Published in final edited form as:

Psychopharmacology (Berl). 2008 June ; 198(3): 351–362.

The effects of chronic versus acute desipramine on nicotine withdrawal and nicotine self-administration in the rat

Neil E. Paterson^{*,†}, Svetlana Semenova^{*}, and Athina Markou

Department of Psychiatry, School of Medicine, University of California at San Diego, La Jolla, California 92093-0603, USA

Abstract

Rationale—Nicotine withdrawal is characterized by depression-like symptomatology that may be mediated by dysregulations in norepinephrine transmission. These aversive aspects of nicotine withdrawal and the rewarding effects of nicotine play major roles in maintaining nicotine dependence.

Objectives—To evaluate the effects of desipramine (DMI), a preferential norepinephrine reuptake inhibitor and antidepressant, on preclinical models of nicotine dependence in rats.

Methods—A rate-independent current-intensity discrete-trial threshold intracranial self-stimulation procedure was used to assess brain reward function during nicotine withdrawal induced by cessation of nicotine infusion via subcutaneous osmotic minipumps (3.16 mg/kg/day, base). Nicotine withdrawal was also measured by somatic signs of withdrawal. DMI was administered acutely (2 or 5 mg/kg, salt) during nicotine/saline withdrawal. In other naïve rats, chronic DMI treatment via minipump (15 mg/kg/day, salt) began after 7 days of nicotine/saline exposure and continued during administration of nicotine/saline for 14 days and during nicotine/saline withdrawal. Additional rats acquired intravenous nicotine- or food-maintained responding, were prepared with DMI/vehicle-containing minipumps, and self-administered nicotine or food during 12 days of DMI/vehicle exposure.

Results—Acute DMI administration had no effect on threshold elevations observed in nicotine-withdrawing rats. Chronic DMI administration prevented the reward threshold elevations and the increased somatic signs of nicotine withdrawal. Although chronic DMI significantly decreased nicotine self-administration, it also decreased food-maintained responding.

Conclusions—The results suggest that norepinephrine reuptake inhibitors may be effective anti-smoking treatments that reduce the anhedonic depression-like and somatic components of nicotine withdrawal, and may alter the rewarding effects of nicotine and food.

Keywords

nicotine; withdrawal; self-administration; brain stimulation reward; somatic signs; desipramine; norepinephrine; rats

Nicotine dependence in the form of tobacco smoking (Stolerman and Shoaib 1991; Stolerman and Jarvis 1995) presents a major global health burden (Proctor 2004). The negative affective consequences of early nicotine withdrawal are recognized as playing significant roles in relapse

Corresponding Author: Athina Markou, Ph.D., Department of Psychiatry, 0603, School of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0603 USA, Tel: +858-534-1572, Fax: +858-534-9917, E-mail: amarkou@ucsd.edu.

[†]Current address: Department of Psychiatry Semel Institute for Neuroscience & Human Behavior University of California at Los Angeles Los Angeles, California 90095-1563, USA

^{*}These authors contributed equally to this work.

during quit attempts (West et al. 1989; Piasecki et al. 2003; but see Hughes et al. 1991), and in the maintenance of compulsive nicotine use (Solomon and Corbit 1973; Koob et al. 1989; Markou et al. 1998). Some affective signs of early nicotine withdrawal resemble certain aspects of major depressive disorder, such as anhedonia defined as diminished ability to experience pleasure (Markou et al. 1998; American Psychiatric Association 1994). Studies have identified complex dysregulations in norepinephrine neurotransmission within multiple brain regions in major depression (Ressler and Nemeroff 2000). Nevertheless, major depression is also associated with perturbations in multiple other neurotransmitter systems, such as serotonin (Neumeister et al. 2004). The neurobiological similarities between nicotine withdrawal and major depression (Markou et al. 1998) raise the possibility that both disease states share dysregulations in noradrenergic neurotransmission (Quattrochi et al. 2000).

The antidepressant medications nortriptyline and bupropion, both of which inhibit norepinephrine reuptake, attenuate nicotine withdrawal in humans. Nortriptyline is proposed (Prochazka et al. 1998; Wagena et al. 2005) and bupropion is established (Hurt et al. 1997; Jorenby et al. 1999) as effective anti-smoking medications. However, preclinical evidence for an ameliorative effect of noradrenergic manipulations on nicotine withdrawal is limited. Both nortriptyline and bupropion, in addition to their norepinephrine reuptake inhibition properties, exhibit pharmacological effects that may mediate their anti-smoking properties. Specifically, nortriptyline preferentially blocks norepinephrine reuptake (Frazer 2001), but also inhibits serotonin reuptake and blocks the serotonin 2 receptor (Sanchez and Hyttel 1999). Bupropion inhibits the dopamine and the norepinephrine transporters (Ferris et al. 1983; Damaj et al. 2004) and blocks nicotinic acetylcholine receptors (Fryer and Lukas 1999; Slemmer et al. 2000). Behavioral studies in rats suggest that the dopamine-elevating (Paterson et al. 2007) and nicotinic acetylcholine receptor antagonist (Glick et al. 2002; Bruijnzeel and Markou 2003) properties of bupropion may contribute to the anti-smoking effects of bupropion. Nonetheless, bupropion exhibits low levels of dopamine transporter occupancy at therapeutic doses used for smoking cessation and antidepressant activity (Meyer et al. 2002; Learned-Coughlin et al. 2003), while there is evidence in humans that bupropion increases noradrenergic neurotransmission (Dong and Blier 2001; Gobbi et al. 2003). Thus, it is possible that norepinephrine transporter inhibition, in addition to dopamine transporter inhibition, also contributes to the antidepressant and anti-smoking properties of bupropion and nortriptyline. Desipramine (DMI) is a preferential inhibitor of norepinephrine reuptake inhibition, a weak inhibitor of serotonin reuptake (Richelson and Pfenning 1984) and a nicotinic acetylcholine receptor antagonist (Rana et al. 1993; Izaguirre et al. 1997). In the present studies, chronic DMI was administered at a dose that selectively inhibits norepinephrine, but not serotonin, reuptake in rats (Benmansour et al. 1999, 2004).

In animal models, intracranial self-stimulation (ICSS) thresholds provide a quantitative measure of brain reward function. Elevated reward thresholds are an operational measure of anhedonia (Markou et al. 1993; Geyer and Markou 1995), a core symptom of nicotine withdrawal and major depression (American Psychiatric Association 1994). Nicotine withdrawal may be induced by cessation of chronic nicotine infusion and is associated with elevated reward thresholds and increased somatic signs (Epping-Jordan et al. 1998; Malin et al. 1992).

Several clinically effective antidepressant treatments attenuate brain reward threshold elevations observed during nicotine or amphetamine withdrawal (for review, see Paterson and Markou 2007). For example, acute (Cryan et al. 2003a) or chronic (Paterson et al. 2007) bupropion administration attenuated both the anhedonic and somatic components of nicotine withdrawal in rats. Further, increased serotonergic neurotransmission attenuated threshold elevations associated with either nicotine or amphetamine withdrawal (Harrison et al. 2001). Recently, acute nortriptyline attenuated the somatic component of precipitated nicotine

withdrawal in rats (Wing and Shoaib 2007). To extend these previous findings and to assess the potential role of increased norepinephrine transmission in the antismoking properties of bupropion and nortriptyline, the present study assessed the effects of DMI on the anhedonic and somatic components of nicotine withdrawal. DMI was selected because this compound shares the norepinephrine reuptake inhibition properties of bupropion and nortriptyline, but not their dopaminergic (bupropion) or serotonergic (nortriptyline) effects (Richelson and Pfenning 1984; Benmansour et al. 1999, 2004). Previous studies assessed the effects of noradrenergic manipulations on alternative measures of nicotine dependence-related behaviors (for review, see Lerman et al. 2007), such as nicotine self-administration, but not on the anhedonic component of nicotine withdrawal.

The present studies investigated the effects of chronic DMI on both the reinforcing properties of nicotine in the intravenous nicotine self-administration procedure and the anhedonic and somatic aspects of nicotine withdrawal, because compulsive nicotine use is maintained by both the positive and negative reinforcing effects of nicotine (Solomon and Corbit 1973; Koob et al. 1989; Markou et al. 1998). Thus, concomitant attenuation of both the negative consequences of nicotine withdrawal and the positive reinforcing effects of acute nicotine, observed under identical chronic treatment regimens, would be a highly desirable feature of potential anti-smoking medications. We predicted that norepinephrine reuptake inhibition via chronic DMI administration would decrease nicotine self-administration in rats, similarly to the effects of chronic treatment with reboxetine, a selective norepinephrine reuptake inhibitor (Rauhut et al. 2002). Acute DMI administration has antidepressant-like properties in models of antidepressant-like activity, such as the forced swim test (Lahmame and Armario 1996; Cryan et al. 2003b). Therefore, it was important to assess whether acute DMI administration would also be effective in our animal model of depression-like behavior observed during spontaneous nicotine withdrawal.

Methods and Materials

Subjects

Male Wistar rats (Charles River, Raleigh, NC) weighing 300-350 g upon arrival in the laboratory were group-housed (two per cage) and maintained on a 12-hour reverse light dark cycle (lights on at 2000 h) in a temperature- and humidity-controlled vivarium. For one week after arrival in the vivarium, animals were allowed to habituate to their new environment during which time rats were handled twice. Animals were given *ad libitum* access to food and water, except during testing. All testing occurred during the dark phase of the light-dark cycle. All subjects were treated in accordance with the National Institutes of Health guidelines regarding the principles of animal care (National Institutes of Health 1996) and the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Drugs

(-)-Nicotine hydrogen tartrate (doses expressed as base) and DMI (doses expressed as salt) were purchased from Sigma (St. Louis, MO, USA). Nicotine was dissolved in saline, and DMI was dissolved in sterile water. For preparation of nicotine solutions for self-administration, the pH was adjusted to 7 ± 0.5 with sodium hydroxide (Sigma-Aldrich, St. Louis, MO) and the solution was filtered through a 0.22 μm syringe filter (Fisher Scientific, Pittsburgh, PA) for sterilization purposes. Chronic DMI and nicotine were administered via subcutaneous osmotic minipumps. Acute DMI was administered intraperitoneally (i.p.) in a volume of 4 ml/kg.

Intracranial self-stimulation

Methodological details of surgery, apparatus and the intracranial self-stimulation procedure used are described in detail elsewhere (Harrison et al. 2001). Briefly, subjects were prepared

with stainless steel bipolar electrodes in the posterior lateral hypothalamus [AP: -0.5 mm from bregma; ML: ± 1.7 mm; DV: -8.3 mm from dura; incisor bar set 5 mm above the interaural line (Pellegrino et al. 1979)] under isoflurane/oxygen vapor mixture (1-1.5% isoflurane) anesthesia. Subjects were trained to respond for electrical stimulation under a discrete-trial current-threshold intracranial self-stimulation procedure, modified from Kornetsky and Esposito (1979). Each test session typically lasted 30-40 min and provided two dependent variables for behavioral assessment: threshold and response latency (see Harrison et al. 2001 for details).

Intravenous self-administration and food responding

Methodological details of catheter construction, surgery and acquisition of nicotine- and food-maintained responding have been described elsewhere (Paterson et al. 2004). Briefly, rats were food-restricted and trained to respond for food, progressing from a fixed-ratio 1 time-out 1 s (FR1 TO1) to a FR5 TO20 s schedule of reinforcement, with sessions lasting approximately 30 min. Rats used to assess the effects of chronic DMI administration on food-maintained responding remained on a restricted diet (20 g rat chow per day) and continued to respond for food in 1 h daily sessions. Other rats were prepared with intravenous catheters inserted into the right jugular vein under isoflurane/oxygen vapor mixture (1-1.5% isoflurane) anesthesia and were allowed to self-administer nicotine (0.03 mg/kg/inf, base). Responding on the active lever (previously paired with delivery of a food pellet) resulted in the delivery of the nicotine solution in a volume of 0.1 ml over a 1 s period, and the presentation of a cue light above the active lever that remained lit for 20 sec, during which time responses on the active lever had no consequences (i.e., time-out period). Responding on the inactive lever (introduced during the first self-administration session) had no consequences. Rats received 20 g rat chow per day, at least 1 h after termination of testing.

Osmotic minipump implantation and removal surgery

Rats were anesthetized with an isoflurane/oxygen vapor mixture (1-1.5% isoflurane), and an osmotic minipump (model 2ML1 in Experiment 2; models 2ML2 and 2ML4 in Experiments 3 and 4; Alza Corp, Palo Alto, CA) containing nicotine, saline, DMI or vehicle was implanted subcutaneously (back of the animal parallel to the spine) with the flow-moderator directed posteriorly. Minipump removal was also performed under anesthesia. After pump implantation/removal, the wound was stapled, and an antibacterial preparation was applied to the incision area.

Observation of somatic signs of nicotine withdrawal

Somatic signs of nicotine withdrawal were counted under white light conditions in cylindrical Plexiglas chambers (diameter 15 cm) with sawdust bedding on the floor. Each subject was observed for 10 min by an observer blind to the subjects' treatments. The standard checklist used was adapted from an opiate withdrawal signs checklist (see Epping-Jordan et al. 1998; Malin et al. 1992). The following somatic signs of withdrawal were recorded: body shakes, chews, cheek tremors, escape attempts, eye-blinks, foot licks, gasps, writhes, genital licks, head shakes, ptosis, teeth chattering, and yawns. Ptosis, if present, was recorded only once per minute.

Experimental Design

Experiment 1: Effects of acute DMI administration on ICSS thresholds—After establishment of stable ICSS thresholds (defined as less than 10% variation over five consecutive days), drug-naïve rats ($n = 10$) were injected with DMI (0, 0.5, 1, 2, and 5 mg/kg, i.p., in a volume of 4 ml/kg) 30 min before ICSS testing sessions using a within-subjects Latin square design. Each dose of DMI was administered only when rats had shown a stable three

day baseline between drug injections. This experiment was aimed to determine a dose-response function for the effect of acute DMI on ICSS thresholds under baseline conditions.

Experiment 2: Effects of acute DMI administration on nicotine withdrawal assessed by ICSS thresholds and somatic signs

—After establishment of stable ICSS thresholds (defined as less than 10% variation over five consecutive days), drug-naïve rats were prepared with 7 day osmotic minipumps delivering either nicotine (3.16 mg/kg/day, base; $n = 23$) or saline ($n = 25$). ICSS thresholds continued to be assessed daily. On Day 7, the pumps were removed, and ICSS thresholds were assessed at 6 and 24 h after pump removal, and at 24 h intervals thereafter until thresholds returned to baseline levels. Based on data obtained 6 h after pump removal, rats were assigned to experimental groups, counter-balanced for ICSS thresholds, prior to testing the effects of DMI on nicotine withdrawal. DMI (2 or 5 mg/kg, i.p.; nicotine-exposed: $n = 9$ and $n = 6$, respectively; saline-exposed: $n = 9$ and $n = 7$, respectively) or saline (nicotine-exposed: $n = 8$; saline-exposed $n = 9$) was administered in a volume of 4 ml/kg, 30 min before the ICSS session 24 h after pump removal (i.e., withdrawal day 1).

Experiment 3: Effects of chronic DMI administration on nicotine withdrawal assessed by ICSS thresholds and somatic signs

—After establishment of stable ICSS thresholds (defined as less than 10% variation over five consecutive days), drug-naïve rats were assigned to one of four treatment groups, counter-balanced for baseline ICSS thresholds and body-weights. Rats were prepared with 28-day osmotic minipumps delivering either nicotine (3.16 mg/kg/day, base) or saline. Seven days later, rats were prepared with 14-day minipumps containing sterile water or 15 mg/kg/day DMI, a dose that selectively inhibits norepinephrine reuptake (Benmansour et al. 1999, 2004). Due to the low solubility of DMI, the drug was administered via two consecutively implanted 14-day osmotic minipumps. Thus, there were four treatment groups: saline/vehicle ($n = 9$), saline/DMI ($n = 8$), nicotine/vehicle ($n = 8$), nicotine/DMI ($n = 9$). After 14 days of nicotine/saline and DMI/vehicle co-administration, nicotine/saline minipumps were removed, resulting in nicotine withdrawal, and a second DMI/vehicle minipump replaced the first DMI/vehicle minipump. DMI/vehicle was continuously administered throughout the withdrawal phase. ICSS thresholds were assessed at 8 and 24 h after nicotine/saline pump removal, and at 24 h intervals thereafter until thresholds returned to baseline levels. Somatic signs were counted once during a 10 min observation session, 24 h after removal of the nicotine/saline minipumps.

Experiment 4: Effects of chronic DMI administration on nicotine self-administration and food-maintained responding

—Naive rats ($n = 14$) were allowed to self-administer intravenous nicotine (0.03 mg/kg/inf, base) under a fixed-ratio 5 time-out 20 second schedule of reinforcement. After 6 days, the unit dose of nicotine was decreased to 0.015 mg/kg/inf. Previous studies from our laboratory indicated that this procedure results in reliable acquisition of nicotine self-administration of lower nicotine doses. We selected this relatively low dose of self-administered nicotine because the reinforcement-attenuating effects of pharmacological treatments are more readily observed when animals self-administer lower doses such as 0.01 mg/kg/inf (Paterson et al. 2003, 2004). In the overwhelming majority of previous studies, only decreases in nicotine self-administration are observed after administration of pharmacological manipulations that attenuate the reinforcing effects of nicotine irrespective of the nicotine dose available for self-administration (for review, Lerman et al. 2007). Therefore, the assessment of the effects of DMI on self-administration of multiple unit doses of nicotine was not likely to provide additional information. Another set of rats ($n = 16$) was trained to respond for food under an identical schedule of reinforcement as nicotine self-administration. After establishing stable levels of responding (defined as less than 20% variation over three consecutive days), rats were prepared with osmotic minipumps containing either DMI (15 mg/kg/day, salt; nicotine: $n = 7$; food: $n = 8$) or sterile water (nicotine: $n = 7$;

food: $n = 8$). Rats were allowed to respond for nicotine or food in 1 h sessions daily for an additional 12 day period during exposure to DMI or vehicle via the minipumps.

Data Analyses

ICSS threshold and response latency data are presented as a percent of baseline, with baseline defined as the mean threshold or response latency during the last five days prior to the implantation of the first set of minipumps. Somatic signs data are expressed as total number of signs counted. Nicotine self-administration and food-maintained responding data are expressed as a percent of baseline, with baseline defined as the mean number of rewards earned during the last three days prior to the implantation of the minipumps. Absolute baseline values for all experimental groups are reported in the Results section. In Experiment 1, the effects of acute DMI on ICSS thresholds were analyzed using a one-way analysis of variance (ANOVA), with DMI (4 levels) defined as the within-subjects factor. In Experiment 2, the initial nicotine/saline administration phase data were analyzed using a two-way ANOVA, with Nicotine/Saline (2 levels) defined as the between-subjects factor, and Days (6 levels) defined as the within-subjects factor. The withdrawal phase data were analyzed using a three-way ANOVA, with Nicotine Withdrawal (2 levels) and DMI (3 levels) defined as between-subjects factors, and Days (5 levels) defined as the within-subjects factor. In Experiment 3, the initial nicotine/saline administration phase data were analyzed using a two-way ANOVA, with Nicotine/Saline (2 levels) defined as the between-subjects factor, and Days (7 levels) defined as the within-subjects factor. The co-administration phase data were analyzed using a three-way ANOVA, with Nicotine/Saline and DMI/vehicle (2 levels each) defined as between-subjects factors, and Days (14 levels) defined as the within-subjects factor. Withdrawal phase data were analyzed using a three-way ANOVA, with Nicotine Withdrawal and DMI/vehicle (2 levels each) defined as between-subjects factors, and Days (7 levels) defined as the within-subjects factor. In Experiment 3, somatic signs data were analyzed using two-way ANOVAs, with Nicotine Withdrawal (2 levels) and DMI/vehicle (2 levels) defined as between-subjects factors. Self-administration data in Experiment 4 were analyzed using a three-way ANOVA, with Reinforcer and DMI/vehicle (2 levels each) defined as between-subjects factors, and Days (12 levels) defined as the within-subjects factor. Where indicated by significant main or interaction effects, ANOVAs were followed by Newman-Keuls *post hoc* comparisons (Winer 1971). The level of significance was set at 0.05.

Results

Experiment 1: Effects of acute DMI administration on ICSS thresholds

The absolute baseline ICSS thresholds (mean $\pm$ SEM) were $104.7 \pm 0.9 \mu\text{A}$, and the absolute baseline response latencies (mean $\pm$ SEM) were $3.3 \pm 0.2 \text{ sec}$. DMI significantly elevated brain reward thresholds [$F_{(4,36)} = 4.5, p < 0.005$] that *post hoc* analyses attributed to 5 mg/kg DMI significantly elevating thresholds compared to the vehicle-treated condition (Table 1). DMI administration had no effect on response latencies.

Experiment 2: Effects of acute DMI administration on nicotine withdrawal assessed by ICSS thresholds and somatic signs

The absolute baseline ICSS thresholds (mean $\pm$ SEM) for the various treatment groups were as follows: saline/saline $112.4 \pm 16.2 \mu\text{A}$; saline/DMI-2 115.4 ± 19.3 ; saline/DMI-5 113.7 ± 7.3 ; nicotine/saline $104.9 \pm 15.2 \mu\text{A}$; nicotine/DMI-2 109.9 ± 11.3 ; nicotine/DMI-5 126.6 ± 8.9 . The absolute baseline response latencies (mean $\pm$ SEM) for the various treatment groups were: saline/saline $3.3 \pm 0.1 \text{ s}$; saline/DMI-2 $3.4 \pm 0.1 \text{ s}$; saline/DMI-5 $3.2 \pm 0.1 \text{ s}$; nicotine/saline $3.1 \pm 0.1 \text{ s}$; nicotine/DMI-2 $3.2 \pm 0.1 \text{ s}$; nicotine/-DMI-5 $3.4 \pm 0.2 \text{ s}$. There were no statistically significant differences in baseline thresholds or response latencies among treatment groups.

Nicotine/Saline administration phase—Analysis of the ICSS threshold data obtained during the first six days of nicotine/saline infusion indicated a trend for nicotine to lower ICSS thresholds [$F_{(1,46)} = 3.66, p = 0.06$] and a significant main effect of Days [$F_{(5,230)} = 3.14, p < 0.01$], similar to the initial ICSS threshold-lowering effect of nicotine delivered via minipumps observed previously (Harrison et al. 2001). In addition, nicotine significantly lowered response latencies [$F_{(1,46)} = 37.21, p < 0.001$], as also seen previously (Harrison et al. 2001). There were no other significant main or interaction effects on thresholds or response latencies (data not shown).

Nicotine/Saline withdrawal phase—After removal of the nicotine/saline-containing minipumps, rats that were previously treated with nicotine exhibited elevations in ICSS thresholds [main effect of Nicotine Withdrawal: $F_{(1,42)} = 9.69, p < 0.01$]. Threshold elevations returned to baseline levels over the testing period as indicated by a significant main effect of Days [$F_{(4,168)} = 23.79, p < 0.001$] and a significant Days $\times$ Nicotine Withdrawal interaction [$F_{(4,168)} = 6.03, p < 0.0001$; Table 2). There was no significant main effect of acute DMI treatment and no significant Nicotine Withdrawal $\times$ DMI interaction. There was a significant Days $\times$ DMI interaction [$F_{(8,168)} = 3.75, p < 0.001$], due to a combination of lowered ICSS thresholds in DMI-treated nicotine-exposed subjects on Days 2 and 3 post-pump removal, and elevated ICSS thresholds immediately after acute administration of 5 mg/kg DMI in saline-exposed subjects. There was no significant Days $\times$ Nicotine Withdrawal $\times$ DMI treatment interaction. Analysis of response latency data indicated that response latencies were increased by acute administration of DMI [main effect of Days: $F_{(4,168)} = 7.34, p < 0.001$; Days $\times$ DMI interaction: $F_{(8,168)} = 4.27, p < 0.001$]. *Post hoc* analyses indicated that 5 mg/kg DMI significantly elevated response latencies in saline-exposed controls ($120.9 \pm 7.5\%$) but not in nicotine-withdrawing rats ($114.4 \pm 6.6\%$) compared to vehicle/saline-exposed controls on Day 1 of nicotine/saline withdrawal (Table 2). There were no other significant main or interaction effects on response latencies.

Experiment 3: Effects of chronic DMI on nicotine withdrawal assessed by ICSS thresholds and somatic signs

The absolute baseline ICSS thresholds (mean $\pm$ SEM) for the four treatment groups were: saline/vehicle $102.4 \pm 5.8 \mu\text{A}$; saline/DMI $99.0 \pm 15.8 \mu\text{A}$; nicotine/vehicle $94.1 \pm 14.6 \mu\text{A}$; nicotine/DMI $111.7 \pm 8.9 \mu\text{A}$. The absolute baseline response latencies (mean $\pm$ SEM) for the four groups were: saline/vehicle 3.1 ± 0.2 sec; saline/DMI 3.3 ± 0.1 sec; nicotine/vehicle 3.2 ± 0.1 sec; nicotine/DMI 3.2 ± 0.1 sec. There were no statistically significant differences in baseline thresholds or response latencies among treatment groups.

Nicotine/Saline administration phase—Analysis of the ICSS threshold and response latency data during the first 7 days of nicotine/saline administration indicated no effect of Nicotine/Saline administration on ICSS thresholds or response latencies (no significant main or interaction effects; data not shown).

Nicotine/Saline and DMI/Saline co-administration phase—Analysis of the ICSS threshold data during the 14 days of co-administration of nicotine/saline and DMI/vehicle indicated that ICSS thresholds were the same in all treatment groups (no significant main effects of Nicotine/Saline or DMI/Vehicle or interaction effects of Nicotine/Saline $\times$ DMI/Vehicle or Nicotine/Saline $\times$ DMI/Vehicle $\times$ Days; data not shown). Nonetheless, there was a significant main effect of Days [$F_{(13,390)} = 2.00, p < 0.05$] due to minor fluctuations in ICSS thresholds (within the range of 90–110% of pre-drug baseline) across the 14 days of co-administration of Nicotine/Saline and DMI/Vehicle. Analysis of response latency data indicated no effect of co-administration of Nicotine/Saline and DMI/Vehicle on response

latencies during the 14 days of co-administration (no significant main or interaction effects; data not shown).

Nicotine/Saline Withdrawal phase—Analysis of the ICSS threshold data indicated that nicotine pump removal induced elevations in ICSS thresholds that were attenuated in rats receiving DMI (Nicotine Withdrawal $\times$ DMI/vehicle interaction: $F_{(1,30)} = 5.00, p < 0.05$; main effect of Nicotine Withdrawal: $F_{(1,30)} = 6.34, p < 0.05$; main effect of DMI/Vehicle: $F_{(1,30)} = 7.27, p < 0.05$). There was also a main effect of Days [$F_{(6,180)} = 4.4, p < 0.01$]. There were no Days interaction effects. Newman-Keuls *post hoc* comparisons indicated that ICSS thresholds were significantly elevated in the nicotine/vehicle group compared to the nicotine/DMI group at 8 h and 1 day, and the saline/vehicle group at 8 h and 1, 2 and 3 days after removal of the nicotine-containing pump (Figure 1A). Analysis of response latency data over the withdrawal period indicated that response latencies were unchanged in all treatment groups across the withdrawal period (no significant main or interaction effects; data not shown).

Somatic signs—Analysis of somatic signs data obtained 1 day after nicotine/saline pump removal indicated a significant increase in somatic signs in rats undergoing nicotine withdrawal [main effect of Nicotine Withdrawal: $F_{(1,30)} = 5.55, p < 0.05$]. The somatic component of nicotine withdrawal was attenuated in nicotine-withdrawing subjects treated with chronic DMI (DMI $\times$ Nicotine Withdrawal interaction: $F_{(1,30)} = 7.16, p < 0.05$). There was no significant main effect of DMI. Newman-Keuls *post hoc* comparisons indicated that somatic signs were significantly increased in the nicotine/vehicle group compared to the saline/vehicle control and nicotine/DMI groups. There were no other significant differences between treatment groups (Figure 1B).

Experiment 4: Effects of chronic DMI on nicotine self-administration and food-maintained responding

The absolute baseline number (mean $\pm$ SEM) of nicotine and food reinforcers earned were as follows for the various treatment groups: nicotine-responding vehicle-treated group: 16.9 ± 1.3 infusions; nicotine-responding DMI-treated group 14.3 ± 2.3 infusions; food-responding vehicle-treated group 98.3 ± 9.7 pellets; food-responding DMI-treated group 109.1 ± 10.4 pellets. There were no statistically significant differences in baseline reinforcers between treatment groups within each reinforcer condition. The absolute baseline inactive lever presses (mean $\pm$ SEM) were as follows for the various treatment groups: nicotine-responding vehicle-treated group 7.1 ± 2.0 presses; nicotine-responding DMI-treated group 8.2 ± 3.8 presses; food-responding vehicle-treated group 7.5 ± 2.3 presses; food-responding DMI-treated group 4.5 ± 1.5 presses. There were no significant differences in baseline inactive lever presses between any groups. Chronic DMI treatment significantly decreased both nicotine and food reinforcers earned as indicated by a significant DMI/vehicle $\times$ Days interaction [$F_{(11,286)} = 2.41, p < 0.05$] and significant main effects of DMI/vehicle [$F_{(1,26)} = 95.64, p < 0.001$] and Days [$F_{(11,286)} = 2.13, p < 0.05$]. Newman-Keuls *post hoc* comparisons indicated that the number of nicotine and food reinforcers earned was significantly decreased in the DMI-treated groups compared to the corresponding vehicle-treated groups on Days 2 through 12 of treatment, and on Day 1 in the food-responding group only (Figure 2). Analysis of inactive lever data indicated higher number of inactive lever-pressing in the food-responding groups compared to the nicotine-responding groups [main effect of Reinforcer: $F_{(1,26)} = 4.57, p < 0.05$], independent of treatment or time. There were no other significant main or interaction effects (data not shown).

Discussion

The results of the present study indicated that chronic, but not acute, DMI treatment attenuated the anhedonic and somatic components of nicotine withdrawal, and that chronic DMI treatment

significantly decreased nicotine- and food-maintained responding. The attenuation of nicotine withdrawal by chronic administration of the antidepressant DMI, at a dose (15 mg/kg/day) that selectively inhibits norepinephrine reuptake (Benmansour et al. 1999, 2004), suggests that nicotine withdrawal may be associated with dysregulated noradrenergic neurotransmission. Further, the results suggest that altered norepinephrine transmission via chronic administration of DMI decreased the rewarding effects of both nicotine and food.

The effects of DMI treatment on brain reward function

Acute DMI administration (2 or 5 mg/kg) had no effect on ICSS threshold elevations during nicotine withdrawal. However, consistent with previous studies (Hall et al. 1990; Valentino et al. 1987; Markou et al. 1992), the higher acute DMI dose tested (5 mg/kg) elevated thresholds in drug-naïve control subjects. These results are similar to our previous findings showing that acutely raising serotonin overflow altered ICSS thresholds in opposite directions in control and nicotine-withdrawing rats, probably due to nicotine withdrawal-induced dysregulated serotonergic neurotransmission (Harrison et al. 2001). The present data indicated altered sensitivity of ICSS thresholds to acute DMI in nicotine-exposed versus nicotine-naïve rats, suggesting perturbed noradrenergic neurotransmission during nicotine withdrawal. The nature of the perturbation(s) may include altered function/number of adrenoceptors in nicotine-exposed subjects, or elevated norepinephrine overflow such that a further DMI-induced elevation has no additional effect on brain reward function. Nevertheless, the possibility that acute DMI at doses up to 5 mg/kg is not sufficient to reverse the anhedonic component of nicotine withdrawal cannot be excluded. We chose not to assess the effects of higher DMI doses and instead focused on the effects of chronic DMI administration as having more relevance to clinical treatment regimens.

In contrast to acutely administered DMI, chronic DMI administration (15 mg/kg/day) via subcutaneous minipump prevented the elevations in ICSS thresholds and increases in somatic signs associated with cessation of chronic nicotine infusion. The DMI-treated saline control group did not show changes in ICSS thresholds during 21 days of continuous DMI administration, indicating that chronic DMI alone did not affect brain reward function. Previously, repeated daily DMI (10-20 mg/kg/day) administration had inconsistent effects on ICSS reward thresholds (Fibiger and Phillips 1981; Valentino et al. 1987; Hall et al. 1990). Further, the present data contrast favorably with previously demonstrated effects of acute or chronic bupropion, that reversed or prevented respectively, significant elevations of ICSS thresholds during nicotine withdrawal via a non-specific ICSS threshold-lowering effect (Cryan et al. 2003a; Paterson et al. 2007). In contrast, chronic DMI selectively attenuated the anhedonic and somatic component of nicotine withdrawal at a dose that had no effects in control rats.

Prevention of nicotine withdrawal with chronic DMI treatment is consistent with findings showing that DMI (Markou et al. 1992) or imipramine (Kokkinidis et al. 1980) attenuated elevated ICSS thresholds associated with cocaine or amphetamine withdrawal, respectively. The effects of DMI on cocaine withdrawal were correlated with DMI-induced β -adrenoceptor downregulation (Markou et al. 1992). Downregulation of β -adrenoceptor function may explain the ameliorative effects of chronic DMI on the anhedonic and somatic components of nicotine withdrawal in the present study. Clinical studies indicated that the α_2 adrenoceptor agonist clonidine decreased nicotine withdrawal in humans (Covey and Glassman 1991; Prochazka et al. 1992). DMI also induces changes in α_2 adrenoceptor activity (Nurse et al. 1985; Mateo et al. 1998), and thus α_2 adrenoceptor alterations may have contributed to the ameliorative effects of DMI in the present study.

Alternatively, non-noradrenergic mechanisms could mediate the effects of DMI in the present study. Specifically, DMI may alter dopamine overflow in the mesocorticolimbic pathway.

Although acute DMI had no direct effect on dopamine reuptake in the rat substantia nigra (Hoffman and Gerhardt 1998), prolonged administration of DMI increased dopamine transporter mRNA expression and protein levels in subregions of the mesolimbic pathway (Petrie et al. 1998; Chen and Lawrence 2003). These increases in dopamine transporter levels may be a neuroadaptive response to elevated dopamine overflow induced by DMI. Decreased dopamine signaling in the prefrontal cortex is implicated in drug withdrawal and depression (Ridley et al. 1982; Tremblay et al. 2005). Extracellular dopamine overflow in the prefrontal cortex is regulated by the norepinephrine transporter (Carboni et al. 1990; Yamamoto and Novotney 1998). Thus, chronic norepinephrine transporter inhibition may attenuate behavioral measures of withdrawal by increasing dopaminergic transmission in the prefrontal cortex and/or mesolimbic pathway. In addition to norepinephrine and/or dopamine transporter blockade, non-competitive nicotinic acetylcholine receptor blockade exerted by DMI may also contribute to the observed amelioration of nicotine withdrawal in the present study (Dani and De Biasi 2001). Nevertheless, this is highly unlikely as nicotinic acetylcholine receptor blockade precipitated withdrawal-like threshold elevations and increased somatic signs of nicotine withdrawal in nicotine-treated rats (Epping-Jordan et al. 1998; Watkins et al. 2000), and increased somatic signs of nicotine withdrawal are reversed by nicotine administration (Malin et al. 1992).

The effects of DMI treatment on nicotine self-administration and food intake

Chronic DMI administration significantly decreased responding for nicotine infusions and food pellets in the present study. However, the food pellet reinforcer maintained higher rates of lever-pressing than nicotine delivery. Dews (1955) noted that drugs can have different effects on behaviors emitted at different rates. Nonetheless, the response-suppressant effects of drugs are seen when rates of responding are high, while low rates of responding may be increased by the same drug manipulations (Dews 1955; Phillips et al. 1991). In the present study, nicotine- and food-maintained lever-pressing were similarly decreased. Further, chronic DMI had no effect on response latencies in the ICSS task, a measure of motor function (Markou and Koob 1992), indicating no general motor-suppressant effects of DMI. Thus, the near-identical effects of DMI on nicotine- and food-maintained responding most likely represent similar decreases in the reinforcing effects of nicotine and food rather than nonspecific motor-suppressant or rate-dependent effects of DMI.

DMI-induced suppression of food-maintained responding is consistent with numerous previous studies (Lucki and Frazer 1985; Durcan et al. 1988). Nonetheless, administration of DMI, and other tricyclic antidepressant compounds, leads to weight-gain in the clinic (Fernstrom and Kupfer 1988; Orzack et al. 1986). Thus, it is unclear whether the decreased food-maintained responding observed in the present study predicts similar anorectic effects in human smokers.

In contrast to the present data where DMI had similar effects on nicotine- and food-maintained responding, the selective norepinephrine reuptake inhibitor reboxetine, administered acutely or repeatedly over 14 consecutive days, decreased nicotine self-administration to a greater extent (approximately 60%) than sucrose-maintained responding (approximately 25%) during the first 15 min of a 60 min nicotine self-administration session (Rauhut et al. 2002). However, it is possible that during a longer sucrose test session, as used in the present study for food pellets, reboxetine-induced decreases in responding for sucrose and nicotine would be similar, because reboxetine decreased nicotine self-administration by a maximum of 40% across the 60 min session (Rauhut et al. 2002). Both reboxetine and DMI (Miller et al. 2002; Rana et al. 1993; Izaguirre et al. 1997) are nicotinic acetylcholine receptor antagonists. Nevertheless, DMI has only been tested at the $\alpha 3\beta 4$ nicotinic subtype (Hennings et al. 1997), at which reboxetine was 100-fold more potent than at the $\alpha 4\beta 2$ nicotinic receptor (Miller et al. 2002). It is unknown

whether DMI blocks $\beta 2$ -containing nicotinic acetylcholine receptor subtypes that are critical to the reinforcing effects of nicotine (Picciotto et al. 1998; David et al. 2006) and heavily implicated in the anhedonic component of nicotine withdrawal (Bruijnzeel and Markou 2004; Kenny and Markou 2005). Finally, DMI is a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist (Sernagor et al. 1989; Mjellem et al. 1993), which could contribute to DMI-induced attenuation of nicotine self-administration (Blokhina et al. 2005; Kenny and Markou, unpublished observations). Blockade of either NMDA receptors or $\alpha 3\beta 4$ nAChRs (Glick et al. 2002) would selectively attenuate responding for nicotine- not food-maintained responding.

Summary

The present data suggest that noradrenergic neurotransmission is involved in mediating the anhedonic and somatic aspects of nicotine withdrawal, and may contribute to the reinforcing effects of nicotine and food reward. Future work should address issues of pharmacological specificity due to multiple pharmacological effects of DMI, and expand the characterization of the effects of norepinephrine reuptake inhibitors on the reinforcing properties of self-administered nicotine at different unit doses and under a progressive-ratio schedule.

Acknowledgements

The authors would like to thank Mr. Michael Arends for editorial assistance and Mrs. Jessica Benedict and Mr. Randy Ares for technical assistance. The authors would also like to thank Dr. John F. Cryan for initial work with the effects of acute desipramine during amphetamine withdrawal in our laboratory. NEP was supported by Post-Doctoral Fellowship 14-FT0056 from the Tobacco Related Disease Research Program (State of California) and a research grant from The Peter F. McManus Charitable Trust. This work was supported by National Institute of Mental Health grants R01 MH62527 and U01 MH69062 to AM. The authors report no actual or potential financial conflicts of interest.

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Figure 1.

Effects of chronic DMI (15 mg/kg/day, salt) treatment on ICSS thresholds and somatic signs of nicotine withdrawal. Panel A depicts ICSS thresholds (mean $\pm$ SEM) during the nicotine/saline withdrawal period, expressed as percent of pre-nicotine/saline baseline thresholds. Asterisks ($*p < 0.05$, $**p < 0.01$) indicate significant differences from the saline/vehicle group, and the pound sign ($#p < 0.01$) indicates significant differences from the nicotine/DMI control group. Panel B depicts numbers of somatic signs (mean $\pm$ SEM) 24 h after removal of the nicotine/saline-containing minipumps. Asterisks ($**p < 0.01$) indicate a significant difference from the saline/vehicle control group, and the pound sign ($#p < 0.05$) indicates a significant difference from the nicotine/DMI group ($p < 0.05$).

Figure 2.

Effects of chronic DMI (15 mg/kg/day, salt) on number of nicotine infusions (0.015 mg/kg/inf) and food reinforcers earned, expressed as percent of pre-drug baseline reinforcers earned (mean $\pm$ SEM). Squares and circles represent nicotine and food reinforcers, respectively. The number of nicotine or food reinforcers earned was significantly decreased in DMI-treated compared to saline-treated rats on Days 1-14 for the food group ($*p < 0.05$), and from Day 2 onward for the nicotine group ($**p < 0.01$).

Table 1

ICSS thresholds and response latencies after acute DMI administration (i.p., 30 min before the ICSS session, $n = 10$). Data are presented as percent of baseline ICSS thresholds and response latencies (mean $\pm$ SEM). Asterisks (** $p < 0.01$) indicate significant elevations in ICSS thresholds compared to thresholds after saline administration

DMI (mg/kg)	ICSS threshold (% of baseline)	Response latency (% of baseline)
0	99.21 $\pm$ 2.67	100.99 $\pm$ 1.80
0.5	104.03 $\pm$ 2.68	106.88 $\pm$ 4.84
1	101.88 $\pm$ 3.11	105.32 $\pm$ 2.08
2	107.43 $\pm$ 5.01	109.69 $\pm$ 7.63
5	123.13 $\pm$ 8.17**	103.82 $\pm$ 3.27

ICSS thresholds and response latencies during nicotine withdrawal. Data are presented as ICSS thresholds and response latencies (mean $\pm$ SEM), expressed as percent of pre-pump baseline during the four days of nicotine or saline withdrawal, respectively. ICSS thresholds and response latencies were increased by acute administration of 5 mg/kg DMI in both control and nicotine-withdrawing rats 1 day after the removal of the minipumps, as indicated by a significant main effect of Days and a significant Days $\times$ DMI interaction (see text for details). Asterisk indicates statistically significant difference from the saline/vehicle control group ($p < 0.05$)

Table 2

Time-point	Saline/Vehicle	Saline/2 mg/kg DMI	Saline/5 mg/kg DMI	Nicotine/Vehicle	Nicotine/2 mg/kg DMI	Nicotine/5 mg/kg DMI
ICSS thresholds						
6 h	103.6 $\pm$ 6.8	101.3 $\pm$ 3.9	102.3 $\pm$ 6.0	124.8 $\pm$ 5.8	125.1 $\pm$ 4.7	117.1 $\pm$ 3.0
1 day	101.4 $\pm$ 4.3	100.3 $\pm$ 4.6	117.2 $\pm$ 4.4*	118.9 $\pm$ 8.7	112.3 $\pm$ 5.8	122.4 $\pm$ 8.9
2 days	97.5 $\pm$ 4.7	96.8 $\pm$ 4.2	98.1 $\pm$ 5.2	111.3 $\pm$ 5.9	106.7 $\pm$ 3.2	101.0 $\pm$ 3.7
3 days	99.6 $\pm$ 4.8	95.9 $\pm$ 5.0	99.1 $\pm$ 5.1	102.9 $\pm$ 3.8	106.1 $\pm$ 4.0	95.6 $\pm$ 5.6
4 days	98.8 $\pm$ 3.9	98.6 $\pm$ 4.0	94.0 $\pm$ 5.6	111.3 $\pm$ 3.8	102.1 $\pm$ 3.5	91.1 $\pm$ 5.9
Response latencies						
6 h	100.9 $\pm$ 3.4	98.3 $\pm$ 2.7	103.9 $\pm$ 2.8	106.0 $\pm$ 4.7	107.5 $\pm$ 2.8	102.1 $\pm$ 3.1
1 day	104.6 $\pm$ 1.8	101.4 $\pm$ 2.6	120.9 $\pm$ 7.5*	100.4 $\pm$ 4.4	103.4 $\pm$ 3.8	114.4 $\pm$ 6.6
2 days	101.8 $\pm$ 1.9	99.3 $\pm$ 2.7	105.3 $\pm$ 6.1	97.9 $\pm$ 3.4	102.9 $\pm$ 1.5	100.8 $\pm$ 4.2
3 days	103.5 $\pm$ 1.7	99.0 $\pm$ 4.3	100.2 $\pm$ 4.9	102.5 $\pm$ 3.0	101.8 $\pm$ 1.8	97.2 $\pm$ 5.3
4 days	101.1 $\pm$ 1.9	98.9 $\pm$ 2.7	101.7 $\pm$ 4.6	101.2 $\pm$ 2.4	98.53 $\pm$ 2.5	98.6 $\pm$ 4.7