shakes its body, or moves the hand with quick sideway movements  |
| Writhing posture | It is characterized by the presence of writhes, which are defined as episodes of hyperextension of the neck along with contraction of abdominal muscles so that the abdomen touches the floor. The rat can also elongate the body, yawn, or masticate. While moving, the rat gaits at short steps. |
| Mastication      | The act of masticating and/or swallowing   |
| Jumping          | Leaping off the surface of the cage  |
| Teeth-chattering | The rat rapidly clicks teeth together  |

model 5100 A Coulochem electrochemical detection system consisted of a model 5021 conditioning cell (detector setting +400 mV) followed in sequence by a model 5011 dual electrode analytical cell (cell 1: +100 mV; cell 2: -260 mV). The output signal from the final electrode was amplified by a 5100 A controller and relayed to an integrator (Model 106, Beckman). The detection limit of the system was 0.1 pg/ $\mu$ l.

The mobile phase for the separation of catecholamines and their metabolites was a mixture of 0.075 M Na<sub>2</sub>HPO<sub>4</sub>, 1.2 mM sodium heptanosulfonate, 0.097 mM EDTA, and 8% methanol (v/v) adjusted to pH 3.6. The buffer solution was filtered through a 0.45  $\mu$ m membrane filter and degassed. The flow rate was set to 1.7 ml/min and pressure was around 2000 psi. The mobile phase was recycled for two weeks of continuous use before being replaced with fresh solution. The entire chromatographic system was run at ambient temperature. Peaks of biogenic amines and metabolites were identified by comparing the retention time of each peak in the sample solution with that in the standard solution. The program System Gold 2.01 (Beckman) was used to calculate monoamines levels in each sample. The contents of DA, NA, and 5-HT along with those of the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA) were quantified. Dopamine and serotonin turnovers were estimated by the DOPAC/DA and the 5-HIAA/5-HT ratios, respectively.

**Statistics and Ethics**

Neurochemical data were analysed by using the Student's t-test for comparison of independent groups. Behavioral results were analysed by using two-way ANOVA (group as between factor, naloxone dose as within variable), followed by the Student's t-test for comparison of the two groups at the same dose point. Experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

**RESULTS**

**Neurochemical Data**

Regarding dopamine levels, 6-OHDA lesion induced a profound reduction of mPFC DA (-79%, *p* < .01). Chronic morphine treatment was not accompanied by changes in mPFC DA levels in sham rats vs sham placebo, and lesioned rats showed similar percent changes during morphine dependence to those observed in sham animals, as shown in Table 2. These data indicates the emergence of neuroadaptation to chronic morphine in both sham and lesioned animals. During opiate withdrawal, a huge increase of mPFC dopamine content

**Table 2.** Monoamine Levels (pg/mg Wet Weight of Tissue) in the Medial Prefrontal Cortex in Sham and 6-OHDA-Lesioned Rats, with Placebo or Morphine Pellets (during Chronic Morphine Dependence and Precipitated Withdrawal)

| Group                      | DA                              | DOPAC                         | HVA                             | NA                         | 5-HT                         | 5-HIAA                     | DOPAC/DA                  | 5-HIAA/5-HT |
|----------------------------|---------------------------------|-------------------------------|---------------------------------|----------------------------|------------------------------|----------------------------|---------------------------|-------------|
| Sham placebo               | 128.5 ± 33.1                    | 32.4 ± 12.1                   | 211.2 ± 24.5                    | 184.8 ± 35.5               | 505.5 ± 78.2                 | 401.1 ± 76.6               | 0.25 ± 0.1                | 0.8 ± 0.3   |
| Sham morphine dependence   | 150.7 ± 34.2                    | 45.5 ± 13.3                   | 232.2 ± 21.1                    | 187.3 ± 16.5               | 565.2 ± 43.2                 | 398.8 ± 29.2               | 0.3 ± 0.1                 | 0.7 ± 0.5   |
| Sham withdrawal            | 3653.3 ± 505.8***               | 989.6 ± 210.1***              | 945.6 ± 180.8**                 | 391.9 ± 41.5**             | 1162.3 ± 281.2**             | 2239.3 ± 670.2**           | 0.27 ± 0.2                | 1.9 ± 0.4** |
| 6-OHDA placebo             | 26.8 ± 12.2++                   | 19.8 ± 10.2+                  | 166.8 ± 28.5+                   | 199.3 ± 42.2               | 395.7 ± 40.1+                | 379.2 ± 65.5               | 0.7 ± 0.2++               | 0.9 ± 0.4   |
| 6-OHDA morphine dependence | 19.8 ± 14.2**                   | 20.1 ± 5.7**                  | 123.2 ± 18.2**                  | 181.8 ± 17.7               | 423.7 ± 38.1*                | 377.1 ± 26.8               | 1 ± 0.3**                 | 0.9 ± 0.3   |
| 6-OHDA withdrawal          | 410.6 ± 202.3 <sup>oo</sup> ### | 436 ± 182.1 <sup>oo</sup> ### | 510.5 ± 192.2 <sup>oo</sup> ### | 331.4 ± 46.8 <sup>oo</sup> | 639.5 ± 134.6 <sup>o</sup> # | 709 ± 350.1 <sup>o</sup> # | 1.06 ± 0.3 <sup>o</sup> # | 1.1 ± 0.3   |

Mean ± SEM. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001 vs. sham placebo; +*p* < 0.05, ++*p* < 0.01, +++*p* < 0.001 vs. sham morphine dependence; <sup>o</sup>*p* < 0.05, <sup>oo</sup>*p* < 0.01, <sup>ooo</sup>*p* < 0.001 vs. sham withdrawal; #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001 vs. sham morphine dependent group (Student's t-test). Abbreviations: DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; NA, noradrenaline; 5-HT, serotonin; 5-HIAA, 5-hydroxyindole-3-acetic acid.

was observed in sham (+2324.2%,  $p < .001$ ) and lesioned animals (+1973.7%,  $p < .001$ ) vs. corresponding morphine dependent rats although, if absolute values (pg/mg tissue) are considered, the difference between both groups during opiate withdrawal was found to be significant ( $p < .001$ ), being values much lower in lesioned rats (−88.7%). Similar changes were observed for DOPAC and HVA, dopamine metabolites (Table 2).

Prefrontocortical 6-OHDA lesion did not alter mPFC NA levels (Table 2). Chronic morphine treatment was not accompanied by changes in mPFC NA levels in sham rats vs sham placebo, and lesioned rats showed similar changes to those observed in sham animals. These data indicate that chronic morphine does not alter basal noradrenaline levels in the mPFC. During opiate withdrawal, a strong increase of noradrenaline content was observed in sham (+109.2%,  $p < .01$ ) and lesioned animals (+82.3%,  $p < .01$ ) vs. corresponding morphine dependent rats. No significant differences in NA levels were observed between both groups during opiate withdrawal.

Prefrontocortical 5-HT levels were significantly reduced after prefrontocortical 6-OHDA lesion (−21.7%,  $p < .05$ ). Chronic morphine treatment was not accompanied by significant percent changes in mPFC serotonin levels in sham and lesioned rats. During opiate withdrawal, an increase of serotonin content was observed in sham (+ 105.6%,  $p < .01$ ) and lesioned animals (+50.9%,  $p < .05$ ) vs. corresponding morphine dependent rats, although the absolute values were found to be significantly higher in sham than lesioned animals (+44.9%,  $p < .05$ ). Similar changes were observed for 5-HIAA, the serotonin metabolite.

The ratio DOPAC/DA, indicative of dopamine turnover, was found to be similar in every group of sham rats, being significantly enhanced in every group of lesioned rats (placebo, +180%; dependent, +233.3%; withdrawal, +337%,  $p < .01$ ) vs. corresponding sham groups. Serotonin turnover, as estimated through the 5-HIAA/5-HT ratio, was not modified after prefrontocortical lesion or chronic morphine, being significantly en-

hanced in sham rats during opiate withdrawal (+171.4%,  $p < .01$ ), but not in lesioned animals.

Regarding neurochemical changes in the nucleus accumbens, 6-OHDA lesion per se induced an increase in dopamine and metabolites levels in rats with placebo (DA, +35%,  $p < .05$ ; DOPAC, +65.8%; HVA, +66.8%,  $p < .01$ ), indicating that there is an inverse relationship between prefrontocortical and accumbal dopaminergic tone (Table 3). During morphine dependence, the difference between accumbal DA levels of both sham and lesioned groups was further enhanced (+88.7% in lesioned rats,  $p < .01$ ). During opiate withdrawal, a significant reduction of dopamine content was observed in sham (−75.8%,  $p < .01$ ) and lesioned rats (−51%,  $p < .01$ ) vs. corresponding morphine dependent groups, together with an enhancement of DA turnover in sham rats only (+236.8%,  $p < .01$  vs. sham placebo). However, although DA percent changes were similar, dopamine content (pg/mg tissue) in the nucleus accumbens was found to be significantly higher in lesioned than in sham rats during withdrawal (+283.1%,  $p < .01$ ), indicating that the accumbal dopaminergic reduction during withdrawal was lower after 6-OHDA lesion.

### Behavioral Data During Morphine Withdrawal

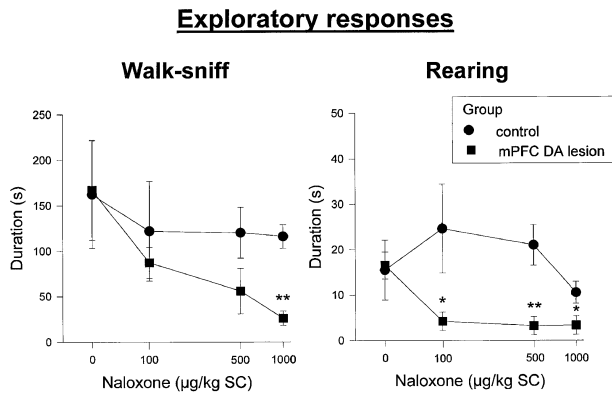
Two-way ANOVA indicated significant interaction effects (3, 48 df) for sniffing duration ( $F = 3.2$ ,  $p < .05$ ), rearing duration ( $F = 4.2$ ,  $p < .05$ ), writhing duration ( $F = 5.7$ ,  $p < .01$ ), writhing latency ( $F = 2.9$ ,  $p < .05$ ), number of mastications ( $F = 12.4$ ,  $p < .0001$ ), number of teeth-chattering ( $F = 19.9$ ,  $p < .0001$ ), and etho-score values ( $F = 11.2$ ,  $p < .001$ ).

As for exploratory responses, post-hoc analyses revealed that sniffing duration was significantly lower in lesioned rats at 1000  $\mu\text{g}/\text{kg}$  naloxone ( $t = 6.9$ ,  $p < .01$ ), and rearing duration was significantly reduced in lesioned rats (100, 500  $\mu\text{g}/\text{kg}$ ,  $p < .01$ ; 1000 $\mu\text{g}/\text{kg}$ ,  $p < .05$ ), indicating that exploration was further reduced after 6-OHDA lesion, as shown in Figure 1. Regarding writhing posture (Figure 2), its duration was enhanced

**Table 3.** Dopaminergic Levels (pg/mg Wet Weight of Tissue) in the Nucleus Accumbens Sham and 6-OHDA-Lesioned Rats, with Placebo or Morphine Pellets (during Chronic Morphine Dependence and Precipitated Withdrawal)

| Group                      | DA                             | DOPAC                         | HVA                           | DOPAC/DA                 |
|----------------------------|--------------------------------|-------------------------------|-------------------------------|--------------------------|
| Sham placebo               | 5810 ± 832.2                   | 1090.8 ± 113.1                | 925.3 ± 223.5                 | 0.19 ± 0.1               |
| Sham morphine dependence   | 4297.7 ± 780.8                 | 1130 ± 143.3                  | 877.7 ± 212.2                 | 0.26 ± 0.1               |
| Sham withdrawal            | 1036.3 ± 515.8**               | 671.5 ± 156.1**               | 801.2 ± 177.8                 | 0.64 ± 0.1**             |
| 6-OHDA placebo             | 7903.3 ± 305.5 <sup>+</sup>    | 1808.8 ± 123.2 <sup>++</sup>  | 1543.4 ± 225.5 <sup>+++</sup> | 0.22 ± 0.1               |
| 6-OHDA morphine dependence | 8108.8 ± 1010**                | 1989.9 ± 212.1**              | 1454.4 ± 199.2*               | 0.24 ± 0.2               |
| 6-OHDA withdrawal          | 3969.8 ± 706.3 <sup>oo##</sup> | 445.1 ± 102.1 <sup>oo**</sup> | 552.3 ± 198.2 <sup>oo#</sup>  | 0.11 ± 0.1 <sup>##</sup> |

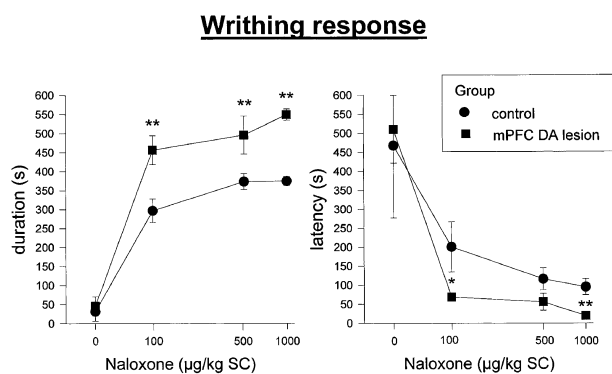
Mean ± SEM. <sup>+</sup> $p < 0.05$ , <sup>++</sup> $p < 0.01$  vs. sham placebo; \* $p < 0.05$ , \*\* $p < 0.01$  vs. sham morphine dependence; # $p < 0.05$ ; ## $p < 0.01$  vs. sham withdrawal; <sup>oo</sup> $p < 0.01$  vs. 6-OHDA morphine dependence group (Student's *t*-test). Abbreviations: DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid.



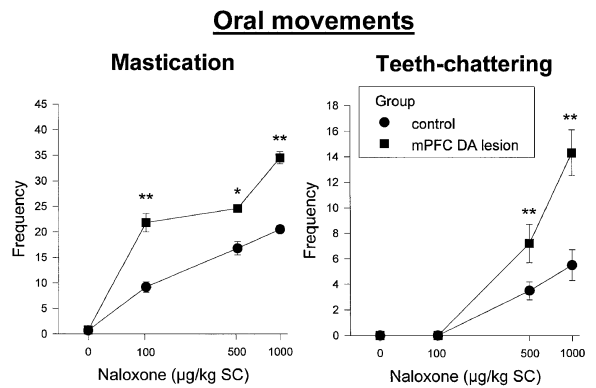
**Figure 1.** Duration of exploratory responses (walk-sniff and rearing) in sham and 6-OHDA-lesioned rats during naloxone-precipitated withdrawal. Mean  $\pm$  SEM. \*  $p < .05$ ; \*\*  $p < .01$  vs. sham (Student's t-test). Exploratory behavior, which is normally reduced during opiate withdrawal, was further reduced in lesioned rats.

in both sham and lesioned rats after naloxone, but this augmentation was significantly higher in mPFC DA-lesioned rats after every dose ( $p < .01$ ), and latency was significantly shortened at 100 and 1000  $\mu\text{g}/\text{kg}$  naloxone ( $p < .05$ ).

The number of oral movements (mastication and teeth-chattering) were enhanced in both sham and lesioned rats after naloxone, but the frequency of mastication was found to be significantly higher after 6-OHDA lesion at every dose (100, 500  $\mu\text{g}/\text{kg}$ ,  $p < .01$ ; 1000  $\mu\text{g}/\text{kg}$ ,  $p < .05$ ), and teeth-chattering frequency at 1000  $\mu\text{g}/\text{kg}$  naloxone ( $p < .05$ ), as illustrated in Figure 3. Ethoscore values were also further enhanced after mPFC lesion (100, 1000  $\mu\text{g}/\text{kg}$ ,  $p < .01$ ; 500  $\mu\text{g}/\text{kg}$ ,  $p < .05$ ), as shown in Table 4. Behavioral study hence indicated a



**Figure 2.** Duration and latency for the first occurrence of writhing posture in sham and 6-OHDA-lesioned rats during naloxone-precipitated withdrawal. Mean  $\pm$  SEM. \*  $p < .05$ ; \*\*  $p < .01$  vs. sham (Student's t-test). Writhing behavior was further enhanced after 6-OHDA lesion, as observed through an augmented writhing duration and shortened latency.



**Figure 3.** Frequency of oral movements (mastication and teeth-chattering) in sham and 6-OHDA-lesioned rats during naloxone-precipitated withdrawal. Mean  $\pm$  SEM. \*  $p < .05$ ; \*\*  $p < .01$  vs. sham (Student's t-test). The frequency of oral movements was further enhanced after 6-OHDA lesion.

significant enhancement of the intensity of some symptoms of opiate withdrawal in lesioned rats as revealed by writhing and oral responses and the global score, in parallel to a reduction of exploratory responses. The remainder physical parameters were not significantly changed (Table 4).

**DISCUSSION**

The neurochemical findings indicated that noradrenaline levels in the mPFC during chronic morphine dependence were similar to that found in naive rats. It is well known that acute morphine decrease extraneuronal levels of noradrenaline (Gunne 1959, 1963; Maynert and Klingman 1962), but chronic morphine treatment is known to return noradrenaline to basal levels suggesting that there is adaptation to long-term chronic exposure to the drug (Gunne 1963; Maynert and Klingman 1962; Sloan and Eisenman 1968; Akera and Brody 1968). Neuroadaptation of dopamine and serotonin activity was also observed after chronic morphine, in accordance with early studies (Gunne 1963; Segal et al. 1972; Puri and Lal 1974; Bhargava and Matwyshyn 1977; Tao and Auerbach 1995). In this context, brain serotonin is known to be enhanced after acute morphine treatment only (Tao and Auerbach 1995).

Regarding lesioned rats with placebo, prefrontocortical dopamine levels fell down to 20.8% (−79.2% reduction) and dopamine turnover was enhanced nearly four-fold, likely as a reactive response due to dopamine lesion in accordance with several authors (Bubser and Schmidt 1990; Hemby et al. 1992; Kurachi et al. 1995). This enhanced dopamine turnover was quite similar in morphine dependent and abstinent lesioned rats, further indicating that the change in DA turnover is di-

**Table 4.** Physical Signs and Etho-Score Values during Opiate Withdrawal in Sham and 6-OHDA-Lesioned Rats

| Parameter             | Group  | Naloxone 0 | Naloxone 100 | Naloxone 500 | Naloxone 1000 |
|-----------------------|--------|------------|--------------|--------------|---------------|
| Wet dog shakes        | Sham   | 0.1 ± 0.1  | 1.3 ± 0.6    | 2.7 ± 0.8    | 5.5 ± 1.8     |
|                       | 6-OHDA | 0.2 ± 0.1  | 2.8 ± 1.3    | 4.2 ± 2.3    | 3.6 ± 1.7     |
| Jumping               | Sham   | 0          | 0            | 0.7 ± 0.4    | 0.3 ± 0.1     |
|                       | 6-OHDA | 0          | 0            | 0            | 0.4 ± 0.2     |
| Animals with diarrhea | Sham   | 0          | 0            | 0.4 ± 0.1    | 3 ± 1.2       |
|                       | 6-OHDA | 0          | 0            | 0.3 ± 0.2    | 2.6 ± 0.9     |
| Chromodachryorrhea    | Sham   | 0          | 0.5 ± 0.1    | 0.8 ± 0.3    | 1.1 ± 0.8     |
|                       | 6-OHDA | 0          | 0.4 ± 0.1    | 0.8 ± 0.3    | 1.6 ± 0.7     |
| Rinorrhea             | Sham   | 0          | 1.2 ± 0.1    | 1.3 ± 0.2    | 2.3 ± 0.7     |
|                       | 6-OHDA | 0          | 0.9 ± 0.1    | 1.0 ± 0.2    | 2.8 ± 0.8     |
| Weight loss           | Sham   | 0          | 0.7 ± 0.2    | 1.8 ± 0.9    | 2.2 ± 0.9     |
|                       | 6-OHDA | 0          | 0.6 ± 0.3    | 1.6 ± 0.7    | 2.4 ± 1.1     |
| Etho-score            | Sham   | 0.2 ± 0.1  | 1.0 ± 0.3    | 2.3 ± 0.5    | 3.5 ± 0.6     |
|                       | 6-OHDA | 0.3 ± 0.2  | 2.7 ± 0.4**  | 3.6 ± 0.4*   | 5.1 ± 0.5**   |

Mean ± SEM. \* $p < 0.05$ , \*\* $p < 0.01$  vs. sham (Student's *t*-test). Abbreviations: Naloxone 0, 100, 500, 1000 correspond to 0, 100, 500, and 1000 µg/kg naloxone doses, respectively.

rectly related to the prefrontocortical lesion. Interestingly, dopamine and metabolite contents were enhanced in the nucleus accumbens after 6-OHDA lesion, suggesting that there is an inverse relationship between mesocortical and mesolimbic dopaminergic systems, as proposed by other authors (Glowinski et al. 1984; Grace 1991; Le Moal and Simon 1991; Bassareo et al. 1995). In this context, it is known that the prefrontal cortex possesses corticofugal excitatory neurons that innervate striatal and DA midbrain cell regions, and that prefrontocortical dopaminergic activity in the prefrontal cortex inhibits these corticofugal neurons (Glowinski et al. 1984; Grace 1991). Morphine dependence did not alter the basal levels of mPFC or accumbal monoamines in lesioned rats, indicating that the chronic neuroadaptation of mesocortical and mesolimbic dopaminergic systems to morphine remains after prefrontocortical dopamine depletion.

During opiate withdrawal of sham rats, naloxone induced a very strong enhancement of prefrontocortical dopamine, noradrenaline and serotonin levels, together with an enhancement of serotonin turnover. Early studies have shown that a strong increase of dopamine and noradrenaline release takes place in the medial prefrontal cortex during naloxone-precipitated withdrawal (Redmond 1987; Rossetti et al. 1993; Bassareo et al. 1995), but an enhancement of mPFC serotonin neurotransmission has not been reported so far. This fact points towards a role of prefrontocortical serotonin in opiate withdrawal. After prefrontocortical 6-OHDA lesion, it is worth noting that, although percent changes in dopamine and serotonin levels were similar in sham and lesioned groups, absolute values of prefrontocortical dopamine and serotonin content were much lower in lesioned rats. These differences were not observed as for noradrenaline changes.

Since some behavioral signs of withdrawal were exacerbated in lesioned rats, the findings indicate that the severity of some symptoms of physical opiate withdrawal is mostly related to the prefrontocortical dopamine and serotonin tone rather than to the noradrenergic one. This latter fact is in agreement with previous results from our group where the complete noradrenergic depletion of the locus coeruleus, source of encephalic NA afferents, fails to modify physical opiate withdrawal (Caillé et al. 1999). In this context, our findings are in contrast with the "classical" hypotheses that behavioral signs of withdrawal are mediated by either an increased activity of NA frontocortical system (Gunne 1959, 1963, Rossetti et al. 1993; Redmond 1987), or a noradrenergic hyperactivity as a whole (Gunne 1959; Montel et al. 1975; Crawley et al. 1979; Laverty and Roth 1980; Brodie et al. 1980; Roth and Redmond 1982; Swann et al. 1983; Rossetti et al. 1993).

The opiate withdrawal syndrome was exacerbated with respect to some behavioral symptoms such as writhing and oral movements (mastication and teeth-chattering), in parallel to an enhanced reduction in the exploratory activity. However, other behavioral responses such as wet dog shakes and jumping, and autonomic signs such as diarrhea, rinorrhea, chromodachryorrhea, and weight loss were not further enhanced after prefrontocortical 6-OHDA lesion. Hence, monoamine changes in the medial prefrontal cortex appear to be related to the intensity of writhing, oral movements and exploration. Noteworthy, oral movements such as licking and mastication are known to be correlated to hyperdopaminergic activity within the medial ventral striatum (nucleus accumbens) or the ventrolateral part of the dorsal striatum (Kelley et al. 1988; Jicha and Salamone 1991; Cools et al. 1993), suggesting that changes in the prefrontal cortex could reactively modify the

dopaminergic activity in the striatum and hence the expression of these signs. Our neurochemical data are consistent with these assumptions, since a reduced dopaminergic content was detected in the nucleus accumbens during opiate withdrawal, but this reduction in accumbal DA content was lower after prefrontocortical 6-OHDA lesion. In this context, a reduction of dopamine release has been reported to take place in the nucleus accumbens during opiate withdrawal (Acquas et al. 1991; Pothos et al. 1991; Acquas and Di Chiara 1992; Rossetti et al. 1992)

These findings hence indicate that prefrontocortical monoaminergic changes play a role in the behavioral expression of opiate withdrawal, and that the intensity of some signs such as writhing, oral movements and exploration are related to the dopaminergic and serotonergic tone of the medial prefrontal cortex rather than to the noradrenergic one. The findings permit the proposal of the hypothesis that enhanced dopamine and serotonin levels within the medial prefrontal cortex would counteract the severity of writhing and oral movements. Furthermore, it seems that there is a positive relationship between dopamine and serotonin activity in the mPFC, as revealed by a parallel enhancement of both amines during withdrawal, and the reactive serotonergic reduction after prefrontocortical 6-OHDA lesion (Gobert et al. 1998). Prefrontocortical noradrenergic system seems to escape the dopaminergic modulation during opiate withdrawal.

Finally, the profound depression of dopaminergic activity in the nucleus accumbens during opiate withdrawal was prevented by prefrontocortical 6-OHDA lesion, indicating that an inverse relationship between mesocortical and mesolimbic dopaminergic systems exists. This fact could account for the increase in oral movements after prefrontocortical lesion, since oral responses are linked to dopaminergic activity in the ventral striatum.

## ACKNOWLEDGMENTS

This work was supported by grants from Spanish DGES (PM97-0127), Junta de Andalucía (CVI-0127), University de Bordeaux 2, the Centre National de la Recherche Scientifique (CNRS), the Conseil Regional d'Aquitaine, and a Spanish-French Integrated Action (HF-97-0220). The authors thank Dr. J. Miñano (Sevilla, Spain) for HPLC measurements.

## REFERENCES

- Acquas E, Carboni E, Di Chiara G (1991): Profound depression of mesolimbic dopamine release after morphine withdrawal in dependent rats. *Eur J Pharmacol* 193:133-134
- Acquas E, Di Chiara G (1992): Depression of mesolimbic dopamine transmission and sensitization to morphine during opiate abstinence. *J Neurochem* 58:1620-1625
- Akera T, Brody TM (1968): The addiction cycle to narcotics in the rat and its relation to catecholamines. *Biochem Pharmacol* 17:675-688.
- Barnett SA (1975): *The Rat: A Study in Behavior*. Chicago, Aldine
- Bassareo V, Tanda G, Di Chiara G (1995): Increase of extracellular dopamine in the medial prefrontal cortex during spontaneous and naloxone-precipitated opiate abstinence. *Psychopharmacology* 122:202-205
- Bhargava HN, Matwyshyn GA (1977): Brain serotonin turnover and morphine tolerance-dependence induced by multiple injections in the rat. *Eur J Pharmacol* 44:25-33
- Bhargava HN (1979): The synthesis rate and turnover time of 5-hydroxytryptamine in brains of rats treated chronically with morphine. *Br J Pharmacol* 65:311-317
- Blasig J, Papeschi R, Gramsch C, Herz A (1976): Central serotonergic mechanisms and development of morphine dependence. *Drug Alcohol Depend* 1:221-239
- Bozarth MA (1994): Physical dependence produced by central morphine infusions: An anatomical mapping study. *Neurosci Biobehav Rev* 18:373-383
- Brodie ME, Lavert R, McQueen EG (1980): Noradrenaline release from slices of the thalamus of normal and morphine-dependent rats. *Naunyn Schmiedebergs Arch Pharmacol* 313:135-138
- Bubser M, Schmidt WJ (1990): 6-Hydroxydopamine lesion of the rat prefrontal cortex increases locomotor activity, impair acquisition of delayed alternation tasks, but does not affect uninterrupted task in the radial maze. *Behav Brain Res* 37:157-168
- Caillé S, Espejo EF, Reneric JP, Cador M, Koob GF, Stinus L (1999): Total neurochemical lesion of noradrenergic neurons of the locus coeruleus does not alter either naloxone-precipitated or spontaneous opiate withdrawal nor does it influence the ability of clonidine to reverse opiate withdrawal. *J Pharmacol Exp Therap* 290:881-892
- Cervo L, Romandini S, Smanin R (1983): Evidence that 5-hydroxytryptamine in the forebrain is involved in naloxone-precipitated jumping in morphine-dependent rats. *Br J Pharmacol* 79:993-996
- Cools AR, Kikuchi de Beltran K, Prinssen EPM, Koshikawa N (1993): Differential role of core and shell of the nucleus accumbens in jaw movements of rats. *Neurosci Res Commun* 13:55-61
- Crawley JN, Laverty R, Roth RH (1979): Clonidine reversal of increased norepinephrine metabolite levels during morphine withdrawal. *Eur J Pharmacol* 57:247-250
- Espejo EF, Stinus L, Cador M, Mir D (1994): Effects of morphine and naloxone on behaviour in the hot plate test: An ethopharmacological study in the rat. *Psychopharmacology* 113:500-510
- Espejo EF, Cador M, Stinus L (1995): Ethopharmacological analysis of naloxone-precipitated morphine withdrawal syndrome in rats: A newly-developed "etho-score." *Psychopharmacology* 122:122-130
- Glowinski J, Tassin JP, Thierry AM (1984): The mesocortico-



- prefrontal dopaminergic neurons. *Trends Neurosci* 7:415–418
- Gmerek DE (1988): Physiological dependence on opioids. In Rodgers RJ, Cooper SJ (eds), *Endorphines, Opiates and Behavioral Processes*. New York, Wiley, pp 25–54
- Gobert A, Rivet JM, Audinot V, Newman-Tancredi A, Cistarelli L, Millan MJ (1998): Simultaneous quantification of serotonin, dopamine and noradrenaline levels in single frontal cortex dialysates of freely-moving rats reveals a complex pattern of reciprocal auto- and heteroreceptor-mediated control of release. *Neuroscience* 84:413–429
- Gold L, Stinus L, Inturrisi CE, Koob GF (1994): Prolonged tolerance, dependence and abstinence following subcutaneous morphine pellet implantation in the rat. *Eur J Pharmacol* 253:45–51
- Grace AA (1991): Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. *Neuroscience* 41:1–24
- Grant EC, Mackintosh JH (1963): A comparison of the social postures of some common laboratory rodents. *Behaviour* 21:246–259
- Gunne LM (1959): Noradrenaline and adrenaline in the rat brain during acute and chronic morphine administration and during withdrawal. *Nature* 184:1950–1951
- Gunne LM (1963): Catecholamines and 5-hydroxytryptamine in morphine tolerance and withdrawal. *Acta Physiol Scand* 58:1–91
- Hayward MD, Duman RS, Nestler EJ (1990): Induction of the c-fos protooncogene during opiate withdrawal in the locus coeruleus and other regions of the brain. *Brain Res* 525:256–266
- Hemby SE, Jones GH, Neill DB, Justice JB (1992): 6-Hydroxydopamine lesions of the medial prefrontal cortex fail to influence cocaine-induced place conditioning. *Behav Brain Res* 49:225–230
- Jicha GA, Salamone JD (1991): Vacuous jaw movements and feeding deficits in rats with ventrolateral striatal dopamine depletion: possible relation to parkinsonian symptoms. *J Neurosci* 11:3822–3829
- Kelley A, Lang CG, Gauthier AM (1988): Induction of oral stereotypy following amphetamine microinjection into a discrete subregion of the striatum. *Psychopharmacology* 95:556–559
- Kurachi M, Yasui SI, Kurachi T, Shibata R, Murata M, Hagiwara H, Tani Y, Kurata K, Suzuki M, Sakurai Y (1995): Hypofrontality does not occur with 6-hydroxydopamine lesions of the medial prefrontal cortex in rat brain. *Eur Neuropsychopharmacol* 5:63–68
- Laverty R, Roth RH (1980): Clonidine reverses the increased norpephrine turnover during morphine withdrawal in rats. *Brain Res* 182:482–485
- Le Moal M, Simon H (1991): Mesocorticolimbic dopaminergic network: Functional and regulatory roles. *Physiol Rev* 71:155–234
- Maldonado R, Stinus L, Gold LH, Koob GF (1992): Role of different brain structures in the expression of the physical morphine withdrawal syndrome. *J Pharmacol Exp Ther* 261:669–677
- Maynert EW, Klingman GI (1962): Tolerance to morphine. I. Effects on catecholamines in the brain and adrenal glands. *J Pharmacol Exp Therap* 135:285–295
- Montel J, Starke K, Taube HD (1975): Morphine tolerance and dependence in noradrenaline neurons of the rat cerebral cortex. *Naunyn Schmiedebergs Arch Pharmacol* 288:415–426
- Pothos E, Rada P, Mak GP, Hoebel BG (1991): Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone-precipitated withdrawal and clonidine treatment. *Brain Res* 566:348–350
- Puri SK, Lal H (1974): Tolerance to the behavioral and neurochemical effects of haloperidol and morphine in rats chronically treated with morphine or haloperidol. *Naunyn Schmiedebergs Arch Pharmacol* 282:151–170
- Redmond DE Jr (1987): Studies of the locus coeruleus in monkeys and hypotheses for neuropsychopharmacology. In Meltzer HY (ed), *Psychopharmacology: The Third Generation of Progress*. New York, Raven Press, pp 967–975
- Rossetti ZL, Longu G, Mercurio G, Gessa GL (1993): Extra-neuronal noradrenaline in the prefrontal cortex of morphine-dependent rats: Tolerance and withdrawal mechanisms. *Brain Res* 609:316–320
- Rossetti ZL, Hmaidan Y, Gessa GL (1992): Marked inhibition of mesolimbic dopamine release: A common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats. *Eur J Pharmacol* 221:227–234
- Roth RH, Redmond DE Jr (1982): Clonidine suppression of noradrenergic hyperactivity during morphine withdrawal: Biochemical studies in rodents and primates. *J Clin Psychiatry* 43:42–46
- Saito H, Murai S, Abe E, Masuda Y, Itoh T (1992): Rapid and simultaneous assay of monoamine neurotransmission and their metabolites in discrete brain areas of mice by HPLC with coulometric detection. *Pharmacol Biochem Behav* 42:351–356
- Segal M, Deneau GA, Seevers MH (1972): Levels and distribution of central nervous system amines in normal and morphine-dependent monkeys. *Neuropharmacology* 11:211–222
- Silverstone PH, Done C, Sharp T (1993): In vivo monoamine release during naloxone-precipitated morphine withdrawal. *Neuroreport* 4:1043–1045
- Slater PJB (1978): Data collection. In Colgan PW (ed), *Quantitative Ethology*. New York, Wiley, pp 7–24
- Sloan JW, Eisenman AJ (1968): Long-persisting changes in catecholamine metabolism following addiction to and withdrawal from morphine. In Wickler A (ed), *The Addictive States*. Baltimore, Williams & Wilkins, pp 96–105
- Swann A, Elsworth JD, Charney DS, Jablons DM, Roth RH, Redmond DE, Mass JW (1983): Brain catecholamine metabolites and behavior in morphine withdrawal. *Eur J Pharmacol* 86:167–175
- Tao R, Auerbach SB (1995): Involvement of the dorsal raphe but not median raphe nucleus in morphine-induced increases in serotonin release in the rat forebrain. *Neuroscience* 68:553–561