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Fluoxetine Combined with a Serotonin-1A Receptor Antagonist Reversed Reward Deficits Observed during Nicotine and Amphetamine Withdrawal in Rats

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The symptom of "diminished interest or pleasure" in rewarding stimuli is an affective symptom of nicotine and amphetamine withdrawal, and a core symptom of depression. An operational measure of this symptom is elevation of brain reward thresholds during drug withdrawal. We report here that acute co-administration of fluoxetine, a selective serotonin reuptake inhibitor, and p-MPPI, a serotonin-1A receptor antagonist, alleviated the diminished interest in brain stimulation reward observed during withdrawal from nicotine or amphetamine in rats (i.e., increased reward). By contrast, the same drug combination treatment did not reduce the somatic signs of nicotine withdrawal indicating symptom-specific neurobiological abnormalities. Surprisingly, the same treatment had opposite effects in control rats where reductions in reward were produced, suggesting that animal models should be based primarily on studying specific deficits that are pathognomic of a psychiatric disorder. The reversal of the affective aspects of drug withdrawal by a treatment that enhances serotonin neurotransmission indicates that decreased serotonergic function may mediate the reward decrements characterizing nicotine and amphetamine withdrawal, and that these symptoms may be homologous to a core symptom of non-drug–induced depressions.

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Cessation of chronic nicotine or amphetamine administration precipitates withdrawal syndromes characterized by affective symptoms, including "diminished interest or pleasure" in rewarding stimuli (i.e., anhedonia) (American Psychiatric Association 1994; Covey et al. 1998; Glassman 1993; Hughes 1992). Interestingly, the symptom of "diminished interest or pleasure" is not only a symptom of drug withdrawal, but also a core symptom of depression and a negative symptom of schizophrenia (American Psychiatric Association 1994; Markou et al. 1998). Brain reward threshold elevation is an operational measure of this symptom because it reflects diminished sensitivity to rewarding electrical stimuli. In rats, withdrawal from drugs of abuse belonging to diverse pharmacological classes, such as nicotine (Epping-Jordan et al. 1998;

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Watkins et al. 2000b), amphetamine (Kokkinidis and Zacharko 1980a, 1980b; Kokkinidis et al. 1980, 1986; Leith and Barrett 1976, 1980; Lin et al. 1999, 2000; Paterson et al. 2000; Wise and Munn 1995), cocaine (Baldo et al. 1999; Kokkinidis and McCarter 1990; Markou and Koob 1991, 1992a; Markou et al. 1992) morphine (Schulteis et al. 1994) and ethanol (Schulteis et al. 1995) elevated brain stimulation reward thresholds.

In addition to the affective aspects of drug withdrawal reflected in threshold elevations, nicotine, opiate, or ethanol withdrawal also lead to alterations in a set of behaviors termed somatic signs. In the case of nicotine, these somatic signs are primarily gasps, writhes, eye blinks, and ptosis (Epping-Jordan et al. 1998; Hildebrand et al. 1997, 1999; Malin et al. 1992). It is unlikely that these somatic signs in the rat reflect the affective component of drug withdrawal. Nevertheless, the study of both threshold elevations and somatic signs permits the investigation of the effects of manipulations on the various aspects of withdrawal.

Based on evidence demonstrating the efficacy of serotonergic antidepressant treatments, reduced cerebrospinal fluid levels of serotonin metabolites, endocrine measures reflecting reduced serotonergic neurotransmission and the exacerbation of depressive symptomatology seen after serotonin (5-HT) depletion in depressed individuals, it is hypothesized that reduced serotonergic neurotransmission underlies at least some aspects or some subtypes of non–drug-induced depressions (for reviews, see Caldecott-Hazard et al. 1991; Caldecott-Hazard and Schneider 1992; Heninger et al. 1996; Markou et al. 1998; Meltzer and Lowy 1988; Willner 1985). The purpose of the present study was to test the hypothesis that reduced serotonergic neurotransmission mediates some of the affective aspects, not only of non-drug-induced depressions, but also of drug-induced depressions. Thus, the present study tested the hypothesis that enhancement of serotonergic neurotransmission through acute administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine (Wong et al. 1995) in combination with a relatively selective 5-HT_{1A} receptor antagonist would alleviate the symptom of "diminished interest or pleasure" observed in rats during nicotine or amphetamine withdrawal. Such an experimental outcome would suggest that the affective aspects of drug withdrawal may be homologous to the core symptom of depression of "diminished interest or pleasure" in rewarding stimuli. It was also hypothesized that this serotonergic drug treatment would not reverse the somatic signs of nicotine withdrawal that most likely do not reflect affective aspects of withdrawal.

The combination treatment of a SSRI together with the relatively selective 5-HT_{1A} receptor antagonist p-MPPI [4-(2′-methoxy-phenyl)-1-[2′-(n-(2″-pyridinyl)-p-iodobenzamido]-ethyl-piperazine] (Allen et al. 1997; Kung et al. 1994a, 1994b, 1995) was selected based on the following considerations. First, the threshold elevations associated with nicotine (Epping-Jordan et al. 1998) and amphetamine (Lin et al. 1999, 2000; Paterson et al. 2000) withdrawal, and the increases in somatic signs seen during nicotine withdrawal (Epping-Jordan et al. 1998) are relatively short lasting phenomena (3–5 days) that do not allow the detection of the effects of pharmacological treatments that may require chronic administration for producing their effect. *In vivo* microdialysis work demonstrated that the administration of a SSRI together with a 5-HT_{1A} receptor antagonist acutely and rapidly elevates forebrain serotonin dialysate levels beyond levels seen after acute treatment with the SSRI alone (Auerbach and Hjorth 1995; Bel and Artigas 1993; Bengtsson and Milano 1996; Hjorth 1993, 1996; Invernizzi et al. 1994; Knobelman et al. 2000; Kreiss and Lucki 1994, 1995; for reviews, see Artigas et al. 1996; Blier and de Montigny 1994). Thus, such a drug combination may have rapid therapeutic effects that may be detectable during the short-lasting drug withdrawal. Second, the combination of a SSRI with pindolol [antagonist at 5-HT_{1A}, 5-HT_{1B}, and β-adrenergic receptors (Assie and Koek 1996; Bourin et al. 1998; Gobert and Millan 1999; Hoyer and Schoeffter 1991; Newman-Tancredi et al. 1998); also reported to act as a partial agonist at 5-HT_{1A} and α-adrenergic receptors (Clifford et al. 1998; Fornal et al. 1999a, 1999b, 1999c; Gobert and Millan 1999; Pauwels and Palmier 1994a, 1994b)] accelerates the onset of antidepressant action of SSRIs in humans (Bordet et al. 1998; Zanardi et al. 1997, 1998), or augments the antidepressant response to SSRIs in terms of both magnitude and duration of the response (Maes et al. 1999; Perez et al. 1997; Tome and Isaac 1998; for review, see McAskill et al. 1998; however, see Berman et al. 1997, 1999; Tome et al. 1997a, 1997b). In the present study, instead of pindolol, which has multiple receptor actions, the relatively selective 5-HT_{1A} receptor antagonist p-MPPI was used. The use of p-MPPI allowed the investigation of the role of serotonergic neurotransmission and antagonism at 5-HT_{1A} receptors in drug withdrawal, without complicating data interpretation with neuroadaptive effects that may occur with chronic SSRI treatment. In summary, acute treatment with a SSRI together with a 5-HT_{1A} receptor antagonist allowed us to rapidly elevate extracellular serotonin levels and delineate the potential role of serotonin neurotransmission in the affective and somatic aspects of drug withdrawal.

MATERIALS AND METHODS

Subjects

Male Wistar rats (Charles River, Hollister, CA) (300–320 g at the start of the experiment) were housed in pairs in

a temperature and humidity controlled environment with a 12 hr light/dark cycle (lights on at 6:00 A.M.). Food and water were available ad libitum in the home cages. All subjects were treated in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals," and the animal facilities and experimental protocols were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care. All testing was conducted during the light phase of the light/dark cycle.

Intracranial Self-stimulation

The intracranial self-stimulation task used is a modified version (Markou and Koob 1992b, 1993) of a discrete-trial current-threshold procedure originally developed by Kornetsky and co-workers (Kornetsky and Esposito 1979). The apparatus, surgery, procedure, and parameters for the lateral hypothalamic intracranial self-stimulation task have been described in detail previously (Harrison et al. 1999; Kornetsky and Esposito 1979; Markou and Koob 1992b, 1993).

Apparatus. The experimental apparatus consisted of 16 Plexiglas chambers (30.5 × 30 × 17 cm) (Med Associates Inc.) encased in sound-attenuating boxes (San Diego Instruments, San Diego, CA). Each operant chamber consisted of a stainless steel grid floor and a metal wheel manipulandum located on one wall, which required a 0.2 N force to rotate it a quarter turn. Gold-contact swivel commutators and bipolar leads connected the animals to the stimulation circuit (Plastics One, Roanoke, VA). Brain stimulation was administered by constant current stimulators (Stimtek 1200, San Diego Instruments, San Diego, CA). The stimulation parameters, data collection and all programming functions were controlled by a microcomputer.

Surgery. The rats were prepared with 11 mm stainless steel bipolar electrodes (Plastics One; diameter = 0.25 mm) in the posterior lateral hypothalamus (AP −0.5 mm from bregma; L ± 1.7 mm; DV −8.3 mm from dura, with the incisor bar set at 5 mm above the interaural line; Pellegrino et al. 1979) under halothane anesthesia (1–1.5% halothane/oxygen mixture) and allowed to recover for at least seven days.

Intracranial Self-stimulation Procedure

The subjects were initially trained to turn the wheel manipulandum on a fixed ratio 1 schedule of reinforcement during which each quarter turn of a wheel manipulandum resulted in the delivery of a contingent electrical reinforcer. The electrical reinforcer had a train duration of 500 msec and consisted of 0.1 msec rectangular cathodal pulses that were delivered with 100 Hz frequency. The current intensity was adjusted for each animal and typically ranged from 100-250 μA. After successful familiarization with this procedure (two sessions of 100 reinforcers in less than 20 min), the rats were gradually trained on the discrete-trial current threshold procedure. In this procedure, at the start of each trial, rats received a non-contingent electrical stimulus. During the following 7.5 sec, the limited hold, if the subjects responded by turning the wheel manipulandum a quarter turn (positive response), they received a second contingent stimulus identical to the previous non-contingent stimulus. During a 2 sec period immediately after a positive response, further responses had no reinforcement or task consequences. If no response occurred during the 7.5 sec limited hold period, a negative response was recorded. The intertrial interval (ITI), which followed the limited hold period, had an average duration of 10 sec (ranging from 7.5–12.5 sec). Responses that occurred during the ITI resulted in a further 12.5 sec delay of the onset of the next trial. During training, the duration of the ITI and delay periods imposed by inappropriate ITI responding were gradually increased until the standard task parameters were reached. Stimulation intensities were varied according to the classical psychophysical method of limits. Thus, the subjects received four alternating series of ascending and descending current intensities starting with a descending series. Within each series the stimulus intensity was altered by 5 μA steps between each set of trials (three trials per set). The initial stimulus intensity was set at 40 μA above the baseline current threshold for each animal. A series was terminated after either 15 stimulus increments (or decrements) had occurred, or after the determination of the threshold for the series (see below). Each test session typically lasted 30 min and provided two dependent variables. After training in the above brain stimulation procedure, rats were tested until stable baseline thresholds and latencies were achieved (±10% over a 5-day period), which typically occurred after two to three weeks of daily baseline testing.

Reward Thresholds. The current threshold for each descending series was defined as the stimulus intensity between the successful completion of a set of trials (positive responses during two or more of the three trials) and the stimulus intensity for the first set of trials, of two consecutive sets, during which the animal failed to respond positively on two or more of the three trials. During the ascending series, the threshold was defined as the stimulus intensity between the unsuccessful completion of a set of trials (negative responses during two or more of the three trials) and the stimulus intensity for the first set of trials, of two consecutive sets, during which the animal responded positively on two or more of the trials. Thus, during each test session, four thresholds were determined and the mean of these values was taken as the threshold for each subject.

Response Latency. The latency between the onset of the non-contingent stimulus at the start of each trial and a positive response was recorded as the response latency. The response latency for each test session was defined as the mean response latency of all trials during which a positive response occurred.

Drugs

d-Amphetamine sulphate (obtained from the National Institute on Drug Abuse, Bethesda, MD) and fluoxetine hydrochloride (Research Biochemicals Inc., Natick, MA) were dissolved in saline and administered intraperitoneally in a volume of 1 ml/kg. 4-(2′-Methoxy-phenyl)-1-[2′-(n-(2″-pyridinyl)-p-iodobenzamido]-ethyl-piperazine hydrochloride (p-MPPI) (Research Biochemicals Inc.) was dissolved in sterile water and sonicated for 10–20 min, and then brought to a pH of approximately 5.2 with 0.1 M NaOH. p-MPPI hydrochloride was administered subcutaneously in a volume of 4 ml/kg. Nicotine hydrogen tartrate (Sigma, St. Louis, MO) was dissolved in saline and administered through subcutaneous osmotic minipumps. Mecamylamine HCl (Sigma) was dissolved in saline and administered subcutaneously in a volume of 1 ml/kg.

Nicotine Administration and Withdrawal

When stable self-stimulation performance had been achieved, osmotic minipumps [model 2ML1 (7 day) Alza Corporation, Palo Alto, CA] filled with either saline (4 groups of $n = 8$) or nicotine hydrogen tartrate dissolved in saline (4 groups of $n = 8$), were implanted subcutaneously under halothane/oxygen anesthesia. The surgical wounds were closed with 9 mm stainless steel autoclips (Becton Dickinson Primary Care Diagnostics, Sparks, MD). The concentration of nicotine was adjusted to compensate for differences in body weight at the time of implantation, resulting in a dose of 9 mg/kg/day nicotine tartrate (or 3.16 mg/kg/day base) for 7 days. During these 7 days, intracranial self-stimulation reward thresholds, response latencies and the body weight of the subjects were recorded daily.

The minipumps were removed on the seventh day after pump implantation. Intracranial self-stimulation reward thresholds, response latencies, the number of somatic signs of withdrawal and body weight were measured at regular intervals thereafter. Subjects were tested in the intracranial self-stimulation (ICSS) procedure at 6, 12, 18, 24, 36, 48, 72, 96, 120, and 144 hr after pump removal. Body weight was measured immediately before each ICSS session. The somatic signs of nicotine withdrawal have been described in detail previously (Epping-Jordan et al. 1998; Hildebrand et al. 1997, 1999; Malin et al. 1992; Watkins et al. 2000b). Briefly, the number of abstinence signs, including gasps, writhes, eye blinks, chewing, teeth chattering, cheek tremor, ptosis, "wet dog" shakes, piloerection, genital grooming, and escapes were recorded during a 10 min period. Somatic signs were assessed immediately after the self-stimulation sessions at 6.5, 12.5, 18.5, 24.5, 36.5, 48.5, 72.5, 96.5, 120.5, and 144.5 hr after pump removal. Acute administration of vehicle, p-MPPI hydrochloride (3 mg/kg), fluoxetine hydrochloride (5 mg/kg), or p-MPPI hydrochloride (3 mg/kg) + fluoxetine hydrochloride (5 mg/kg), occurred prior to the 18-hr test session. This testing time point was selected based on the time course of threshold elevations and somatic signs observed during nicotine withdrawal previously (Epping-Jordan et al. 1998). p-MPPI hydrochloride was administered 135 min prior to test, and fluoxetine hydrochloride was administered 120 min prior to test.

Mecamylamine Challenge. On the seventh day post-pump removal, all subjects were subcutaneously injected with mecamylamine (3.0 mg/kg) 15 min before intracranial self-stimulation testing, which was immediately followed by assessment of somatic signs of withdrawal. The purpose of this mecamylamine challenge was to test whether prior nicotine exposure and withdrawal would lead to potential tolerance or augmentation to the withdrawal effects. The dose of mecamylamine used was selected based on data showing that this dose reliably precipitated nicotine withdrawal symptomatology in rats that were chronically treated with nicotine (Watkins et al. 2000b).

Amphetamine Administration and Withdrawal

The amphetamine administration regimen was a modification of that used originally by Leith and Barrett (1976), and identical to that used by Lin et al. (1999). Briefly, d-amphetamine sulphate (6 groups of $n = 8$) was administered intraperitoneally three times a day (6:00 A.M., 12:00 A.M., 6:00 P.M.) for four days in a rising dose regime starting at 1 mg/kg and stabilizing at 5 mg/kg (i.e., 1, 2, 3, 4, 5, 5, 5, 5, 5, 5, 5, 5 mg/kg; total dose = 50 mg/kg). Another set of rats (6 groups of $n = 8$) was injected at the same time-points with saline. Intracranial self-stimulation reward thresholds and response latencies were determined at 12, 36, 42, 60, 84, 108, 132, and 156 hr after the last amphetamine or saline injection. Acute administration of vehicle, p-MPPI hydrochloride (3 mg/kg), fluoxetine hydrochloride (2.5 or 5 mg/kg), or p-MPPI hydrochloride (3 mg/kg) + fluoxetine hydrochloride (2.5 or 5 mg/kg) was administered prior to the 36-hr test session. This testing time point was selected based on the time course of threshold elevations observed during amphetamine withdrawal previously (Lin et al. 1999). p-MPPI hydrochloride was administered 135 minutes prior to test, and fluoxetine hydrochloride was administered 120 minutes prior to test.

Data Analyses

Chronic Nicotine. Body weight data recorded during the nicotine phase were expressed as a percentage of the weight immediately after minipump implantation, to take into account the weight of the mini-pump. Body weight, intracranial self-stimulation reward threshold and response latency data were analyzed using two-way analyses of variance (ANOVAs) with time after minipump implantation as the within-subject factor and chronic drug treatment (nicotine or saline) as the between-subject factor.

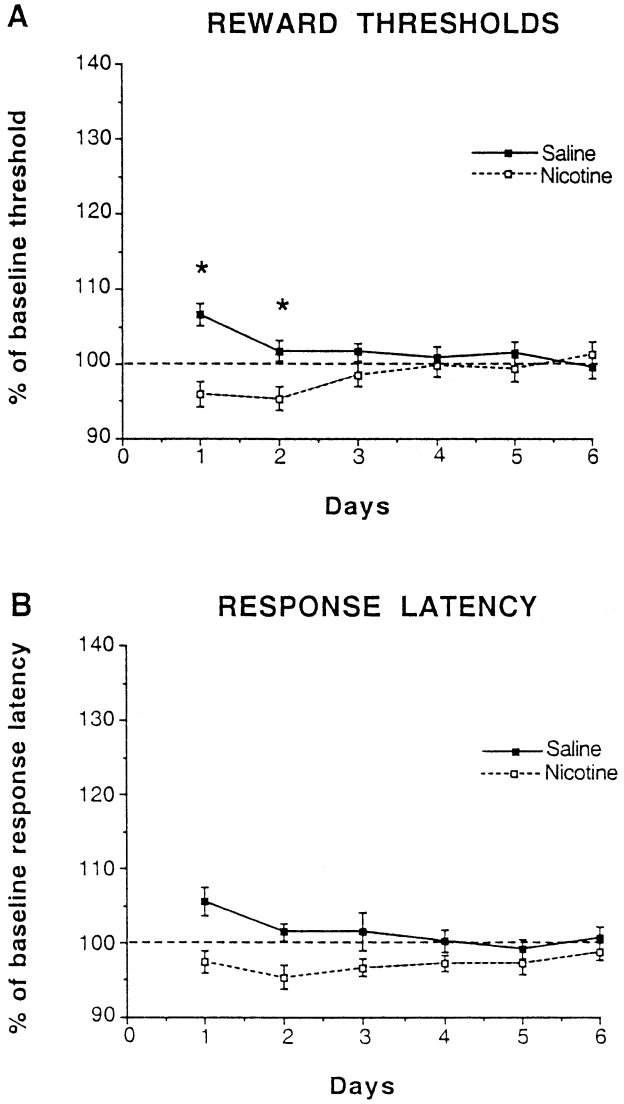

Figure 1. The effects of chronic nicotine administration on reward thresholds and response latencies (mean ± SEM). Reward thresholds were lowered during the first two days of nicotine administration (n = 32) compared to saline-treated control animals (n = 32) **(A)**. Response latencies were reduced also by chronic nicotine administration compared to saline-treated controls (main effect in the ANOVA; see text) **(B)**. Asterisks indicate statistically significant differences between the nicotine and saline groups for specific time points ($p < .05$).

Nicotine and Amphetamine Withdrawal. All reward threshold and response latency data were expressed as a percentage of the mean baseline value assessed during the five days immediately prior to the implantation of the minipumps or the first amphetamine injection. Body weight data recorded during the nicotine withdrawal phase were expressed as a percentage of the body weight immediately after minipump removal. Data were analyzed using three-way mixed factors analyses of variance. The within-subjects factor was time after nicotine, amphetamine or saline treatment, and the two between-subjects factors were chronic drug treatment [drug (nicotine or amphetamine) versus saline] and acute drug treatment administered during withdrawal. Statistically significant interactions were followed by post-hoc Newman-Keuls tests. The level of significance was set at $p < .05$.

Mecamylamine Challenge. Reward threshold, response latency and somatic signs data were compared to the data collected the previous day (time-point 144 hr in withdrawal, when performance had returned to baseline levels for all subjects). The data were analyzed using three-way mixed factors analyses of variance. The within-subjects factor was dose of mecamylamine (0 or 3.0 mg/kg), and the two between-subjects factors were chronic drug treatment (nicotine or saline) and acute drug treatment administered during withdrawal. Statistically significant interactions were followed by post-hoc Newman-Keuls tests. The level of significance was set at $p < .05$.

RESULTS

Chronic Nicotine

There were no statistically significant differences between the mean baseline thresholds or response laten-

Table 1. Body Weight of Rats During Chronic Nicotine (n = 32) or Saline (n = 32) Administration via Osmotic Minipumps

Days Post-pump Implantation	Saline	Nicotine
1	101.472 ± 0.278	100.481 ± 0.201*
2	101.959 ± 0.306	100.205 ± 0.209*
3	102.322 ± 0.456	100.280 ± 0.234*
4	102.775 ± 0.455	100.358 ± 0.208*
5	103.353 ± 0.466	100.692 ± 0.287*
6	103.743 ± 0.446	101.301 ± 0.278*
7	105.111 ± 0.544	102.900 ± 0.347*

Body weight expressed as a percentage of the body weight immediately after mini-pump implantation (mean + SEM).

*Statistically significant differences from saline-exposed group ($p < .05$).

cies of subjects assigned to the saline "withdrawal" group ($n = 32$) [mean thresholds ± SEM: 155.55 ± 10.06 μA; mean latencies ± SEM: 3.34 ± 0.06 sec], and subjects assigned to the nicotine group ($n = 32$) [mean thresholds ± SEM: 160.36 ± 8.04 μA; mean latencies ± SEM: 3.36 ± 0.04 sec] ($p > .1$). Based on the animals' performance during the 6- and 12-hr tests (time-points before the acute drug administration during with-

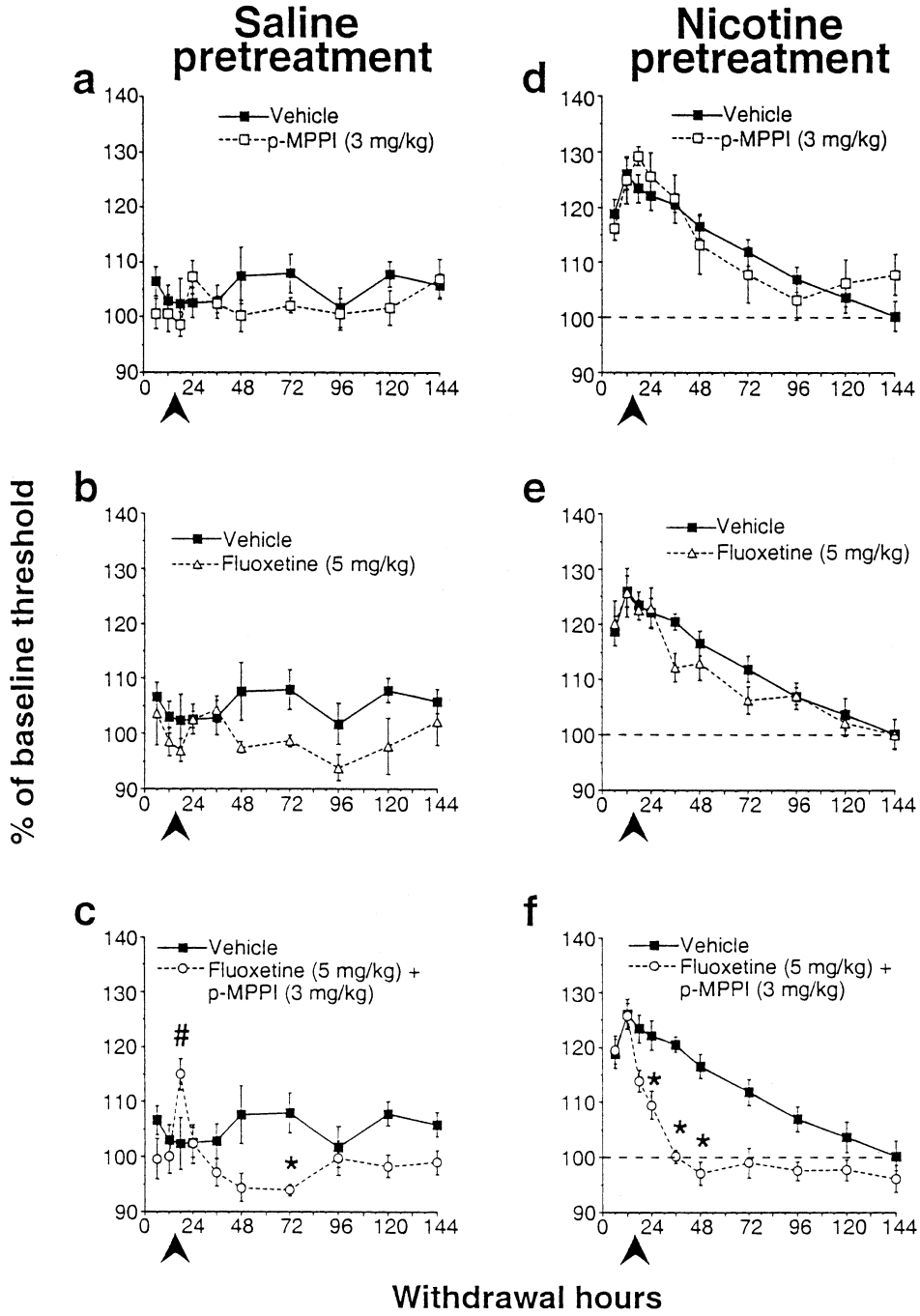

Figure 2. Serotonergic treatment reversed the elevations in brain reward thresholds observed during nicotine withdrawal. Nicotine withdrawal resulted in elevated reward thresholds (mean ± SEM) **(d, e, f)**, while saline-treated subjects' thresholds were stable **(a, b, c)**. p-MPPI **(a, b)** or fluoxetine **(b, e)** had no effect on thresholds of saline- or nicotine-treated rats. p-MPPI + fluoxetine lowered the threshold elevations of nicotine withdrawing rats **(f)**, while the same treatment elevated thresholds of saline-treated subjects **(c)**. The same vehicle-treated saline "withdrawing" animals are presented **(a, b, c)**, and the same vehicle-treated nicotine withdrawing animals are presented (d, e, f) ($n = 8$/group). Arrows indicate the time-point at which one of the various treatments was administered. Asterisks indicate statistically significant differences from the corresponding "control" group ($p < .05$). The pound sign indicates statistically significant differences from the group's predrug baseline.

drawal) subjects were assigned to treatment groups so that original withdrawal effects on threshold elevations were equal across groups.

Reward Thresholds. Reward thresholds of nicotine-exposed animals were significantly lower than those of saline-exposed animals [$F(1,62) = 5.983$, $p < .05$] during pump-exposure. Post-hoc analysis of a significant days X drug interaction revealed that the thresholds of nicotine-exposed animals were lower than those of saline-exposed control animals for the first two days after minipump implantation [$F(5,310) = 6.166$, $p < .01$] (see Figure 1a).

Response Latencies. Response latencies of nicotine-exposed rats also were shorter than those of saline-exposed control rats during pump-exposure [$F(1,62) = 7.917$, $p < .05$]. A trend for a statistically significant days $\times$ drug interaction [$F(5,310) = 2.134$, $p = .0612$] indicated that the difference in response latencies between nicotine and saline controls was greatest during the first two days of treatment (see Figure 1b).

Body Weight. The percent body weights of the nicotine-exposed rats were lower than those of the saline control rats during the nicotine phase [$F(1,62) = 21.551$, $p < .01$]. The percent body weight of both nicotine-exposed and control rats increased during the seven days of pump pressure [$F(6,372) = 77.247$, $p < .01$]. However, a significant days $\times$ drug interaction [$F(6,372) = 5.36$, $p < .01$] revealed that the body weight of the saline control rats increased steadily over time, whereas the nicotine-exposed rats did not gain weight during the first five days of nicotine-exposure. Consequently, the percent body weights of the saline-exposed rats were consistently greater than those of the nicotine-exposed rats during the seven day pump exposure ($p < .05$) (see Table 1).

Nicotine Withdrawal

Reward Thresholds. Withdrawal from chronic nicotine administration reliably increased reward thresholds compared to saline-treated rats [$F(1,56) = 57.87$, $p < .0001$] (see Figure 2). Analysis of the significant time $\times$ chronic treatment $\times$ acute treatment interaction [$F(27,504) = 2.55$, $p < .0001$] revealed the following. Nicotine-exposed rats treated with vehicle during withdrawal had elevated thresholds at withdrawal hr 6, 12, 18, 24 and 36, and returned to baseline levels at 48 hr post-pump removal compared to saline-exposed rats treated with vehicle. Administration of p-MPPI (3 mg/kg) or fluoxetine (5 mg/kg) administered alone had no significant effect on thresholds of either saline- or nicotine-exposed subjects (Figure 2a, b, d, e). By contrast, nicotine-exposed rats treated with p-MPPI + fluoxetine had significantly lower thresholds than the nicotine-exposed vehicle-treated subjects at 24, 36 and 48 hr post-nicotine (Figure 2f); that is, thresholds returned to baseline levels 8 hours after the acute combination drug treatment, and 24 hr before nicotine-exposed vehicle-treated subjects' thresholds returned to baseline levels.

Saline-exposed subjects treated with vehicle during withdrawal exhibited stable thresholds over the duration of the experiment (Figure 2a). Administration of p-MPPI or fluoxetine alone had no significant effect on reward thresholds of saline-exposed rats (Figure 2a, b). By contrast, in saline-exposed rats, the co-administration of p-MPPI + fluoxetine elevated thresholds compared to the previously stable performance of this group at the time-point immediately after this acute drug administration (Figure 2c), an effect that was seen repeatedly (see below). Thresholds returned to baseline levels by the following test session that was 8 hours after the acute drug treatment.

Response Latencies. Although there were no statistically significant interactions [$F = 0.94$–1.48, $p > .1$], there was a main effect of chronic drug treatment [$F(1,56) = 7.64$, $p < .01$], and a main effect of time [$F(9,504) = 7.05$, $p < .0001$] on response latencies. These main effects indicate longer response latencies in nicotine-exposed rats, and in both groups at 24 hr post-pump removal (see Figure 3).

Somatic Signs. Nicotine-exposed animals exhibited an increased number of somatic signs of withdrawal for

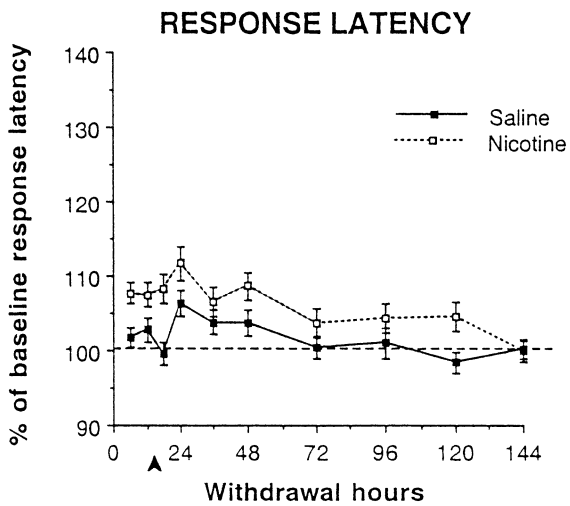

Figure 3. The effects of nicotine withdrawal and serotonergic treatment on response latencies (mean ± SEM). Nicotine withdrawal resulted in longer response latencies (n = 32: main effect in the ANOVA), while saline-treated subjects' (n = 32) response latencies were relatively stable. Vehicle, p-MPPI, fluoxetine, or p-MPPI + fluoxetine had no effect on response latencies of either saline- or nicotine-treated rats (data from individual groups not shown). The arrow indicates the time-point at which one of the various treatments was administered.

72 hr after pump removal compared to saline-exposed rats, reflected in a significant chronic drug treatment × time interaction [$F(9,504) = 14.78$, $p < .0001$] (Figure 4). None of the acute drug treatments administered during withdrawal had a significant effect on the number of somatic signs in either saline- or nicotine-exposed subjects ($p > .1$).

Body Weight. During nicotine withdrawal, animals that received the p-MPPI + fluoxetine combination treatment had significantly lower percent body weights than the animals in other treatment groups [$F(3,56) = 2.951$, $p < .05$]. A significant hours × pump interaction [$F(9,504) = 6.63$, $p < .01$] revealed that only the nicotine withdrawing animals significantly increased body weight only between 96–120 hr and 120–144 hr post-pump removal. The percent body weight of the nicotine-exposed rats was therefore greater than that of saline-exposed animals at time points 120 and 144 hr post-pump removal (see Table 2).

Mecamylamine Challenge

Mecamylamine had no significant effect on reward thresholds [$F(1,56) = 2.974$, n.s.]; (0 mg/kg mean ± SEM = 102.287 ± 9.132; 3.0 mg/kg mean ± SEM = 104.288 ± 9.352). Response latencies of all subjects were increased by the administration of mecamylamine [$F(1,56) = 5.598$, $p < .05$], (0 mg/kg mean ± SEM = 100.109 ± 0.954 ; 3.0 mg/kg mean ± SEM = 102.973 ± 1.229). Mecamylamine also increased the number of somatic signs displayed by all subjects [$F(1,56) = 308.443$, $p < .01$], (0 mg/kg mean ± SEM = 2.453 ± 1.967; 3.0 mg/kg mean ± SEM = 13.172 ± 4.914), thus not showing differential sensitivity of nicotine- and saline-exposed rats to this dose of mecamylamine.

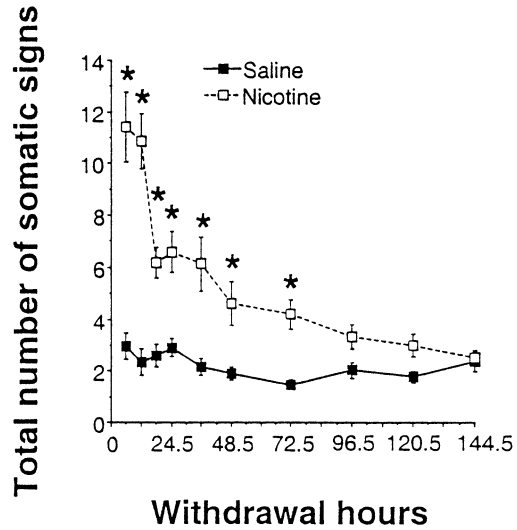

Figure 4. Somatic signs of withdrawal in nicotine and saline withdrawing rats (mean ± SEM). Termination of chronic nicotine administration increased the total number of somatic withdrawal signs ($n = 32$) compared to the saline-treated control group ($n = 32$). Neither vehicle, p-MPPI, fluoxetine, nor p-MPPI + fluoxetine affected the number of somatic withdrawal signs in either saline- or nicotine-treated rats (data from individual groups not shown). Somatic sign data are from the same subjects presented in Figures 1, 2, and 3. Asterisks indicate statistically significant differences from the saline-treated group ($p < .05$).

Amphetamine Withdrawal

There were no statistically significant differences between the mean baseline thresholds or response latencies of subjects assigned to the saline "withdrawal" group ($n = 48$) [mean thresholds ± SEM: 158.86 ± 8.67 μA; mean latencies ± SEM: 3.36 ± 0.044 sec], and subjects assigned to the amphetamine withdrawal group ($n = 48$) [mean thresholds ± SEM: 146.03 ± 4.26 μA; mean latencies ± SEM: 3.30 ± 0.04 sec] ($p > .1$). Based on the animals' performance during the 12-hr test (time-point before the acute drug administration during withdrawal), subjects were assigned to treatment groups so that original withdrawal effects on threshold elevations were equal across groups.

Thresholds. Amphetamine-exposed subjects exhibited elevated brain reward thresholds relative to saline-treated rats after the final amphetamine injection [$F(1,84) = 106.76$, $p < .0001$) (Figure 5). Analysis of the significant time × chronic treatment × acute treatment interaction [$F(35,588) = 2.56$, $p < .0001$] revealed the following. Amphetamine-exposed animals treated with vehicle prior to the 36-hr time-point had elevated thresholds at withdrawal hours 12, 36, 44, 60, and 84, and returned to baseline levels at 108 hr after the last amphetamine injection compared to saline-exposed rats treated with vehicle. p-MPPI (3 mg/kg) or fluoxetine

Table 2. Body Weight of Saline ($n = 32$) and Nicotine ($n = 32$) Exposed Rats During Nicotine Withdrawal

Hours Post-pump Removal	Saline	Nicotine
6	99.282 ± 0.171	99.076 ± 0.172
12	98.996 ± 0.197	98.815 ± 0.188
18	100.295 ± 0.202	100.435 ± 0.255
24	99.302 ± 0.141	99.198 ± 0.288
36	98.523 ± 0.284	98.479 ± 0.276
48	100.030 ± 0.213	99.783 ± 0.242
72	100.083 ± 0.163	100.088 ± 0.349
96	99.853 ± 0.158	100.448 ± 0.270
120	100.488 ± 0.186	101.485 ± 0.323*
144	100.759 ± 0.185	102.151 ± 0.272*

Body weight expressed as a percentage of the body weight immediately after mini-pump removal (mean + SEM).

*Statistically significant differences from saline-exposed group ($p < .05$).

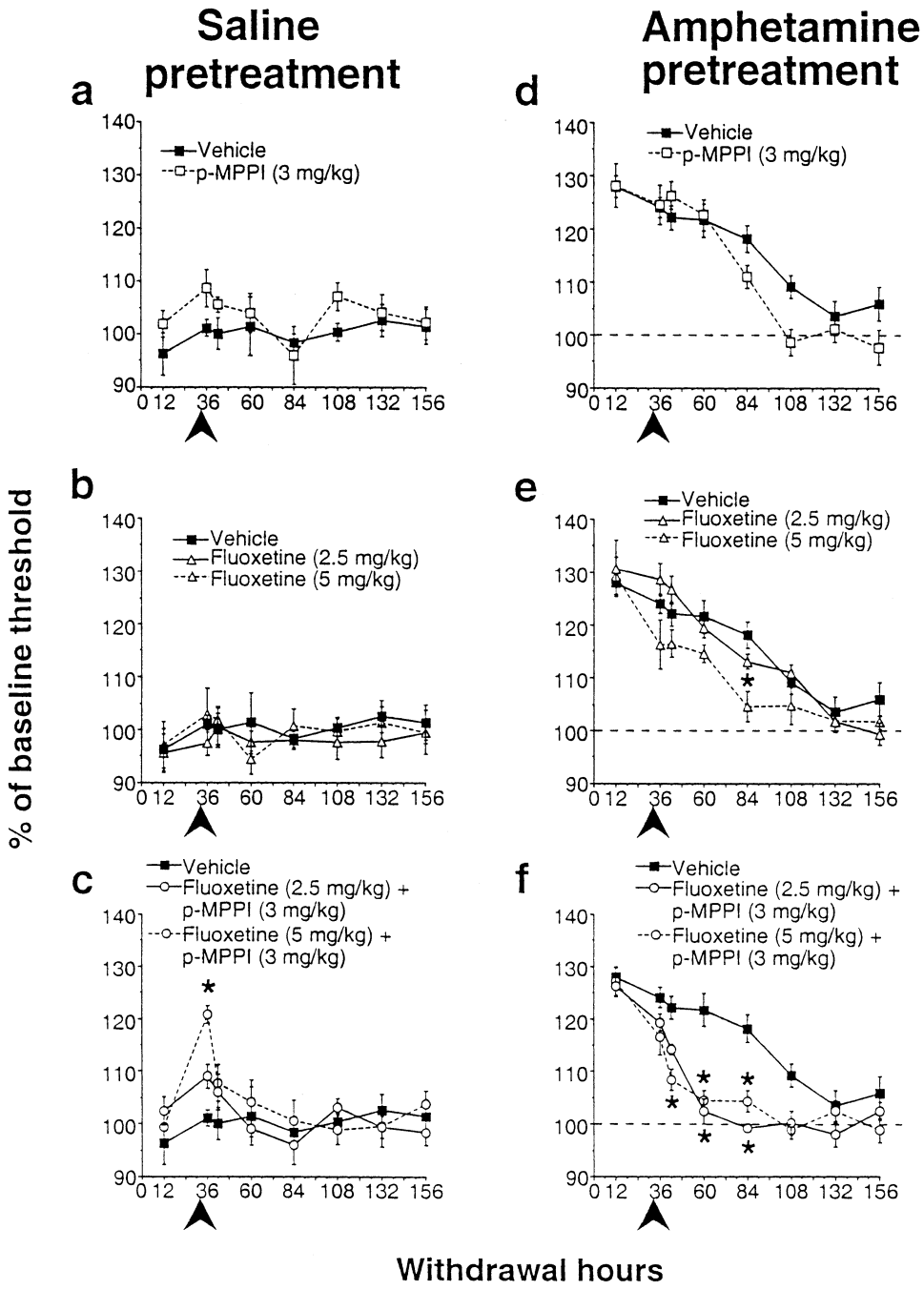

Figure 5. Serotonergic treatment reversed the elevations in brain reward thresholds observed during amphetamine withdrawal. Amphetamine withdrawal resulted in elevated reward thresholds (mean ± SEM) **(d, e, f)**, while saline-treated subjects' thresholds were stable **(a, b, c)**. p-MPPI **(a, d)** or 2.5 mg/kg fluoxetine **(b, e)** had no effect on thresholds of saline- or amphetamine-treated rats. 5 mg/kg fluoxetine reduced the duration of the amphetamine-induced threshold elevations by 24 hr compared to the vehicle-treated control group. p-MPPI + fluoxetine (2.5 or 5 mg/kg) lowered the threshold elevations of amphetamine withdrawing rats in a dose-related manner **(f)**, while the same treatment elevated thresholds of saline-treated subjects **(c)**. The same vehicle-treated saline "withdrawing" animals are presented **(a, b, c)**, and the same vehicle-treated amphetamine withdrawing animals are presented (d, e, f) ($n = 8$/group). Arrows indicate the time-point at which one of the various treatments was administered. Asterisks indicate statistically significant differences from the corresponding "control" group ($p < .05$).

(2.5 mg/kg) administered alone had no significant effect on the thresholds of either saline- or amphetamine-exposed subjects (Figure 5a, b, d, e). Nevertheless, the higher fluoxetine dose (5 mg/kg) reduced the duration of the amphetamine withdrawal-induced elevations of thresholds by 24 hr (Figure 5e). Most importantly, administration of p-MPPI and either of the two doses of fluoxetine prior to the 36-hr time point reduced the du-

ration of the threshold elevations in amphetamine-exposed rats (Figure 5f). More specifically, amphetamine-exposed rats treated with p-MPPI + 2.5 mg/kg fluoxetine had significantly lower thresholds than amphetamine-exposed vehicle-treated subjects at 60 and 84 hours post-amphetamine (24 hr after the acute treatment), while p-MPPI + 5 mg/kg fluoxetine returned thresholds to baseline levels at the 42-hr time-point (8 hr after the acute treatment) (Figure 5f). That is, 2.5 mg/kg fluoxetine in combination with p-MPPI returned thresholds to baseline levels 48 hours earlier than vehicle treatment, while 5 mg/kg fluoxetine in combination with p-MPPI returned thresholds to baseline levels 72 hours earlier than vehicle treatment (Figure 5f).

As expected, saline-exposed subjects treated with vehicle exhibited stable thresholds over the duration of the experiment (Figure 5a). Administration of p-MPPI or either of the two doses of fluoxetine alone had no significant effect on reward thresholds of saline-exposed rats (Figure 5a, b). As seen in the nicotine withdrawal experiment, in saline-treated rats, the co-administration of p-MPPI + the higher dose of fluoxetine (5 mg/kg) elevated thresholds compared to the previously stable thresholds of this group and the saline-exposed vehicle-treated group's thresholds immediately after the acute drug administration (Figure 5c). Thresholds returned to baseline levels by the following test session that was 8 hours after the acute drug treatment. Other experiments in unperturbed non-withdrawing control rats confirmed that 10 mg/kg p-MPPI combined with 5 mg/kg fluoxetine also significantly elevated thresholds (data not shown), providing the third demonstration of decrease in reward after this drug combination when subjects are not in drug withdrawal.

Response Latencies. There was a main effect of acute drug treatment during withdrawal [$F(5,84) = 2.88$, $p < .05$], a main effect of time [$F(7,588) = 5.87$, $p < .0001$], and a significant time X acute drug treatment interaction [$F(35,588) = 1.96$, $p < .001$]. These effects reflect longer response latencies in both saline and amphetamine-exposed rats immediately after the co-administration of p-MPPI and either of the two fluoxetine doses (36 hr time-point). Further, the effects of p-MPPI + the higher dose of fluoxetine (5 mg/kg) on response latencies were still observed at the 42 hour time-point of withdrawal which was 8 hr after the administration of the combination drug treatment (see Figure 6).

DISCUSSION

During chronic nicotine or saline administration, animals treated with nicotine had lower brain reward thresholds than saline controls. The small threshold elevation of saline-exposed rats on the first day of exposure to the minipumps may be due to residual discomfort from the minipump surgery that occurred the previous day. Similar small and transient threshold elevations after minipump surgery have been observed previously (Paterson et al. 2000). This effect was not observed in nicotine-exposed rats, possibly reflecting a mild analgesic effect of nicotine. Analgesic effects of nicotine that show rapid tolerance have been shown

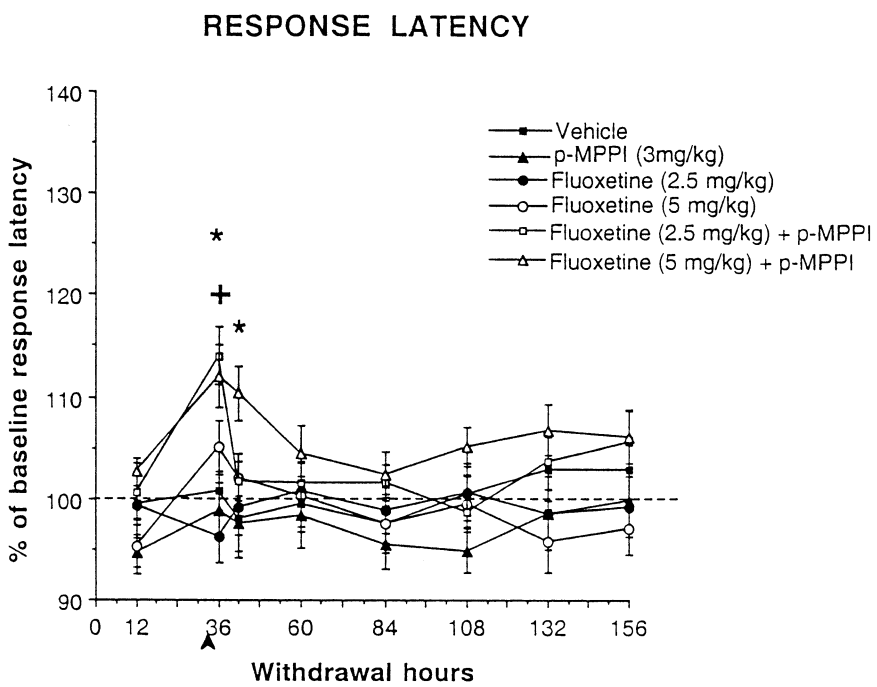

Figure 6. The effects of amphetamine withdrawal and serotonergic treatment on response latencies (mean ± SEM). Co-administration of p-MPPI and either of two doses of fluoxetine (2.5 or 5 mg/kg) resulted in longer response latencies in both saline- and amphetamine-treated rats compared to vehicle-treated subjects ($n = 16$/group). The arrow indicates the time-point at which one of the various treatments was administered. Asterisks indicate statistically significant differences between the 5 mg/kg fluoxetine + p-MPPI and the vehicle control group ($p < .05$). The plus sign indicates statistically significant differences between the 2.5 mg/kg fluoxetine + p-MPPI and the vehicle control group ($p < .05$).

previously with bolus injections of nicotine (1–3 mg/kg, free base) or with continuous nicotine exposure through tobacco smoke or minipumps containing almost double the nicotine dose (6 mg/kg/day free base = approximately 17 mg/kg/day salt) used in the present study (Marubio et al. 1999; Mousa et al. 1988; Yang et al. 1992). On the second day of minipump exposure, nicotine-exposed animals still had lower thresholds than saline controls, indicating a small reward-enhancing effect of continuous slow subcutaneous infusion of nicotine. This result is consistent with increases in brain stimulation reward seen after acute bolus nicotine administration (Bauco and Wise 1994; Clarke and Kumar 1984; Huston-Lyons and Kornetsky 1992). Nevertheless, in the present study, this small reward-enhancing effect of nicotine was no longer evident on the third day of minipump exposure possibly reflecting tolerance to the reward-enhancing effects of slowly infused nicotine. This rapid tachyphylaxis is consistent with experimental and anecdotal reports in humans indicating that the first cigarette of the day is the most pleasurable, in part because of acute tolerance that develops to the subjective effects of nicotine over the day as the smoker consumes additional cigarettes (for review, see Russell 1989). The effects of continuous nicotine infusion on response latencies followed a similar pattern of results to that of thresholds. Nicotine-exposed rats were faster in responding than saline-exposed rats, and there was a trend for this effect to be larger during the first two days of exposure to nicotine. Finally, consistent with previous reports, chronic nicotine administration suppressed body weight gain compared to the weight gain of saline-exposed animals (Carroll et al. 1989; Grunberg et al. 1986; Levin et al. 1987).

After removal of the minipump, animals previously exposed to nicotine gained weight on the last two days of measurement (120 and 144 hr post-pump removal) in comparison to both their own baseline body weight and that of saline-exposed animals. Previous studies indicated similar, but more dramatic, effects of nicotine withdrawal on body weight after longer nicotine exposure than that used in the present study (Levin et al. 1987; Winders and Grunberg 1989). None of the acute serotonergic drug treatments affected this pattern of weight gain in either saline- or nicotine-exposed rats.

In replication of previous findings (Epping-Jordan et al. 1998; Kokkinidis and Zacharko 1980a, 1980b; Kokkinidis et al. 1986; Leith and Barrett 1976, 1980; Lin et al. 1999, 2000; Paterson et al. 2000; Watkins et al. 2000b; Wise and Munn 1995), both nicotine and amphetamine withdrawal resulted in decreased reward reflected in elevated brain reward thresholds relative to saline-treated rats' and to their own pre-drug baseline thresholds. As anticipated (Epping-Jordan et al. 1998; Hildebrand et al. 1997, 1999; Malin et al. 1992; Watkins et al. 2000b), nicotine withdrawal also led to significant increases in somatic signs. Most importantly, the results indicated that the co-administration of fluoxetine, a selective serotonin reuptake inhibitor, and p-MPPI, a 5-HT$_{1A}$ receptor antagonist, alleviated the diminished interest in rewarding stimuli observed during withdrawal from nicotine or amphetamine in rats. Further, the reversal of the threshold elevations associated with amphetamine withdrawal was dose-related because the co-administration of the 5-HT$_{1A}$ antagonist with the highest fluoxetine dose returned thresholds to baseline levels 24 hr earlier than the co-administration of p-MPPI with the lowest fluoxetine dose. Neither p-MPPI nor the low dose of fluoxetine alone had an effect on nicotine or amphetamine withdrawal-induced threshold elevations; while the highest dose of fluoxetine when administered alone reduced the duration of the amphetamine, but not nicotine, withdrawal-induced elevations of thresholds by 24 hr. This rapid restoration of the sensitivity to electrical stimulation (i.e., relative increase in reward) may be attributable to increased serotonin function in forebrain structures, such as the frontal cortex, the hippocampus and the striatum (e.g., Bel and Artigas 1993; Dreshfield et al. 1996, 1997; Invernizzi et al. 1994; Gobert and Millan 1999; Rutter et al. 1994). The present data are consistent with the hypothesis that the rapid onset of clinical antidepressant action of SSRIs when combined with pindolol (Bordet et al. 1998; Tome et al. 1997a, 1997b; Zanardi et al. 1997, 1998; however, see Berman et al. 1997, 1999) is partly attributable to pindolol's 5-HT$_{1A}$ antagonist properties. Nevertheless, other receptors, such as the 5-HT$_{1B}$ and α-adrenergic receptors, for which pindolol has antagonist and/or partial agonist properties, and other neuromechanisms may also contribute to pindolol's augmentation of SSRI antidepressant effects.

By contrast, in saline-treated rats, this combination treatment resulted in a small, but statistically significant elevation of reward thresholds (i.e., decrease in reward) compared to the previously stable performance of these groups, an effect that was seen repeatedly (see below). Considering the relatively short-lasting elevation of reward thresholds seen in saline-treated subjects after the administration of the drug combination, which is typical of acute drug treatments, the permanent reversal of the reward threshold elevations of nicotine- and amphetamine-withdrawing subjects after a single treatment is note-worthy and somewhat surprising. It is possible that an acute drug treatment was sufficient to permanently reverse the threshold elevations for the following reasons. The combination of fluoxetine's long half-life (up to 7 hr with detection of fluoxetine levels up to 30 hr after systemic administration of doses similar to the ones used here; Caccia et al. 1990) and the relatively short-lasting threshold elevation seen especially during nicotine withdrawal may have allowed the gradually declining enhancement of serotonergic neu-

rotransmission to coincide with the gradual return of reward thresholds to baseline levels. Further, the doses administered in the present study are much higher than those that would be tolerated by humans on the first administration (although such comparisons are hard to make) rendering it more likely that the drug treatment would have an acute effect. Further, it has been argued that antidepressants may produce immediate improvement of *some* symptoms in humans, but this acute effect may be hard to detect statistically because the improvement may be initially small and seen only in some, but not all, symptoms (Detke et al. 1997; Frazer 1994). Another interesting possibility is that once reversal of a deficit occurs in a relatively "healthy" animal in which the deficit was transiently induced by a manipulation, a "healthy" system may not readily regress to the previous imbalance.

Considering that chronic drug treatment is required in most cases before a therapeutic antidepressant effect is observed in humans, the demonstration of the reversal of a reward deficit with a single acute drug treatment in the present study may be considered a limitation of the proposed animal model. It could be argued that under such conditions the mechanisms leading to the reversal of the behavioral deficit are not the same as the ones leading to the clinical therapeutic effect (Detke et al. 1997). Even though such arguments have merit, there are counter-arguments and reasons why the study of the acute effects of drugs, when guided by good working hypotheses, still could promote our understanding of drug withdrawal, depressive symptomatology, and mode of action of antidepressant compounds. The present study was designed to test the hypothesis that activation of a specific neurobiological mechanism (i.e., enhanced serotonergic neurotransmission) would reverse a reward deficit. The findings offer one of the few experimental demonstrations that the enhanced serotonin neurotransmission induced by such drug treatments leads to observable behavioral changes with relevance to depressive symptomatology. For example, the *acute* administration of the 5-HT_{1A} partial receptor agonist buspirone enhanced the antidepressant-like actions of a low *acute* dose of a SSRI that had no effect on the forced swim stress when administered on its own (Redrobe and Bourin 1998). By contrast, the addition of a 5-HT_{1A} receptor antagonist to the chronic treatment with a SSRI did *not* accelerate the onset of antidepressant-like action seen after chronic treatment with the SSRI alone neither in the olfactory bulbectomized rat model of depression (Cryan et al. 1998, 1999) nor in the forced swim test (Moser and Sanger 1999). Finally, the study of the *acute* effects of drugs in the forced swim test, a postulated animal model of depression (e.g., for review, see Lucki 1997), and in deficits of the prepulse inhibition of startle, a postulated animal model of gating deficits seen in several psychiatric disorders (e.g., for review, see Swerdlow and Geyer 1998), has led to a plethora of neurobiological findings with relevance to both healthy and psychopathological states. The ability of the two above-mentioned behavioral paradigms to predict the therapeutic efficacy of antidepressant and antipsychotic compounds, respectively, demonstrates the predictive validity of these models, which is a starting point in the development of any model (Geyer and Markou 1995). Similarly, the reversal of the affective, but not the somatic, aspects of drug withdrawal with a drug treatment that enhances serotonergic neurotransmission indicates some predictive and discriminant validity of the present paradigm as an animal model of the symptom of "diminished interest or pleasure".

Ample evidence indicates that brain stimulation reward thresholds are a valid and reliable measure of reward that is minimally affected by performance manipulations (Markou and Koob 1992b, 1993). Further, extensive previous work with the identical brain stimulation reward procedure used in the present study demonstrated that reward thresholds do *not* co-vary with response latencies (e.g., Harrison et al. 1999; Lin et al. 1999, 2000; Macey et al. 2000; Markou and Koob 1991, 1992b; Paterson et al. 2000). Similarly, the present results also demonstrated a lack of co-variation of threshold elevations with increased response latencies (see Results section and Figure 3 and 6). Thus, threshold elevations seen during drug withdrawal cannot be attributed to effects of the various manipulations on performance.

The absence of an effect of doses of fluoxetine up to 5 mg/kg on reward thresholds in control subjects in the present and previous studies in our laboratory (Lin et al. 1999) appears to be in contrast to another report demonstrating elevations in thresholds after 2.5–20 mg/kg fluoxetine using the same intracranial self-stimulation procedure (Lee and Kornetsky 1998). The reasons for this discrepancy are not clear, although there are some methodological differences between the two studies. In the present study, electrodes were placed in the medial forebrain bundle at the level of the posterior lateral hypothalamus, whereas the majority of placements in the Lee and Kornetsky (1998) study were in the ventral tegmental area. Moreover, in the present study, fluoxetine was administered 2 hr, while in the Lee and Kornetsky study 1 hr prior to test. Finally, administration of 10 mg/kg fluoxetine 2 hr before the test did result in threshold elevations in our laboratory (Harrison and Markou, unpublished observations). Thus, the differences in results appear to be quantitative (i.e., dose) rather than qualitative. Taken together, these findings suggest that higher acute doses of fluoxetine than those used in the present study may counteract threshold elevations associated with drug withdrawal.

The present data suggest that reduced serotonergic neurotransmission may be one of the neurobiological abnormalities underlying the symptom of "diminished interest or pleasure" seen in both amphetamine and nicotine withdrawal in humans (American Psychiatric Association 1994; Covey et al. 1998; Glassman 1993; Hughes 1992; Markou et al. 1998). This hypothesis is consistent with the observation that withdrawal from other drugs of abuse, such as cocaine (Parsons et al. 1995) or ethanol (Weiss et al. 1996), also is characterized by decreased serotonergic neurotransmission as reflected in decreased *in vivo* dialysate serotonin levels. Further, considering the strong evidence implicating reduced serotonergic neurotransmission as one of the neurochemical abnormalities associated with depression (for reviews, see Caldecott-Hazard et al. 1991; Caldecott-Hazard and Schneider 1992; Heninger et al. 1996; Markou et al. 1998; Meltzer and Lowy 1988; Willner 1985), it can be hypothesized that there is homology between the symptom of "diminished interest or pleasure" seen in individuals withdrawing from drugs of abuse and the same symptom seen in depressed patients. Decreased dopaminergic neurotransmission is another neurobiological abnormality common to withdrawal from a variety of drugs of abuse, including nicotine (Hildebrand et al. 1999; Parsons et al. 1995; Weiss et al. 1996).

Based on the above hypothesis of homology between symptoms seen across psychiatric diagnostic categories, it can be postulated that reward deficits associated with withdrawal from drugs of abuse may be an animal model of the symptom of "diminished interest or pleasure" with construct, convergent, and predictive validities (Geyer and Markou 1995). In addition to reward threshold elevations, amphetamine withdrawal also is characterized by decreased breaking-points under a progressive ratio schedule for a sucrose solution reinforcer, and decrements in anticipatory and motivational measures for sexual reinforcement in rats (Barr and Phillips 1999; Barr et al. 1999). Taken together, these results provide converging evidence that drug withdrawal is characterized by decreased motivation and "diminished interest or pleasure" for a variety of rewarding stimuli and situations, a condition resembling a core feature of non-drug-induced depressions (i.e., construct validity) (American Psychiatric Association 1994).

Although termination of nicotine administration induced the anticipated somatic signs of withdrawal, none of the drug treatments altered the number of somatic signs observed during nicotine withdrawal. This dissociation of the effects of the drug combination treatment on the affective (i.e., reward threshold elevations) versus the somatic signs of withdrawal is evidence for symptom-specific neurobiological abnormalities, and demonstrates the discriminant validity of this animal model (Geyer and Markou 1995). If, as suggested, the affective symptoms of withdrawal are critical to the development of drug dependence and in causing relapse during the early stages of abstinence (Koob et al. 1993; Markou et al. 1998), then reversal of such symptoms with serotonergic antidepressant treatments may reduce the probability of relapse. Indeed, there is evidence that antidepressant treatment results in improvement of mood and reduction of drug use in depressed cocaine, opiate and nicotine abusers (for reviews, see Hughes et al. 2000; Kosten et al. 1998; Markou et al. 1998; Nunes et al. 1998; Watkins et al. 2000a).

The administration of fluoxetine together with a 5-HT_{1A} receptor antagonist also had dissociable effects on the reward function of withdrawing and non-withdrawing rats. The restoration of the sensitivity of the brain reward system to the electrical stimuli (i.e., relative increase in reward) after the drug treatment in withdrawing rats contrasts with the small decrease in brain stimulation reward induced by the same drug treatment in saline-treated rats. This result is consistent with previous studies of "normal" unperturbed rats in which increasing serotonergic neurotransmission reduced response rates for brain stimulation reward reflecting a decrease in reward (McClelland et al. 1989; Olds 1994). These data from "normal" subjects may seem incompatible with the hypothesis that antidepressants elevate mood and alleviate reward deficits in depressed individuals through enhancement of serotonergic neurotransmission. This apparent disparity may be explained by the present results suggesting that the effects of antidepressant treatments on brain reward function may be dependent on the "hedonic" state of the subjects at the time of treatment (Ahmed and Koob 1998; Koob and Le Moal 1997). The present observation also may explain why previous attempts to show antidepressant-induced alterations in "mood" in unperturbed rats have provided largely negative results (Fibiger and Phillips 1981; Hall et al. 1990; Lin et al. 1999; Moreau et al. 1992; Markou et al. 1992). In the present study and in confirmation of previous work (Lin et al. 1999), administration of low fluoxetine doses alone did not elevate thresholds, paralleling the lack of effect of antidepressants on mood in healthy individuals. Thus, the development of animal models of depression, and potentially of other psychiatric disorders, should be based primarily on modeling and studying deficits that are pathognomic of the disorder; because the predictive validity of models that study healthy "normal" animals may be limited and could lead to erroneous conclusions.

In summary, the present results indicated that acute administration of a drug combination that enhances serotonergic neurotransmission alleviated the diminished interest in brain stimulation reward observed during withdrawal from nicotine or amphetamine in rats. By

contrast, the same drug combination treatment did not reduce the somatic signs of nicotine withdrawal indicating symptom-specific neurobiological abnormalities. Taken together with extensive previous observations about depression, the present findings suggest that the affective aspects of both drug- and non-drug-induced depressions may be mediated, at least partly, by reduced serotonergic neurotransmission. Thus, there may be homology between the affective symptoms of drug withdrawal and the core symptom of "diminished interest or pleasure" characterizing non-drug-induced depressions. Surprisingly, the same serotonergic drug combination treatment had opposite effects in control rats where reductions in reward were produced. This last observation has important implications about the development of animal models for studying the neurobiology of psychiatric disorders, such as drug withdrawal and depression, and predicting the clinical efficacy of novel treatments.

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