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# Contrasting actions of acute or chronic paroxetine and fluvoxamine on morphine withdrawal-induced place conditioning

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**Abstract**

The acute and chronic effects of paroxetine and fluvoxamine on naloxone withdrawal-induced place aversion in morphine dependent rats were investigated. Acutely administered fluvoxamine (25 mg/kg s.c. given 30 min prior to naloxone withdrawal pairing) and chronic daily paroxetine (10 mg/kg s.c.) coadministration with a morphine induction protocol, both attenuated morphine withdrawal place aversion. Conversely, acutely administered paroxetine (up to 25 mg/kg s.c.) or chronic daily fluvoxamine (10 mg/kg s.c.) coadministration with morphine did not modify subsequent withdrawal place aversion. Previous radioligand binding studies indicate that fluvoxamine has opioid-displacing properties. It is suggested therefore that acute fluvoxamine may have decreased withdrawal aversion, probably through serotonin and also, in part, via an opioid-like mechanism whereas chronic paroxetine decreased withdrawal aversion by a serotonergic mechanism, but it is not clear whether opioid systems play any role in the action of paroxetine.

*Keywords:* Opioid dependence; Paroxetine; Fluvoxamine; Place conditioning; 5-HT (5-hydroxytryptamine, serotonin) reuptake inhibitor, specific; Morphine

## 1. Introduction

Analgesia and dependence are both useful as well as negative clinical features of opioid activity respectively. Attempts have been made over the years to produce analgesics devoid of rewarding or dependence-inducing properties, or to develop agents capable of treating opioid withdrawal (Gold, 1993). In this respect, methadone and clonidine are used in the treatment of opioid withdrawal but they are associated with substantial side effects (Gold et al., 1980; Reid, 1977; Hanson et al., 1973).

Traditionally, studies of the neurochemical basis of reward and reinforcement have focused upon noradrenaline, dopamine, and opioid systems themselves. However, there is considerable evidence that brain 5-hydroxytryptamine (5-HT) systems play an important role in the above processes (Cervo et al., 1981; Ronback et al., 1984; Romandini et al., 1984; Higgins et al., 1991; Carboni et al., 1988). Anatomically, 5-HT projections from both dorsal and median raphe nuclei project to the ventral tegmental area and the nucleus accumbens (Azmitia, 1978) and it has been argued that 5-HT and dopamine systems interact through these pathways.

Recently the work of Akaoka and Aston-Jones (1991, 1993) has indicated that hyperactivity of locus coeruleus neurons during opiate withdrawal is mediated by augmented excitatory amino acid input. In addition, *d*-fenfluramine, fluoxetine and sertraline, all of which enhance serotonergic neurotransmission, diminish such opioid withdrawal-induced locus coeruleus hyperactivity (Akaoka and Aston-Jones, 1993). These data led to the proposal that indirectly acting serotonergic agonists may be useful in the treatment of opiate abuse by attenuating locus coeruleus hyperactivity (Akaoka and Aston-Jones, 1993). We examined the hypothesis that serotonin agonists may reduce the effects of withdrawal from opiate treatment by studying the effect of paroxetine and fluvoxamine, two specific serotonin reuptake inhibitors, on negative motivational consequences of opioid withdrawal as measured by place conditioning.

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## 2. Materials and methods

### 2.1. Subjects

Male Sprague-Dawley rats, weighing 150–180 g at the start of place conditioning experiments, were employed. Animals were allowed food and water ad libitum and they were maintained at a temperature of 22.0 ± 1.0°C on a 12/12 h light/dark cycle, the experiments being performed between 10.00 and 16.00 h.

### 2.2. Drugs

Morphine hydrocloride (AAH Pharmaceuticals, UK) and naloxone hydrochloride (Sigma, UK) were dissolved in sterile apyrogenic saline. Fluvoxamine maleate (Duphar, Netherlands) and paroxetine (Smith Kline Beecham, UK) were dissolved in distilled water containing Tween 80 (5%). Naloxone hydrochloride and morphine hydrochloride were administered intraperitoneally (i.p.) and all other agents were injected subcutaneously (s.c.).

### 2.3. Induction of morphine dependence and withdrawal

Morphine dependence was induced using an 8-day dosing schedule, the following doses being administered on successive days: 2.5, 5, 10, 60, 80, 100, 100, 100 mg/kg i.p. and withdrawal was precipitated using naloxone at a dose level of 1.0 mg/kg i.p. since this dose has been shown previously to produce a quantifiable abstinence syndrome in rats (Davies et al., 1983).

### 2.4. Place conditioning apparatus

Each box used for place conditioning measured 90 × 40 × 40 cm (length × width × height) and consisted of a central compartment of 10 cm length and 40 cm width with a white painted floor raised by 2 cm. The other two compartments measured 40 × 40 cm, one having grey sides and floor (roughened), the other being painted in black/white stripes with a smooth floor. Guillotine doors were used to separate the three compartments for pairing and counterbalancing. During observation periods, activity was assessed using remote video recording, and the boxes were placed in sound-attenuated conditions under white light (4 lux).

### 2.5. Withdrawal place conditioning procedure

Rats (eight per group) were habituated to the unpartitioned boxes for 15 min on two successive days. On the third day, the initial baseline preference for any one side was timed over a 15 min period following a 5 min initial habituation phase. Animals were then dosed once daily from days 4–11 with the morphine dependence schedule. Thereafter, on days 12 or 13 of the protocol, animals were either naloxone-treated (24 h after a morphine dose) and paired with initially most preferred side on one day, or saline treated (counterbalanced – 24 h after a morphine dose) and paired with initially least-preferred side on the other day. On day 14 (post-condition test day) each animal was placed on the central platform with doors removed and allowed 5 min habituation to eliminate any influence of increased initial locomotor activity, then subsequently the times in each compartment were measured over a period of 15 min. Data were analysed by calculating the difference between pre-conditioned preference (3rd day) and post-conditioned (test day) values ($\Delta$t) for each individual animal and this was designated 'aversion time' (s) (see Figs. 1, 2 and 3).

### 2.6. Statistical analysis

In conditioned place aversion experiments, results are expressed as differences in time ($\Delta$t) spent on the side between pre- and post-withdrawal conditioning tests (aversion time). Results were analysed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey test and $P < 0.05$ was considered as significantly different.

## 3. Results

### 3.1. Evaluation of pre-conditioning place preference

After a 2-day habituation, the overall experimental pretest time (day 3) spent on the preferred side was 475.65 ± 13 s whilst time on non-preferred side was 193.49 ± 9.8 s. These global values were not significantly different from those in each individual group.

### 3.2. Comparison of conditioned place aversion induced by naloxone following chronic morphine, fluvoxamine or paroxetine

Using the 8-day morphine dosing schedule, along with the withdrawal place conditioning protocol, naloxone (1 mg/kg i.p.) induced place aversion in morphine-dependent rats. Thus, naloxone given to animals pretreated with saline was not inherently aversive compared to the corresponding saline controls ($P > 0.05$), but significantly increased ($P < 0.05$) the aversion time in morphine-treated animals (Fig. 1). Treatment for 8 days with fixed daily doses of either fluvoxamine (10 mg/kg s.c.) or paroxetine (10 mg/kg s.c.) alone yielded no significant change in aversion time following naloxone pairing (Table 1).

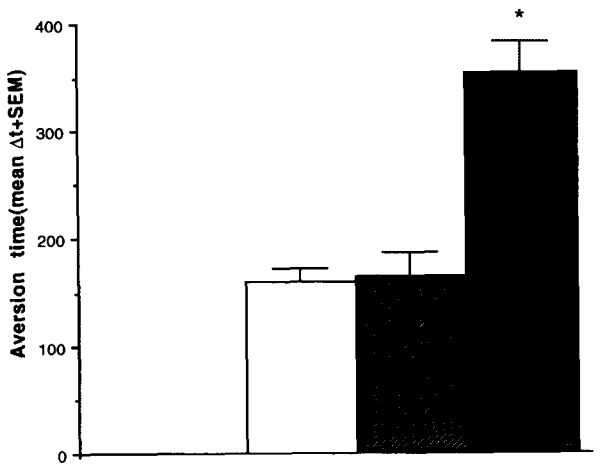


Fig. 1. Comparison of the aversive effect of naloxone (1 mg/kg i.p.) withdrawal, with saline counterbalancing, on day 12 or 13 of the place conditioning procedure in morphine-dependent or saline-treated rats. Mean aversion time ($\Delta t$ (s) between pre- and post-withdrawal conditioning test) was determined on day 14 in the following groups ($n = 8$): Open column: Saline schedule on days 4–11 and saline on day 12 or 13. Hatched column: Saline schedule on days 4–11 and naloxone with saline counterbalancing on day 12 or 13. Filled column: Morphine schedule on days 4–11 and naloxone with saline counterbalancing on day 12 or 13. $^*P < 0.05$ compared to other groups.

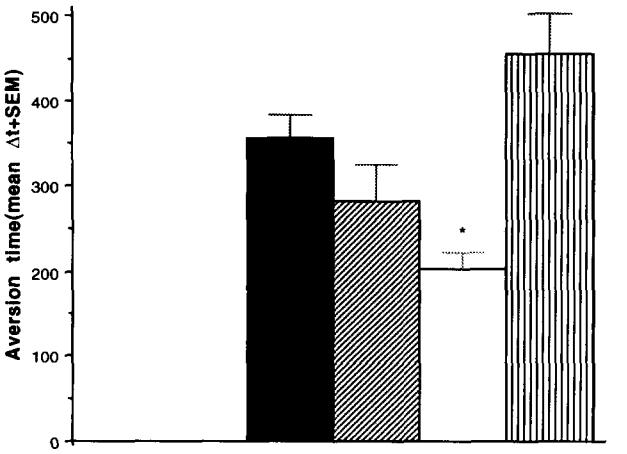


Fig. 2. Effects of acute and chronic fluvoxamine on naloxone (1 mg/kg i.p.)-induced morphine withdrawal place aversion on day 14 in the following animal groups ($n = 8$): Filled column: morphine schedule on days 4–11, then 30 min vehicle pretreatment followed by naloxone (1 mg/kg i.p.) withdrawal with saline counterbalancing, on day 12 or 13. Hatched column: morphine schedule, then fluvoxamine (10 mg/kg s.c.) 30 min pretreatment followed by naloxone with saline counterbalancing. Open column: morphine schedule, then fluvoxamine (25 mg/kg s.c.) 30 min pretreatment followed by naloxone with saline counterbalancing. Striped column: morphine schedule coadministered with daily fluvoxamine (10 mg/kg s.c.), then naloxone with saline counterbalancing. $^*P < 0.05$ compared to morphine schedule plus naloxone with saline counterbalancing.

### 3.3. Effects of acute and chronic fluvoxamine on conditioned withdrawal place aversion in morphine-dependent rats

Fluvoxamine (10, 25 mg/kg s.c.) administered acutely 30 min before naloxone withdrawal conditioning, in morphine-dependent rats dose dependently reduced the aversion time. However, this effect attained significance only at the higher fluvoxamine dose ($P < 0.05$, Fig. 2). Chronic co-administration of fluvoxamine (10 mg/kg) 3 h before daily morphine over 8 days did not induce any significant change in aversion time (Fig. 2.).

### 3.4. Effects of acute and chronic paroxetine on conditioned withdrawal place aversion in morphine-dependent rats

Neither dose of paroxetine (10 or 25 mg/kg s.c.), administered acutely 30 min before naloxone withdrawal conditioning, significantly modified ($P < 0.05$) aversion time in morphine-dependent animals (Fig. 3). However, chronic combined daily injection of paroxetine (10 mg/kg s.c.), 3 h before morphine during the

Table 1
Effects of chronic daily fluvoxamine (10 mg/kg s.c.) and paroxetine (10 mg/kg s.c.) on aversion time following naloxone pairing

| 8-Day treatment + single naloxone pairing | Aversion time (s) (mean $\Delta t \pm$ S.E.M.) |
|---|---|
| Vehicle | 165.0 ± 21.8 |
| Fluvoxamine (10 mg/kg s.c.) | 177.5 ± 27.8 |
| Paroxetine (10 mg/kg s.c.) | 151.7 ± 17.2 |

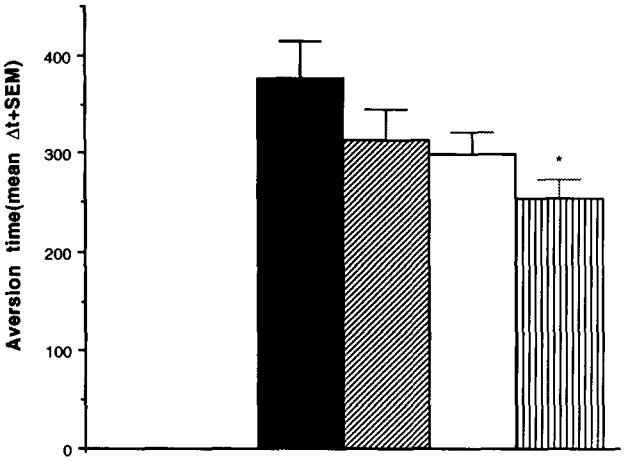


Fig. 3. Effects of acute and chronic paroxetine on naloxone (1 mg/kg i.p.)-induced morphine withdrawal place aversion time (s) on day 14 in the following animal groups ($n = 8$): Filled column: morphine schedule on days 4–11, then 30 min vehicle pretreatment followed by naloxone (1 mg/kg i.p.) withdrawal, with saline counterbalancing, on day 12 or 13. Hatched column: morphine schedule, then paroxetine (10 mg/kg s.c.) 30 min pretreatment followed by naloxone with saline counterbalancing. Open column: morphine schedule, then paroxetine (25 mg/kg s.c.) 30 min pretreatment followed by naloxone with saline counterbalancing. Striped column: morphine schedule coadministered with daily paroxetine (10 mg/kg s.c.), then naloxone with saline counterbalancing. $^*P < 0.05$ compared to morphine schedule plus naloxone with saline counterbalancing.

8-day schedule produced a significant decrease in aversion time ($P < 0.05$).

It should be noted that at the 25 mg/kg dose level, chronic fluvoxamine or paroxetine in combination with morphine exhibited evidence of toxicity, so, for ethical reasons, this higher dose had to be excluded from the chronic studies. Moreover, at the 10 mg/kg dose, a 3 h time differential was chosen between fluvoxamine or paroxetine dosing and morphine administration in order to avoid toxicity.

## 4. Discussion

We have shown in this study that naloxone at a dose of 1 mg/kg inherently failed to produce single trial place aversion in non-dependent vehicle-treated rats. This finding accords with those of Spanagel et al. (1994) who studied place aversion with the same dose of naloxone. These results are also consistent with those of Higgins et al. (1991) who tested doses up to 0.5 mg/kg of naloxone without observing aversive activity. In contrast, others have reported negative motivational properties with naloxone in this particular model (Mucha and Iversen, 1984) where place aversion was noted with naloxone (0.5 mg/kg) only following at least three pairings to the conditioning compartment. It is therefore evident that conflicting data have been reported on the aversive aspects of naloxone and this might be ascribed to differences in laboratory conditions and/or experimental protocols. In morphine-dependent rats we observed significant place aversion following a single pairing with naloxone-induced withdrawal and this concurs with other reports in the literature (Spanagel et al., 1994; Higgins et al., 1991).

Previous studies have indicated that serotonergic pathways in the brain may be involved in opioid withdrawal (Cervo et al., 1981; Ronback et al., 1984; Romandini et al., 1984; Higgins et al., 1991; Carboni et al., 1988). Recent reports have also shown that hyperactivity of locus coeruleus neurons is an important brain substrate of opiate withdrawal (Gold, 1993; Akaoka and Aston-Jones, 1991, 1993). Thus, pretreatment with fluoxetine or sertraline, before naloxone, significantly attenuated withdrawal-induced hyperactivity of locus coeruleus neurons (Akaoka and Aston-Jones, 1993). In the present experiments, acutely administered fluvoxamine decreased opioid withdrawal place aversion and it is possible that this might be attributable, at least in part, to a reduction in locus coeruleus hyperactivity. The exact mechanism of this effect is not clear but it has been suggested that increased 5-HT neurotransmission is a major component of locus coeruleus hyperactivity reduction (Akaoka and Aston-Jones, 1991, 1993), since these compounds have specific activity on 5-HT reuptake. This concept accords with our findings on acute fluvoxamine and chronic paroxetine both of which reduced opioid withdrawal aversion time even though they did not exhibit aversive or negative motivational effects after naloxone pairing.

In the case of paroxetine it is possible that the acute effect on 5-HT reuptake was minimal whilst chronic administration had a more pronounced action on 5-HT mechanisms to reduce naloxone-precipitated withdrawal place aversion. This is supported by the reports that prolonged pretreatment with 5-HT reuptake blockers may increase the presynaptic release of 5-HT more than a single treatment (Chaput et al., 1991; Bel and Artigas, 1993; Rutter et al., 1994; Invernizzi et al., 1994). Since we were unable to examine the chronic activity of paroxetine at the higher dose in combination with morphine, we cannot totally exclude the contingency that chronic treatment with paroxetine may alter opioid activity.

Fluvoxamine has been shown to displace opioid binding in brain tissue possibly by interaction with membrane sites which alter receptor conformation (Somoza et al., 1981). Furthermore, acute opioids directly inhibit electrical activity in the locus coeruleus (Christie, 1991), via $\mu$-opioid receptors which increase potassium efflux to produce hyperpolarisation. It is conceivable, therefore, that acute fluvoxamine invokes an opioid-like response to counteract locus coeruleus hyperactivity evoked during withdrawal and this may have a negative influence on the expression of naloxone-induced withdrawal place aversion.

Following chronic administration of fluvoxamine, it is possible that its opioid-like effect would increase naloxone withdrawal to cancel 5-HT-induced reduction of place aversion.

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