Monoamine Oxidase Inhibition Dramatically Prolongs the Duration of Nicotine Withdrawal-Induced Place Aversion

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Background: Long-lasting effects of withdrawal from nicotine are hypothesized to contribute to relapse and persistence of tobacco habits, and significant evidence supports a role of monoamine oxidase inhibitors (MAOI) contained in cigarette smoke as potent modulators of the rewarding effects of tobacco.

Methods: With quantification of somatic signs of withdrawal and the place aversion conditioning paradigm, we assessed the effects of MAOI pretreatment on both somatic and aversive motivational components of mecamylamine-induced nicotine withdrawal in rats rendered dependent on nicotine by the subcutaneous implantation of osmotic minipumps (vehicle or nicotine tartrate 9 mg/kg/day).

Results: In nicotine-infused rats, mecamylamine induced a place aversion that lasted 6 weeks. When nicotine-infused rats were also treated with a MAOI, mecamylamine-induced conditioned place aversion persisted for at least 8 months of abstinence. The MAOI treatment slightly decreased ratings of somatic signs induced by mecamylamine administration but had no effect on the threshold or the magnitude of mecamylamine-induced conditioned place aversion.

Conclusions: These results show that MAOI pretreatment induces a long-lasting conditioned placed aversion associated with nicotine withdrawal, possibly through a potentiation of learning and memory process, and provides some indications on protracted abstinence that might be useful for delineating the neurobiological substrate of relapse.

Key Words: Conditioned place aversion, nicotine, rats, phenelzine somatic withdrawal, tranylcypromine

It is widely accepted that a majority of habitual tobacco smokers become dependent upon nicotine present in tobacco smoke and that this accounts for the problems many smokers experience when they try to quit (1,2). Although it is clear that the primary reinforcing effects of the drug trigger the initiation of drug consumption, once dependence is initiated the negative consequences of drug abstinence might motivate the continued administration of drug to prevent the appearance of a withdrawal syndrome (3,4).

Nicotine withdrawal might be evoked after cessation of chronic nicotine exposure (5–8) and is characterized by both somatic and affective negative symptoms. Although the somatic symptoms of nicotine withdrawal might contribute to smoking behavior, it has been hypothesized that affective signs are of greater motivational significance in contributing to relapse and continued use (9,10). Clinical studies indicate that the negative affective states experienced during drug withdrawal can become associated with previously neutral environmental stimuli and that these conditioned stimuli gain motivational significance in the maintenance of drug use and relapse during periods of abstinence (11–13).

In rodent models of nicotine withdrawal, somatic signs such as abdominal constrictions, facial fasciculation, and ptosis can be observed (6–8). The aversive state associated with withdrawal can be modeled with the conditioned place aversion paradigm. Indeed, it has been shown that precipitated nicotine withdrawal in rats, with the nicotinic receptor antagonist mecamylamine, produces a place aversion to a previously neutral environment paired with precipitated nicotine withdrawal (14,15). Nevertheless, remarkably little is known about the duration of the motivational aspects of nicotine withdrawal.

Tobacco smoke is known to contain a number of compounds among which monoamine oxidase inhibitors (MAOIs) have been the focus of special interest (16–18). Current smokers have lower brain MAO A and B activity, which normalizes during prolonged abstinence (19–22). Moreover, we have recently demonstrated that MAOI treatment increases the motivation to self-administer nicotine in rats and might thus contribute to the development of tobacco addiction (23). The purpose of the present study was to determine the effects of chronic MAOI treatment on both the intensity of the somatic signs and the aversive motivational state associated with precipitated nicotine withdrawal in rats and the duration of conditioned place aversion, once established, with repeated measures of conditioned place aversion during nicotine abstinence. We report that MAOI treatment dramatically prolonged the aversive state associated with nicotine withdrawal but had little effect on the somatic signs of nicotine withdrawal.

Methods and Materials

Animals

A total of 258 male Sprague Dawley rats (Charles River, Lyon, France) weighing 101–125 g at the beginning of the experiment were used. Animals were housed in groups of four and maintained in rooms at 20°–22°C with a reverse light/dark cycle (lights off from 7:00 AM to 7:00 PM). Animals had ad libitum access to food and water throughout the experiment. Experiments were performed in accordance with the declaration of Helsinki, the European Communities Council Directives (86/609/EEC, Novem-

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ber 24, 1986) and the French Directives concerning the use of laboratory animals (décret n° 87-848, October 19, 1987).

Drugs

(-)Nicotine hydrogen tartrate, mecamylamine hydrochloride, and the two mixed irreversible MAO inhibitors (MAOI-A/B) tranylcypromine hydrochloride and phenelzine sulfate were purchased from Sigma Aldrich (St. Louis, Missouri) and were dissolved in isotonic sodium chloride (.9% w/w saline in water). Treatments with MAOI began the 1st day of pump implantation and were administered twice a day (9:00 AM and 7:00 PM) intraperitoneally (1 mL/kg body weight) at the following doses expressed as free base: tranylcypromine 1.5 mg/kg/day, and phenelzine 2 mg/kg/day. Control rats received vehicle. Mecamylamine (2 or 4 mg/kg; [15]) was injected subcutaneously. Doses of MAOIs were chosen on the basis of previously determined dose–response relationships on locomotor activity for which no psychostimulant effects had been detected (23). The MAOI treatments began the 1st day of pump implantation and occurred every day until the end of the conditioning phase in the place aversion experience or at the end of the somatic evaluation of nicotine withdrawal.

Induction of Nicotine Dependence

Osmotic minipumps (Alzet, model 2 ML2 [14 days]; Alza Corporation, Palo Alto, California) filled with either saline (n = 63) or nicotine tartrate dissolved in saline (n = 195) were implanted subcutaneously under halothane oxygen mixture (1%–3% halothane) anesthesia. The concentration of nicotine was adjusted to compensate for differences in body weight to deliver a dose of 9 mg/kg/day (3.16 mg/kg/day, free base) for 14 days.

Conditioned Place Aversion

The apparatus and place aversion paradigm used to produce a reliable conditioned place aversion have been described in detail elsewhere (24). Briefly, the apparatus consisted of three rectangular boxes (40 × 33 × 34 cm, 120° to each other), distinguished by distinctive visual and tactile cues and accessible from a triangular central compartment. Treatments with MAOI began the 1st day of pump implantation and occurred twice a day (9:00 AM and 7:00 PM) until the end of the conditioning phase.

In the preconditioning phase (day 3 after implantation), animals were allowed to freely explore the apparatus for 20 min. For each rat, the two compartments with the most similar time allotments were chosen. One side was randomly chosen to be paired with mecamylamine (D0) and the other side with vehicle (S0). The third compartment was not paired with any injection (N0). After compartment assignment there were no differences between the time spent in the drug- and saline-paired compartments during the preconditioning phase (D0 vs. S0). This procedure eliminated possible bias before conditioning.

The conditioning phase consisted of five pairings over 5 consecutive days (days 4, 5, 6, 7, and 8 after implantation). In the morning, before being confined to their preselected saline-paired compartment for 20 min, rats received an injection of saline. In the afternoon, rats received mecamylamine immediately before being confined to the preselected mecamylamine-paired compartment for 20 min.

The testing phase consisted of a 20-min free exploration of the entire apparatus, without any MAOI treatment. The first test was conducted 24 hours after conditioning (day 10 after implantation, test 1 [D1, S1, N1]). The same day, minipumps were removed in all rats. Tests for place aversion conditioning were then conducted every 2 weeks, at days 14–224 after conditioning ([D14, S14, N14] to [D224, S224, N224]). The difference (D-D0) in time spent in the mecamylamine-paired compartment during the testing phase (D) and the preconditioning phase (D0) served as a measure of place aversion.

Ratings of Somatic Signs of Withdrawal

Forty-eight naive rats were implanted with minipumps filled with either saline (n = 12) or nicotine tartrate dissolved in saline (n = 36). Treatments with MAOI began the 1st day of pump implantation and occurred twice a day (9:00 AM and 7:00 PM) until the end of the experiment. Animals were habituated to the observation glass box (31 × 29 × 40 cm) in which the rat could move freely for 15 min for 3 consecutive days.

On days 4–10 after implantation, the effects of various doses of mecamylamine on somatic signs were examined. Mecamylamine doses (0, .29, .57, 1.14, 1.72, 2.29 and 3.43 mg/kg SC [15]) were presented according to a within-subjects Latin square design (repeated measures). Five minutes after the antagonist injection, each animal was placed in the observation glass box and filmed for 10 min with a video recording device. The frequency and time of occurrence of the following signs were recorded: body shakes, chews, cheek tremors, foot licks, gasps, genital licks, head shakes, scratches, teeth chattering, writhes, and yawns (6). The categories of "abdominal constrictions" included gasps and writhes; "facial fasciculation" included cheek tremors, chews, and teeth chattering; and "miscellaneous other signs" included shakes, escape attempts, licks, scratches, and yawns (15). If present continuously, ptosis was only counted once/minute. The total number of somatic signs/10-min observation period was defined as the sum of individual occurrences of the aforementioned withdrawal signs.

Data Analyses

Induction of place aversion conditioning, evaluating 1 day after the end of the conditioning phase, was analyzed with a four-way analysis of variance (ANOVA) with place aversion (comparison between the time spent in the antagonist-paired compartment after vs. before conditioning; D vs. D0) as a within factor and nicotine treatment (nicotine-infused or vehicle-infused), MAOI treatments (vehicle, tranylcypromine, or phenelzine), and mecamylamine doses (2 or 4 mg/kg) as between-subjects factors, followed by Newman-Keuls post hoc tests when necessary. Long-term place aversion conditioning was analyzed with a four-way ANOVA with nicotine treatment, MAOI treatments as between-subjects factors, and place aversion and repeated tests (17 levels) as within factors, followed by Newman-Keuls post hoc tests when necessary.

Somatic withdrawal data were analyzed with a three-way repeated measures ANOVA with nicotine treatment, MAOI treatment and mecamylamine doses (6 levels) as between-subjects factors, followed by Newman-Keuls post hoc tests when necessary.

Results

Induction of Place Aversion Conditioning

The induction of place aversion conditioning was evaluated 1 day after the end of the conditioning phase (Table 1). Overall ANOVA indicated a significant effect of place aversion [F (1,183) = 50.8, $p < .001$], nicotine treatment [F(1,183) = 9.7, $p < .01$], and mecamylamine treatment [F (1,183) = 15.9, $p < .001$] and a significant place aversion × mecamylamine interac-

Table 1. Effects of Vehicle, Tranylcypromine, and Phenelzine on Conditioned Place Aversion Induced by Subcutaneous Administration of Mecamylamine in Vehicle-Infused and Nicotine-Infused Animals

		Place Aversion (change in time)	
Nicotine (mg/kg/day)	MAOI (mg/kg)	Mecamylamine 2 mg/kg	Mecamylamine 4 mg/kg
0	Vehicle	− 62 ± 35 (8)	− 117 ± 41 (7)*
0	Tranylcypromine	39 ± 69 (8)	− 88 ± 26 (7)*
0	Phenelzine	− 37 ± 24 (8)	− 121 ± 44 (7)*
9	Vehicle	− 94 ± 48 (9)	− 98 ± 24 (21)**
9	Tranylcypromine	− 48 ± 25 (20)	− 122 ± 28 (22)***
9	Phenelzine	4 ± 29 (33)	− 101 ± 12 (45)***

Values represent the mean ± SEM difference in time spent in the antagonist-paired compartment after vs. before conditioning.

*$p < .05$, **$p < .01$, ***$p < .001$, time spent in the antagonist-paired compartment after vs. before conditioning (Newman-Keuls). Between parentheses, number of subjects/group.

tion [$F(1,183) = 16.4$, $p < .001$], showing that only the dose of 4 mg/kg of mecamylamine induced a reliable place aversion in rats (Newman-Keuls test, $p < .001$). However, there was no mecamylamine × nicotine treatment interaction [$F(1,183) = 1.4$, not significant (NS)], indicating that mecamylamine induced a place aversion to a similar degree in vehicle- and nicotine-infused animals. Moreover, there was no MAOI effect [$F(1,183) = .2$, NS], no nicotine treatment × MAOI interaction, and no aversion × MAOI interaction [$F(2,183) = .01$, NS, and $F(2,183) = .07$, NS, respectively], indicating that MAOI treatments did not affect the acute effects of mecamylamine-induced place aversion.

Long-Term Place Aversion Conditioning

Because only the 4-mg/kg dose of mecamylamine produced a significant place aversion, only animals ($n = 91$) injected with this dose were used to study the time-course evolution of place aversion in abstinent rats (Figure 1).

The ANOVA analysis revealed that the overall place aversion conditioning was significantly more pronounced in nicotine-infused than in vehicle-infused animals [nicotine treatment effect, $F(1,88) = 4.04$, $p < .05$] and that the evolution of this place aversion conditioning is different among the two treatment groups [time × nicotine dependence interaction, $F(15,1444) = 1.58$, $p < .05$]. In placebo minipump abstinent rats (Figure 1A), mecamylamine induced a place aversion that decreased rapidly [time effect, $F(15,288) = 4.3$, $p < .001$] and was not affected by MAOI treatments [time × MAOI interaction, $F(30,288) = .68$, NS]. Indeed, place aversion was detected no more than 2 weeks after the last conditioning day (D14, Newman-Keuls test, $p < .05$, for each treatment).

In contrast, abstinent nicotine-infused rats (Figure 1B) displayed a long-lasting place aversion [time effect, $F(15,1056) = 4.3$, $p < .001$], for which the duration was affected by the MAOI treatments [MAOI effect, $F(2,66) = 5.13$, $p < .01$; and time × MAOI interaction, $F(30,1056) = 1.47$, $p < .05$], indicating that MAOI treatments selectively increased the persistence of the place aversion conditioning in nicotine-infused animals.

In nicotine-infused rats treated with vehicle, the mecamylamine-induced place aversion persisted up to 6 weeks (D42, $p < .05$), whereas it lasted up to 20 weeks of abstinence in nicotine-dependent animals treated with tranylcypromine (D154, $p < .01$) and was still observed at the end of our study (i.e., 8 months) in nicotine-dependent rats treated with phenelzine (D224, $p < .001$) (Figure 1B). Post hoc comparisons revealed that there was a significant difference in mecamylamine-induced place aversion between nicotine-infused rats treated with phenelzine and those treated with vehicle at 78, 98, 182, 210, and 224 days of abstinence ($p < .05$ at 78, D98, and D182; and $p < .01$ at D210 and D224). Despite repeated testing, the intensity of the place aversion in phenelzine-treated rats measured after 32 weeks of abstinence was similar to the intensity observed 1 day after the end of the conditioning phase (D1-D0 = −101 ± 17; D210-D0 = −114 ± 34, and D224-D0 = −135 ± 27 sec).

Somatic Signs of Withdrawal

Subcutaneous administration of mecamylamine produced a higher increase of overall number of somatic signs in nicotine-infused compared with vehicle-infused rats, as revealed by a significant effect of nicotine treatment [$F(1,42) = 51.87$, $p < .001$] (Figure 2). There was also a significant effect of mecamylamine doses [$F(5,210) = 87.41$, $p < .001$] and a significant nicotine treatment × mecamylamine doses interaction [$F(5,210) = 8.07$, $p < .001$]. Newman-Keuls post hoc tests indicated that nicotine-infused animals displayed significantly more somatic signs of withdrawal than vehicle-infused rats at mecamylamine doses above .57 mg/kg (nicotine-infused vs. vehicle-infused; doses of 1.14 mg/kg, $p < .01$; 1.72 mg/kg, $p < .001$; 2.29 mg/kg, $p < .001$; 3.43 mg/kg, $p < .01$). Post hoc tests comparing mecamylamine

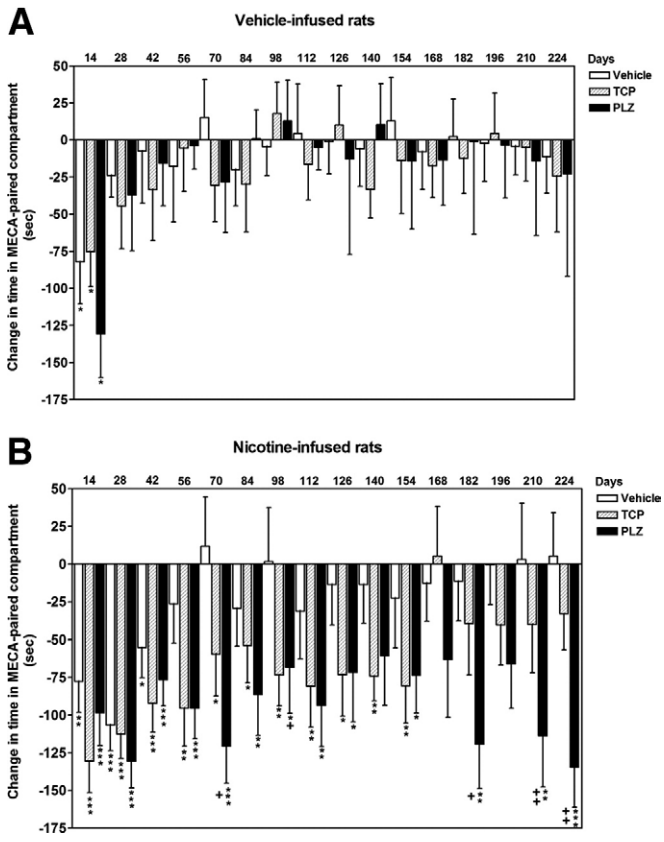

Figure 1. Effects of vehicle (white bars), tranylcypromine (hatched bars, 1.5 mg/kg/day), and phenelzine (black bars, 2 mg/kg/day) on the time-course of place aversion in **(A)** vehicle-infused rats and **(B)** nicotine-infused rats treated with 4 mg/kg of mecamylamine after removal of the minipumps. Animals were tested every 2 weeks (drug-free) at days 14–224. Values represent the mean ± SEM difference in time spent in the antagonist-paired compartment after versus before conditioning. *$p < .05$, **$p < .01$, time spent in the antagonist-paired compartment after versus before conditioning (Newman-Keuls). $^{+}p < .05$, $^{++}p < .01$, significant difference when compared with vehicle treated rats (Newman-Keuls).

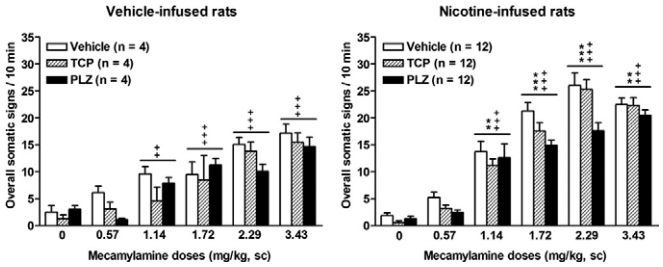

Figure 2. Effects of vehicle (white bars), tranylcypromine (hatched bars, 1.5 mg/kg/day), and phenelzine (black bars, 2 mg/kg/day) on overall somatic nicotine withdrawal signs (mean ± SEM) precipitated by mecamylamine in vehicle-infused and nicotine-infused animals during a 10-min observation period. $^{**}p < .01$, $^{***}p < .001$, nicotine-dependent vs. non-dependent rats (Newman-Keuls). $^{+++}p < .001$, mecamylamine vs. vehicle administration (Newman-Keuls).

doses indicate the 1.14- and 3.43-mg/kg doses are significantly different from vehicle injection ($p < .01$ and $p < .001$, respectively).

Moreover, there was a significant effect of MAOI treatment [$F(2,42) = 6.3$, $p < .01$; vehicle vs. tranylcypromine, $p < .01$; vehicle vs. phenelzine, $p < .001$] but no nicotine treatment × MAOI interaction [$F(5,210) = .85$, NS] and no mecamylamine dose × nicotine treatment × MAOI interaction [$F(10,210) = 8.07$, NS], illustrated by MAOI treatments slightly decreasing ratings of overall somatic signs of withdrawal in both nicotine- and vehicle-infused rats.

The analysis of the individual categories of somatic signs (Table 2) revealed that, compared with vehicle-infused animals, nicotine-infused animals displayed more intense abdominal constrictions [nicotine treatment effect: $F(1,42) = 16.93$, $p < .001$; nicotine treatment × dose interaction: $F(5,210) = 7.94$, $p < .001$] and facial fasciculation [nicotine treatment effect: $F(1,42) = 26.49$, $p < .001$; nicotine treatment × dose interaction: $F(5,210) = 3.57$, $p < .01$] Moreover, a significant effect of MAOI treatment was only found in the facial fasciculation category [$F(2,42) = 20.66$, $p < .001$; vehicle vs. tranylcypromine, $p < .01$; vehicle vs. phenelzine, $p < .001$].

Discussion

In the present study, we explored the effects of MAOI treatments on the aversive motivational and somatic aspects of nicotine withdrawal in rats. Our results show that, whereas chronic MAOI treatments have no effect on the magnitude of mecamylamine-induced place aversion, they dramatically and selectively induce a long-lasting conditioned place aversion in nicotine-abstinent rats. Indeed, significant aversions were evident for up to 5 and 8 months in nicotine-infused rats treated with tranylcypromine and phenelzine, respectively. The specificity of these treatments to prolong place aversion duration in nicotine-infused animals was further supported by the finding that these compounds did not increase ratings of somatic nicotine withdrawal signs. Moreover, in phenelzine-treated nicotine-infused rats, repeated testing had no effect on the intensity of place aversion, which remained stable and unaltered over the duration of the entire experiment, indicating that these animals did not show extinction.

As previously observed (14,15), the present results confirmed the observation that mecamylamine induced conditioned place aversion in rats. Surprisingly, the 4-mg/kg dose of mecamylamine induced a significant place aversion in vehicle-infused animals. Differences in the schedule used in the two

Table 2. Effects of Vehicle ($n = 18$), Tranylcypromine (1.5 mg/kg/day, $n = 18$), and Phenelzine (2 mg/kg/day, $n = 18$) on Individual Categories of Somatic Signs (mean ± SEM) Precipitated by Mecamylamine in Vehicle-Infused and Nicotine-Infused Animals During a 10-min Observation Period

Signs	Nicotine (mg/kg/day)	MAOI (mg/kg)	Mecamylamine doses (mg/kg, sc)					
			0	.57	1.14	1.72	2.29	3.43
Abdominal Constrictions	0	Vehicle	.2 ± .2	.8 ± .5	1.0 ± 1.0	1.3 ± .9	.3 ± .3	.5 ± .5
	0	Tranylcypromine	0	0	0	0	.3 ± .3	.5 ± .5
	0	Phenelzine	.7 ± .7	.3 ± .3	2.3 ± 1.9	2.0 ± 1.4	0	.7 ± .7
	9	Vehicle	.2 ± .2	.3 ± .2	2.2 ± .6^{+}	5.5 ± 1.4^{*+++}	6.2 ± 1.2^{***+++}	.2 ± .2
	9	Tranylcypromine	0	.2 ± .1	1.4 ± .5^{+}	3.3 ± 1.1^{*+++}	7.3 ± 1.6^{***+++}	2.3 ± 1.0
	9	Phenelzine	.1 ± .1	.1 ± .1	2.3 ± .4^{+}	3.5 ± .7^{*+++}	3.5 ± .9^{***+++}	.9 ± .6
Facial fasciculation	0	Vehicle	2.7 ± 1.4	4.0 ± 1.5	2.8 ± .9	.3 ± .3	3.3 ± .5	2.3 ± 1.3
	0	Tranylcypromine	1.2 ± .7	2.2 ± 1.0	1.8 ± .9	2.0 ± 1.2	2.0 ± 1.1	1.8 ± .3
	0	Phenelzine	1.0 ± .4	.3 ± .3	1.5 ± .9	1.3 ± .8	.5 ± .5	0
	9	Vehicle	1.9 ± .7	3.1 ± .8	4.5 ± 1.3^{+}	5.5 ± 1.3^{**+++}	7.8 ± 1.2^{**+++}	6.6 ± 1.1^{**+++}
	9	Tranylcypromine	.4 ± .3	1.8 ± .6	2.1 ± .6^{+}	3.8 ± .9^{**+++}	4.8 ± .7^{**+++}	3.8 ± .7^{**+++}
	9	Phenelzine	.8 ± .2	1.2 ± .4	2.1 ± .6^{+}	2.6 ± .7^{**+++}	2.3 ± .8^{**+++}	3.2 ± .9^{**+++}
Miscellaneous others signs	0	Vehicle	6.2 ± 1.5	7.5 ± 1.7	5.7 ± 1.3	6.2 ± 1.4	9.7 ± 2.2^{+++}	9.0 ± 1.1
	0	Tranylcypromine	5.0 ± .9	7.2 ± .5	7.0 ± 1.2	10.5 ± 3.7	9.5 ± 1.0^{+++}	9.2 ± 1.9
	0	Phenelzine	4.5 ± .9	5.0 ± 1.8	6.0 ± .8	8.2 ± .8	7.0 ± 1.8^{+++}	10.0 ± 1.9
	9	Vehicle	7.1 ± .7	6.7 ± .9	6.7 ± 1.1	8.2 ± 1.1	9.8 ± 1.4^{+++}	9.3 ± .9
	9	Tranylcypromine	4.1 ± .7	3.9 ± .6	4.9 ± .6	6.4 ± .8	7.1 ± 1.2^{+++}	8.3 ± 1.1
	9	Phenelzine	5.8 ± .9	6.1 ± .8	5.4 ± .7	5.2 ± .7	7.2 ± .8^{+++}	7.5 ± .5
Ptosis	0	Vehicle	0	.7 ± .7	2.5 ± .5^{+++}	5.2 ± 1.7^{+++}	7.0 ± .7^{+++}	10.0 ± 0
	0	Tranylcypromine	0	0	1.2 ± 1.2^{+++}	2.5 ± 1.6^{+++}	7.2 ± .2^{+++}	9.3 ± .8
	0	Phenelzine	.5 ± .5	0	3.2 ± .2^{+++}	5.7 ± .8^{+++}	5.7 ± 1.2^{+++}	10.0 ± 0
	9	Vehicle	.3 ± .3	.3 ± .3	4.0 ± .9^{+++}	6.3 ± .4^{+++}	7.3 ± .6^{+++}	9.3 ± .3
	9	Tranylcypromine	0	.3 ± .3	4.8 ± .8^{+++}	6.8 ± .5^{+++}	8.5 ± .4^{+++}	10.0 ± 0
	9	Phenelzine	.2 ± .2	.2 ± .2	3.0 ± .7^{+++}	5.2 ± .6^{+++}	6.5 ± .7^{+++}	10.0 ± 0

$^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, nicotine-infused vs. vehicle-infused rats (Newman-Keuls). $^{+}p < .05$, $^{++}p < .001$, mecamylamine vs. vehicle administration (Newman-Keuls).

studies could account for this discrepancy. First, whereas only three pairings were done in the Watkins study (15), we used five pairings to obtain a robust place aversion that would allow us to examine the evolution of this place aversion across repeated testing. Second, the order of vehicle and mecamylamine injections was not counterbalanced across subjects in the present study. Because of this fixed order of vehicle and mecamylamine administrations, it is possible that there was an association between the cues paired with mecamylamine injections and a time of day artefact that could have led to place aversion in vehicle-infused rats.

Moreover, in contrast to the study of Watkins *et al.* (15), mecamylamine induced some somatic signs in vehicle-infused rats in the present work. Mecamylamine is a noncompetitive nonselective nicotinic acetylcholine receptor antagonist and is acting both centrally and peripherally (25). Thus, it is possible that mecamylamine injection by itself might produce a discomfort that is expressed behaviorally both by some somatic signs and conditioned place aversion in the vehicle-infused animals.

However, the severity of withdrawal signs was higher in nicotine- than in vehicle-infused animals. At the lowest dose of mecamylamine (.57 mg/kg), few behavioral signs, such as miscellaneous signs, were observed, and there was no significant difference between nicotine- and vehicle-infused animals. In addition, scores of this category of signs were similar to those obtained after vehicle injection. Thus, at a low dose of mecamylamine, the physical manifestations observed might represent signs of stress that develop in the experimental condition. At the 1.14-mg dose of mecamylamine, other signs such as abdominal constrictions and facial fasciculation appeared, and the scores of these signs are significantly higher in nicotine-infused animals compared with vehicle-infused rats. Moreover, mecamylamine dose-dependently increased abdominal constrictions and facial fasciculation in nicotine-infused rats, whereas no dose-dependent effect was found in vehicle-infused animals. Thus, in nicotine-infused animals, mecamylamine induced somatic signs that were selectively observed for abdominal constrictions and facial fasciculation categories, indicating that these two categories of somatic signs were specific for nicotine withdrawal.

Furthermore, the results showed that chronic MAOI treatment slightly decreased the severity of the overall somatic manifestations of mecamylamine-precipitated nicotine withdrawal. Although it is clear that both central and peripheral cholinergic systems have been hypothesized to modulate the somatic withdrawal syndrome, other neurotransmitter systems also might be involved. For example, the aversive manifestations of nicotine withdrawal have been related to the inhibition of the dopamine release (26,27). Monoamine oxidase inhibitor administration has been reported to increase extracellular dopamine levels (28), which could explain the ability of MAOI treatment to attenuate the somatic signs of nicotine withdrawal in the present work.

It is generally assumed that craving for nicotine and the dysphoric mood that accompanies nicotine withdrawal play a much more important role in nicotine recidivism than the relatively mild somatic symptoms of nicotine withdrawal (29). Accordingly, we studied nicotine withdrawal with the conditioned place aversion paradigm. We found that in contrast to the relatively low doses of mecamylamine (1.14 mg/kg) required to induce somatic signs of withdrawal, significantly higher doses (4 mg/kg) were required to induce conditioned place aversion. Similarly, low doses of dihydro-β-erythroidine (DHβE; 4 mg/kg), a competitive and selective nicotinic acetylcholine receptor antagonist, increased the number of somatic signs, whereas higher doses (10 mg/kg) induced conditioned place aversion (8,15). This phenomenon seemed fundamentally different from other drugs of abuse, such as opiates, for which the doses of naloxone required to induce conditioned place aversion were much lower than those producing somatic signs of withdrawal (30).

Moreover, it has been shown that relatively high doses of mecamylamine (4–6 mg/kg) or DHβE (10 mg/kg) were required to produce conditioned place aversion compared with lower doses of mecamylamine (.57 mg/kg) or DHβE (2 mg/kg) required to precipitate elevations in brain reward thresholds (8,15). This observation suggests that dissociation might exist in the underlying mechanisms mediating conditioned place aversions compared with those mediating elevations in brain reward thresholds observed during nicotine withdrawal.

It has recently been shown that, consistent with the present work, conditioned cues repeatedly paired with nicotine withdrawal significantly decrease the activity of brain reward systems, mimicking the reward deficit observed during unconditioned nicotine withdrawal (31). These results suggest that withdrawal-paired conditioned stimuli gained negative affective valence and such cues could contribute to a low-level reward dysfunction that could make drugs or stimuli associated with drugs even more motivationally significant (32).

Relapse to smoking can occur after many months of abstinence, when acute withdrawal symptoms cannot account for reinstatement of drug-seeking behavior, suggesting that nicotine withdrawal induced long-lasting adaptations in the brains of smokers. Therefore, we studied the duration of conditioned place aversion, once established, with repeated measures of conditioned place aversion. Mecamylamine-induced place aversion was dramatically longer lasting in nicotine-infused than in vehicle-infused rats without modifying the intensity of the aversion. Indeed, in vehicle-infused animals place aversion lasted 2 weeks, whereas it persisted for 6 weeks in nicotine-infused rats treated with vehicle. This result, together with the recent finding that nicotine self-administration induces a long-lasting reward hypersensitivity for at least 36 days after nicotine intake had ceased (33), suggest that both nicotine and nicotine withdrawal induced persistent neuroadaptations that might induce relapse even after long periods of abstinence.

Moreover, MAOI treatment in the present study dramatically increased the duration of place aversion in nicotine-infused animals. Indeed, place aversion remained with tranylcypromine and phenelzine during the entire post-dependence period tested when rats no longer had chronic nicotine administration (abstinence). It has been shown that, consistent with this finding, low baseline MAO activity significantly predicted the intensity of craving and anxiety reported after smoking cessation, suggesting that there was an association between severity of affective symptoms after cigarette withdrawal and extent of MAO inhibition (34).

Monoamine oxidase inhibitor treatments have previously been shown to prolong behavioral sensitization to nicotine and enhance the rewarding effects on nicotine (35–36,23). However, here we show that MAOI treatments also prolonged place aversion conditioning associated with nicotine withdrawal in drug-free animals. One hypothesis to explain these results is that MAOI treatments during the conditioning phase might have facilitated memory consolidation or storage of withdrawal-related stimuli and its manifestation continued to be present months after animals were drug free (5 and 8 months for tranylcypromine and phenelzine, respectively). Indeed, by increasing functional levels of monoamines, it is possible that MAOI treatments interact with the conditioning of environmental

stimuli and augment the persistence of negative reinforcement derived from those stimuli. Whether such drug-induced memories are encoded and processed in a similar way as other emotional memories or have some unique drug neuropharmacological imprints remains to be determined.

Another explanation resides in the fact that these MAOI treatments have been shown to alter nicotine metabolism (37), thereby amplifying the nicotine dose the rats received. However, we have previously shown that no difference in the pharmacokinetics of nicotine and cotinine was found after a single administration of tranylcypromine and phenelzine (23). Thus, it seems unlikely that MAOI-influenced nicotine metabolism could account for MAOI-increased place aversion in nicotine-infused rats.

Together, the present results suggest that, in nicotine-infused animals treated with MAOI, conditioned place aversion persists for 5–8 months after nicotine abstinence. Such a long-lasting motivational effect might serve as a powerful negative stimulus motivating the continued administration of the drug or relapse after abstinence. Animal models of the aversive state associated with nicotine withdrawal might be important tools for understanding the neurobiological bases of nicotine dependence and for developing more effective treatment strategies to facilitate nicotine abstinence.

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1. Benowitz NL, Jacob P (1990): Nicotine metabolism in nonsmokers. *Clin Pharmacol Ther* 48:473–474.
2. Stolerman IP, Shoaib M (1991): The neurobiology of tobacco addiction. *Trends Pharmacol Sci* 12:467–473.
3. Doherty K, Kinnunen T, Militello FS, Garvey AJ (1995): Urges to smoke during the first month of abstinence: Relationship to relapse and predictors. *Psychopharmacology* 119:171–178.
4. Kenny PJ, Markou A (2001): Neurobiology of the nicotine withdrawal syndrome. *Pharmacol Biochem Behav* 70:531–549.
5. Shiffman SM, Jarvik ME (1976): Smoking withdrawal symptoms in two weeks of abstinence. *Psychopharmacology* 50:35–39.
6. Malin DH, Lake JR, Newlin-Maultsby P, Roberts LK, Lanier JG, Carter VA, *et al.* (1992): A rodent model of nicotine abstinence syndrome. *Pharmacol Biochem Behav* 43:779–784.
7. Hildebrand BE, Nomikos GG, Bondjers C, Nisell M, Svensson TH (1997): Behavioral manifestations of the nicotine abstinence syndrome in the rat: Peripheral versus central mechanisms. *Psychopharmacology* 129: 348–356.
8. Epping-Jordan MP, Watkins SS, Koob GF, Markou A (1998): Dramatic decreases in brain reward function during nicotine withdrawal. *Nature* 393:76–79.
9. Koob GF, Markou A, Weiss F, Schulteis G (1993): Oppponent process and drug dependence: neurobiological mechanisms. *Semin Neurosci* 5:351–358.
10. Markou A, Kosten TR, Koob GF (1998): Neurobiological similarities in depression and drug dependence: A self-medication hypothesis. *Neuropsychopharmacology* 18:135–174.
11. Wikler A (1973): Dynamics of dependence: Implications of a conditioning theory for research and treatment. *Arch Gen Psychiatry* 28:611–616.
12. Childress AR, Ehrman R, McLellan AT, MacRae J, Natale M, O'Brien CP (1994): Can induced moods trigger drug-related response in opiate abuse patients? *J Subst Abuse Treat* 11:17–23.
13. Schulteis G, Stinus L, Risbrough VB, Koob GF (1998): Clonidine blocks acquisition but not expression of conditioned opiate withdrawal in rats. *Neuropsychopharmacology* 19:406–416.
14. Suzuki T, Ise Y, Tsuda M, Maeda J, Misawa M (1996): Mecamylamine-precipitated nicotine-withdrawal aversion in rats. *Eur J Pharmacol* 314: 281–284.
15. Watkins SS, Stinus L, Koob GF, Markou A (2000): Reward and somatic changes during precipitated nicotine withdrawal in rats: Centrally and peripherally mediated effects. *J Pharmacol Exp Ther* 292:1053–1064.
16. Poindexter EH, Carpenter RD (1962): The isolation of harmane and norharmane from tobacco and cigarette smoke. *Phytochemistry* 1:215–221.
17. Breyer-Pfaff U, Wiatr G, Stevens I, Gaertner HJ, Mundle G, Mann K (1996): Elevated norharman plasma levels in alcoholic patients and controls resulting from tobacco smoking. *Life Sci* 58:1425–1432.
18. Rommelspacher H, Meier-Henco M, Smolka M, Kloft C (2002): The levels of norharman are high enough after smoking to affect monoamine oxidase B in platelets. *Eur J Pharmacol* 441:115–125.
19. Berlin I, Said S, Spreux-Varoquaux O, Olivares R, Launay JM, Puech AJ (1995): Monoamine oxidase A and B activities in heavy smokers. *Biol Psychiatry* 38:756–761.
20. Fowler JS, Volkow ND, Wang GJ, Pappas N, Logan J, MacGregor R, *et al.* (1996): Inhibition of monoamine oxidase B in the brains of smokers. *Nature* 379:733–736.
21. Fowler JS, Volkow ND, Wang GJ, Pappas N, Logan J, Shea C, *et al.* (1996): Brain monoamine oxidase A inhibition in cigarette smokers. *Proc Natl Acad Sci U S A* 93:14065–14069.
22. Berlin I, Anthenelli RM (2001): Monoamine oxidases and tobacco smoking. *Int J Neuropsychopharmacol* 4:33–42.
23. Guillem K, Vouillac C, Azar MR, Parsons LH, Koob GF, Cador M, Stinus L (2005): Monoamine oxidase inhibition dramatically increases the motivation to self-administer nicotine in rats. *J Neurosci* 25:8593–8600.
24. Stinus L, Le Moal M, Koob GF (1990): Nucleus accumbens and amygdala are possible substrates for the aversive stimulus effects of opiate withdrawal. *Neuroscience* 37:767–773.
25. Taylor P (1980) Ganglionic stimulating and blocking agents. In: Gilman AG, Goodman LS, Gilman A, editors. *The Pharmacological Basis of Therapeutics*, *6th ed.* New York: MacMillan Company, 211–219.
26. Schechter MD, Meechan SM (1994): Conditioned place aversion induced by dopamine release inhibition. *Eur J Pharmacol* 260:133–137.
27. Rada P, Jensen K, Hoebel BG (2001): Effects of nicotine and mecamylamine-induced withdrawal on extracellular dopamine and acetylcholine in the rat nucleus accumbens. *Psychopharmacology* 157: 105–110.
28. Baker GB, Hiob LE, Dewhurst WG (1980): Effects of monoamine oxidase inhibitors on release of dopamine and 5-hydroxytryptamine from rat striatum in vitro. *Cell Mol Biol Incl Cyto Enzymol* 26:183–186.
29. Hughes JR, Gust SW, Skoog K, Keenan RM, Fenwick JW (1991): Symptoms of tobacco withdrawal. A replication and extension. *Arch Gen Psychiatry* 48:52–59.
30. Schulteis G, Markou A, Gold LH, Stinus L, Koob GF (1994): Relative sensitivity to naloxone of multiple indices of opiate withdrawal: A quantitative dose-response analysis. *J Pharmacol Exp Ther* 271:1391–1398.
31. Kenny PJ, Markou A (2005): Conditioned nicotine withdrawal profoundly decreases the activity of brain reward systems. *J Neurosci* 25: 6208–6212.
32. Koob GF, Le Moal M (1997): Drug abuse: Hedonic homeostatic dysregulation. *Science* 278:52–58.
33. Kenny PJ, Markou A (2006): Nicotine self-administration acutely activates brain reward systems and induces a long-lasting increase in reward sensitivity. *Neuropsychopharmacology* 31:1203–1211.
34. Rose JE, Behm FM, Ramsey C, Ritchie JC Jr. (2001): Platelet monoamine oxidase, smoking cessation, and tobacco withdrawal symptoms. *Nicotine Tob Res* 3:383–390.
35. Villegier AS, Blanc G, Glowinski J, Tassin JP (2003): Transient behavioral sensitization to nicotine becomes long-lasting with monoamine oxidases inhibitors. *Pharmacol Biochem Behav* 76:267–274.
36. Villegier AS, Salomon L, Granon S, Changeux JP, Belluzzi JD, Leslie FM, Tassin JP (2006): Monoamine oxidase inhibitors allow locomotor and rewarding responses to nicotine. *Neuropsychopharmacology* 31:1704–1713.
37. Zhang W, Kilicarslan T, Tyndale RF, Sellers EM (2001): Evaluation of methoxsalen, tranylcypromine, and tryptamine as specific and selective CYP2A6 inhibitors in vitro. *Drug Metab Dispos* 29:897–902.