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Monoamine Oxidase Inhibitors Allow Locomotor and Rewarding Responses to Nicotine

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Although nicotine is generally considered to be the main compound responsible for the addictive properties of tobacco, experimental data indicate that nicotine does not exhibit all the characteristics of other abused substances, such as psychostimulants and opiates. For example, nicotine is only a weak locomotor enhancer in rats and generally fails to induce a locomotor response in mice. This observation contradicts the general consensus that all drugs of abuse release dopamine in the nucleus accumbens, a subcortical structure, and thus increase locomotor activity in rodents. Because tobacco smoke contains monoamine oxidase inhibitors (MAOIs) and decreases MAO activity in smokers, we have combined MAOIs with nicotine to determine whether it is possible to obtain a locomotor response to nicotine in C57Bl6 mice. Among 15 individual or combined MAOIs, including harmane, norharmane, moclobemide, selegiline, pargyline, clorgyline, tranylcypromine and phenelzine, only irreversible inhibitors of both MAO-A and -B (tranylcypromine, phenelzine, and clorgyline + selegiline) allowed a locomotor response to nicotine. The locomotor stimulant interaction of tranylcypromine and nicotine was absent in β2-nicotinic acetylcholine receptor subunit knockout mice. Finally, it was found that, whereas naïve rats did not readily self-administer nicotine (10 μg/kg/injection), a robust self-administration of nicotine occurred when animals were pretreated with tranylcypromine (3 mg/kg). Our data suggest that MAOIs contained in tobacco and tobacco smoke act in synergy with nicotine to enhance its rewarding effects.

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INTRODUCTION

Drugs of abuse, such as D-amphetamine, cocaine, morphine, or heroin, share the ability to cause addiction in humans and to increase release of dopamine (DA) in the nucleus accumbens (Di Chiara and Imperato, 1988; Vezina *et al*, 1989; Pontieri *et al*, 1995; Koob, 1998; Robbins and Everitt, 1999). The same drugs induce locomotor hyperactivity, behavioral sensitization, conditioned place preference, and self-administration in rodents (Vezina *et al*, 1994; Pontieri *et al*, 1995; Biala and Langwinski, 1996; Carboni *et al*, 2003; Li *et al*, 2003). Tobacco is a potent reinforcing agent in humans, and nicotine is generally considered to be the major compound responsible for its addictive properties (Dani and Heinemann, 1996; Balfour *et al*, 2000; Di Chiara, 2000). However, animal experiments indicate some discrepancies between the effects of nicotine and those of other drugs of abuse. For example, the stimulation of DA release in the nucleus accumbens after several nicotine injections remains controversial (Vezina *et al*, 1992; Balfour *et al*, 1998; Di Chiara, 2000), and repeated nicotine treatments in rats induce a behavioral sensitisation, which vanishes quicker than for other drugs of abuse (Ksir *et al*, 1985; Villégier *et al*, 2003). Similarly, although self-administration of nicotine is observed in rodents (Corrigall and Coen, 1989; Donny *et al*, 1995; Martellotta *et al*, 1995; Shoaib *et al*, 1997; Caggiula *et al*, 2001) and monkeys (Goldberg *et al*, 1981; Wakasa *et al*, 1995), its development seems slower and weaker than for other drugs of abuse (Manzardo *et al*, 2002), and often needs facilitation by the use of food restriction (Corrigall *et al*, 2002; Rauhut *et al*, 2002) or cocaine pre-treatment (Picciotto *et al*, 1998). These differences suggest either that nicotine possesses both aversive and rewarding properties (Risinger and Brown, 1996; Shoaib *et al*, 2002), or that the addictive effects of tobacco are not only due to nicotine. A recent report suggests that agents in tobacco smoke can enhance nicotine self-administration (Belluzzi *et al*, 2005).

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One of the most striking differences between the effects of nicotine and those of other drugs of abuse concerns its locomotor effects. Although psychostimulants and opiates induce a substantial locomotor hyperactivity both in rats and mice, nicotine is a weak locomotor stimulant in rats and generally fails to induce locomotor hyperactivity in mice at any dose (Marks *et al*, 1983; Freeman *et al*, 1987; Kita *et al*, 1988; Damaj and Martin, 1993; Smolen *et al*, 1994; Itzhak and Martin, 1999; Sparks and Pauly, 1999; Gaddnas *et al*, 2000; Castane *et al*, 2002).

Tobacco and tobacco smoke are known to contain a number of compounds, among which monoamine oxidase inhibitors (MAOIs) have been the focus of special interest (Poindexter and Carpenter, 1962; Breyer-Pfaff *et al*, 1996; Rommelspacher *et al*, 2002). Many authors have found that tobacco smokers, when compared to nonsmokers, have MAO-A and -B activities that are reduced by up to 40% (Oreland *et al*, 1981; Yu and Boulton, 1987; Berlin *et al*, 1995; Fowler *et al*, 1996a, b). Inhibition of monoamine oxidases (MAOs) by tobacco smoke does not result from the actions of nicotine (Carr and Basham, 1991), but from that of other compounds that are also present in other psychotropic plants (Uebelhack *et al*, 1998; Rommelspacher *et al*, 2002). We have recently shown that MAOI pretreatment allows the maintenance of locomotor behavioral sensitization to nicotine in rats (Villégier *et al*, 2003), thus suggesting a role of MAOIs in the addictive properties of tobacco. We have therefore analyzed the consequences of different MAOI pretreatments on the locomotor response to nicotine in the mouse. First, different individual or combined MAOIs were injected into mice and their locomotor responses were analyzed for 380 min. In another series of experiments, nicotine was injected at different times after MAOI(s) injection and locomotor effects of nicotine were calculated as the difference between responses with and without nicotine. Among the different MAOIs tested, tranylcypromine was found particularly effective in increasing locomotor response to nicotine. Tranylcypromine is a 'cyclized' amphetamine with an exposed amino group making that compound an irreversible mixed MAOI 5000 times as potent an MAOI as amphetamine (Zirkle and Kaiser, 1964).

To verify that locomotor effects of nicotine were related to the stimulation of nicotinic acetylcholine receptors (nAChRs), locomotor activity was monitored in the presence of tranylcypromine in mice knockout for the $\beta 2$ subunit ($\beta 2$-nAchR-KO). This subunit is part of the $\alpha 4\beta 2$ nAChR, one of the most frequently found nAChR oligomers in mammalian brain (Pidoplichko *et al*, 1997; Charpantier *et al*, 1998).

Finally, self-administration of nicotine was examined in rats pretreated with tranylcypromine.

MATERIALS AND METHODS

Subjects

Mice. Animals were adult male mice, on genetic background C57Bl6, weighing 35–45 g when experiments were conducted. Generation of the $\beta 2$-nAChR subunit KO mice and their genetic background (C57Bl6) are as described previously by Picciotto *et al* (1995). Mice were maintained on a 12 h light/12 h dark cycle (light on between 0700 and 1900) at constant temperature (22°C), with food and water *ad libitum*. Animals were housed by groups of four and were habituated to their home cages for at least 1 week before the experiments. Mice were used only once and were treated in accordance with the Guide for Care and Use of Laboratory Animals established by the National Institutes of Health and with the European Community Council Directive 86/609/EEC.

Rats. Animals were Sprague–Dawley rats (Charles River Laboratories). After surgery, all animals were single-housed and maintained on a 12-h light/dark cycle (lights on at 0700) with food and water available *ad libitum*. Rats were allowed at least 3 days of postoperative recovery before any treatment began. All tests were performed during the light part of the light–dark cycle. All experimental procedures were performed in compliance with NIH Guide for Care and Use of Laboratory Animals (NIH No 85–23, rev. 1985) and approved by the UCI Institutional Animal Care and Use Committee.

Drugs

(−)-Nicotine hydrogen tartrate, tranylcypromine hydrochloride, pargyline hydrochloride, *R*-(−)-deprenyl hydrochloride (selegiline), clorgyline, phenelzine sulfate were purchased from Sigma Aldrich (France). Moclobemide was from Roche Pharma (France). Drugs were dissolved in saline (NaCl, 0.9%) and the pH adjusted to 7.4 with NaOH. Harmane and norharmane hydrochloride were from Sigma Aldrich (France). They were dissolved in distilled water with a few drops of 0.1 M lactic acid in order to get a pH > 5. Doses are expressed as salts for all compounds except for nicotine which is expressed as base. Nicotine was injected subcutaneously (1.5 ml/kg per injection for mice) or intravenously (i.v.) (10 μg/kg in 20 μl per injection for rats), whereas other treatments were injected intraperitoneally (i.p.) (3 ml/kg per injection).

Measurement of Locomotor Activity

Mice were introduced into a circular corridor (4.5 cm width, 17 cm external diameter) crossed by four infrared beams (1.5 cm above the base) placed at every 90° (Imetronic, Pessac, France). Locomotor activity was counted when animals interrupted two successive beams and thus had travelled a quarter of the circular corridor (1/4 turn). In each session, mice were placed in the locomotor apparatus for 90 min to record spontaneous activity before drug or vehicle treatment (MAOI or saline).

To study the locomotor effects of various MAOIs, mice received an injection of MAOI and locomotor response was recorded during a subsequent 380-min. period. To study the effect of MAOI pretreatment on nicotine's locomotor effects, nicotine or saline were injected 100, 180, and 210 min following tranylcypromine, phenelzine, and clorgyline + selegiline administrations, respectively. These times were chosen because they corresponded to the delay from the time of injection to the onset of MAOI-induced locomotor effects. In the case of the other MAOIs, which did not elicit any locomotor effect, nicotine or saline was

injected between 100 and 210 min following MAOI administration. These injection times also marked the beginning of the 120-min period for measurement of the locomotor response to nicotine reported in Figure 2a and b. Tests were performed between 12:00 and 18:00 in stable conditions of temperature and humidity.

Surgery

Surgical implantation of intravenous catheters. Catheter construction has been described previously (Manzardo *et al*, 2002). Animals were anesthetized with Equithesin (0.25 ml/100 g, i.p.) and a chronic catheter was surgically implanted into the right external jugular vein using a method similar to that described by Caine *et al* (1993). The catheter was passed subcutaneously from the animal's back to the jugular vein where the tubing was inserted. The polyethylene assembly was mounted on the animal's back. The wounds were closed with wound clips, antiseptic ointment was applied to the wounds, and Baytril (0.1 ml/150 g, i.m.) was injected to prevent infection. Antiseptic ointment was applied to the exposed cannula housing after each surgery to prevent infection, and a metal cap was attached to prevent damage to the cannula. The animals were kept in a warm cage for postsurgical observation until they emerged from anesthesia.

The catheters were flushed daily with 0.2 ml sterile heparinized saline solution (0.3 ml of 1000 U/ml heparin in 30 ml saline) to maintain catheter patency. On test days, heparinized saline was injected before and after the self-administration session. After the final daily flush, the injection tubing was heat-sealed near the top of the cannula and left on in order to maintain a closed system and prevent clogging of the catheter in the home cage. The exposed cannula was protected by a threaded aluminum standoff. After the final daily test session, Propofol (0.6 ml/kg) was injected through the catheter to test the patency of the i.v. catheter as indicated by rapid (5–10 s) anesthesia. Data from all animals not demonstrating rapid anesthesia were discarded.

Self-Administration

Drug self-administration. At 3–5 days after surgery, rats were tested in self-administration chambers with two nose-poke holes side-by-side in the door. A 10-ml syringe was mounted in an infusion pump, located outside the test chamber, and connected by polyethylene tubing to a feed-through swivel located above the test chamber. The other side of the feed-through swivel was connected to the infusion cannula on the animal's back with polyethylene tubing covered by a steel spring to prevent puncture from biting. The syringe was filled with enough solution to provide a maximum of 200 injections. During each 1.1-s 20-μl infusion, the signal light over the hole associated with drug injection went on for a 1.1-s period and, immediately after the drug infusion, responses were counted but had no effect for a 60-s period. All experimental parameters and data collection were controlled by a multichannel computer system (MED Associates, Inc., St Albans, VT).

Acquisition of nicotine self-administration after a pretreatment with tranylcypromine. A nose-poke response was used to measure drug self-administration. This response relies on the animals' natural olfactory exploration to provide adequate initial levels of responding. Priming at the start of each session was not employed because of possible aversive effects of non-contingent injections in naïve animals. A total of 27 rats were used in the experiment. Tests for acquisition of self-administration commenced without prior response training and consisted of five daily 3-h sessions with a fixed-ratio one (FR 1) reinforcement schedule. Each nose-poke at the reinforced hole (R) delivered nicotine (10 μg/kg/injection) or saline vehicle. To control for nonspecific activating effects of drugs, nonreinforced nose-pokes (NR) at a second adjacent hole were counted, but they had no programmed consequences. At 60 min before each session, rats received a pretreatment with tranylcypromine (3 mg/kg, i.p.) or saline vehicle (1 ml/kg, i.p.).

Statistical Analysis of the Data

Mice. Results presented are means ± SEM of data obtained with 8–10 animals. Data were subjected to analysis of variance (ANOVA) using two-way ANOVA for MAOI treatment × time (Figure 1b and comparison between Figure 3b and 3c), or for nicotine dose × time (Figure 2a), three-way ANOVA for MAOI treatment × nicotine dose × time (Figure 3b and c) and genotype × MAOI treatment × time (comparison between Figures 3b and 4) with repeated measures on 10 min intervals. Significant main effects or interactions were tested separately with ANOVAs and Bonferroni- or Dunnett's-corrected *post hoc* comparisons (Figures 1b, 2, 3 and 4). Time was considered as a within-subject factor and pharmacological treatments corresponded to independent groups of animals and were considered as between-subject factors. All data analyses were performed using GraphPad Prism 4.0 software (San Diego, CA) or SYSTAT 10 statistical software. Statistical significance was set at $P<0.05$.

Rats. For group differences over days, daily total 3-h self-injections for each group over the 5-day acquisition period were analyzed by three-way ANOVA for MAOI treatment × nicotine dose × day with repeated measures on day (Figure 5a). Daily reinforced (R) and nonreinforced (NR) scores were analyzed with a three-way ANOVA on nicotine dose × reinforced/nonreinforced responses × day with repeated measures on day. Significant main or interaction effects were further analyzed by one-way ANOVA for treatment for each day with Bonferroni- or Dunnett's-adjusted *post hoc* comparisons.

RESULTS

Effects of Mixed or Selective, Reversible or Irreversible, MAOI(s) on Mouse Locomotor Activity

A total of 15 individual or combined MAOI treatments were tested for their effects on mouse locomotor activity (Figure 1a). The MAOIs used were either mixed (inhibition of MAO-A and -B) or selective (inhibition of MAO-A or -B), and reversible (Rev) or irreversible (Irr). Significant locomotor hyperactivity, beginning at 100 ($t=190$), 210

a

T	Substances	Doses (mg/kg)	Properties	
			Rev / Irr	A / B
1	tranylcypromine	10	Irr	A and B
2	clorgyline	10	Irr	A
	selegiline	3	Irr	B
3	phenelzine	80	Irr	A and B
4	pargyline	100	Irr	B
	moclobemide	20	Rev	A
5	harmane	5	Rev	A
	selegiline	3	Irr	B
6	pargyline	100	Irr	B
7	selegiline	3	Irr	B
8	clorgyline	10	Irr	A
	norharmane	5	Rev	B
9	harmane	5	Rev	A
	norharmane	5	Rev	B
10	norharmane	30	Rev	B
11	norharmane	5	Rev	B
12	harmane	15	Rev	A
13	harmane	5	Rev	A
14	moclobemide	20	Rev	A
15	clorgyline	10	Irr	A

b

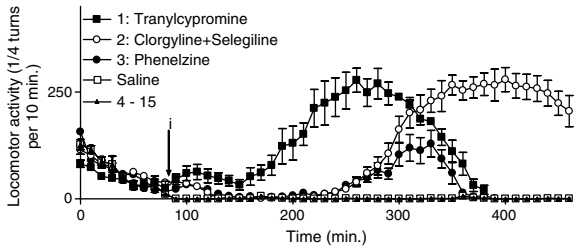

Figure 1 Locomotor activity induced by different MAOIs: mice were introduced in the locomotor apparatus for 90 min before the injection of MAOIs exhibiting different specificities (reversible A and/or B (revA and/or revB) and irreversible A and/or B (irrA and/or irrB)). (a) The table shows the 15 different MAOI treatments (T) used to evaluate their effects on mouse locomotor activity. Locomotor response was recorded during a subsequent 380-min period. (b) Treatments 1, 2, and 3 elicited a significant locomotor hyperactivity, starting 100 ($t=190$), 210 ($t=300$), and 180 ($t=270$) min after injection of MAOI, respectively, whereas other treatments (4–15) did not elicit any locomotor effect at any time when compared to saline; $N=8$–12 animals per group.

($t=300$), and 180 ($t=270$), min. following MAOI injection, was observed for (1) tranylcypromine (+2577% when compared with saline during the 120 min between $t=190$ and 310, $F(11,154)=6.557$, $P<0.001$), (2) clorgyline + selegiline (+2285%, when compared with saline during the 120 min between $t=300$ and 420, $F(11,154)=12.150$, $P<0.001$), and (3) phenelzine (+767% when compared with saline during the 120 min between $t=270$ and 390, $F(11,154)=7.443$, $P<0.001$), respectively (Figure 1b). Other treatments (combinations 4–15) did not induce significant locomotor activity at any time when compared with saline-treated animals, even when higher or lower doses were injected.

Effects of Different Individual or Combined MAOIs on Nicotine-Induced Locomotor Activity

Figure 2a shows locomotor activity following a saline injection in mice pretreated with MAOIs. Saline was injected at $t=190$, 270, 300 min for tranylcypromine, phenelzine, clorgyline + selegiline, and between $t=190$ and $t=300$ for combinations 4–15, respectively. As previously explained in Material and Methods, these times were chosen because they corresponded to the onset of MAOI-induced locomotor effects. These times also corresponded to the start of the 120-min period used to assess the locomotor response scores shown in Figure 2a and b. Figure 2b summarizes the locomotor responses induced by nicotine under the same conditions. Data are presented as total activity obtained in presence of MAOI(s) + nicotine (1 mg/kg) minus the activity score obtained in the corresponding MAOI(s) + saline condition. Significant differences in locomotor responses between MAOI(s) + nicotine and MAOI(s) + saline occurred only for those MAOI treatment conditions that induced significant locomotor enhancement in the absence of nicotine (+69% $t(1,14)=3.123$ $P<0.001$, +22% $t(1,14)=2.196$ $P=0.0454$, and +80% $t(1,25)=2.126$ $P=0.0436$ with or without nicotine, for combinations 1, 2, and 3, respectively). These data indicate that only pretreatment with nonselective, irreversible MAOIs (tranylcypromine or phenelzine) or a combination of selective, irreversible inhibitors of MAO-A and B (clorgyline + selegiline) produced a significant nicotine-induced locomotor hyperactivity. Pretreatments with reversible MAOIs, whether selective or nonselective, did not trigger a locomotor response to nicotine at any dose tested (Figures 1a and 2b).

Effects of Tranylcypromine on Nicotine-Induced Locomotor Activity

Figure 3a shows that there was almost no locomotor response when nicotine (1 mg/kg) was injected following saline pretreatment ($F(1,14)=4.388$ $P=0.055$ nicotine *vs* saline). In contrast, there was a significant MAOI treatment × nicotine dose × time interaction ($F(11,308)=2.093$, $P=0.021$), indicating a significant increase in locomotor activity when nicotine (1 mg/kg) was injected following pretreatment with tranylcypromine (10 mg/kg) (4014 ± 380 *vs* $2385\pm309\frac{1}{4}$ turns per 120 min in the presence or absence of nicotine, respectively) (Figure 3b). The effect of nicotine lasted approximately 100 min and appeared biphasic, with a peak occurring in the first 10 min (Figure 3b). When the dose of tranylcypromine was raised to 20 mg/kg, locomotor response was significantly higher than that obtained with tranylcypromine 10 mg/kg (significant MAOI dose × time interaction $F(11,154)=4.626$; $P<0.001$), but the addition of nicotine (1 mg/kg) still induced a significantly higher locomotor response than tranylcypromine alone (4912 ± 421 *vs* $3682\pm241\ \frac{1}{4}$ turns per 120 min in the presence or absence of nicotine, respectively, and a significant MAOI treatment × nicotine dose × time interaction; $F(11,308)=2.533$, $P=0.004$) (Figure 3c). Dose–response analysis for nicotine indicates that, in animals pretreated with 10 mg/kg tranylcypromine, the maximal nicotine-induced locomotion was induced at 1 mg/kg (Figure 3d).

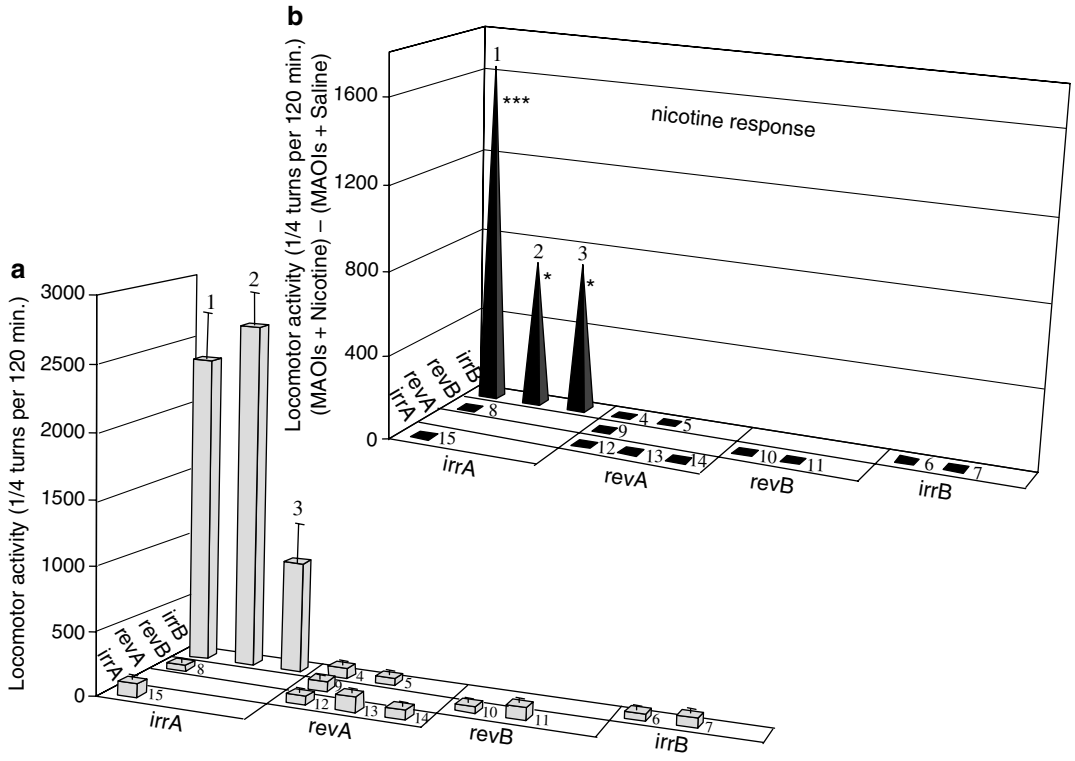

Figure 2 Locomotor activity induced by nicotine in the presence of MAOIs: according to data shown in Figure 1b, nicotine or saline injections were performed 100, 210, and 180 min following treatment injection for conditions 1, 2, and 3, respectively, and between 100 and 210 min following treatment injection for conditions 4–15. Diagrams a and b represent nicotine *vs* saline's effects on MAOI-induced locomotor effects. Locomotor response to MAOIs + saline (a) or ((MAOIs + nicotine)−(MAOIs + saline)) (b) was measured for 120 min following saline or nicotine administration. *$P<0.05$; ***$P<0.001$, significantly different from corresponding saline; $N=8$–12 animals per group.

Effects of Nicotine in Mice Knockout for the β2 nAchR Subunit

As previously described (Villégier *et al*, 2004), it was found that β2-nAChR-KO mice were very reactive to saline injections (Figure 4). However, nicotine injection (1 mg/kg) completely inhibited this locomotor response. Moreover, tranylcypromine (10 mg/kg) alone induced a significantly higher locomotor response in β2-nAChR-KO mice than in wild-type (WT) littermates (4359 ± 302 *vs* $2385\pm309\frac{1}{4}$ turns per 120 min, for β2-nAChR-KO and WT mice, respectively, significant genotype × MAOI treatment × time interaction $F(11,308)=2.728$ $P=0.002$ and significant genotype × time interaction $F(11,154)=6.067$ $P<0.001$; Figures 3b and 4). However, this higher locomotor response to 10 mg/kg tranylcypromine observed in β2-nAChR-KO mice was not significantly different from that of WT mice treated with 20 mg/kg tranylcypromine, as shown by the absence of significant tranylcypromine dose × genotype × Time interaction $F=(11,308)=1.63$, $P=0.089$) (Figures 3c and 4).

Nicotine did not increase locomotor activity in β2-nAChR-KO mice pretreated with tranylcypromine 10 mg/kg (Figure 4); on the contrary, the nicotine-induced decrease of locomotor activity observed in saline pretreated animals was still present in tranylcypromine-treated β2-nAChR-KO animals (significant MAOI treatment × nicotine dose × time interaction $F(11,330)=2.093$, $P=0.02$). This effect was significant for the first two 10 min intervals following nicotine injection (time = 190: 393 ± 38 (saline) *vs* 249 ± 40 (nicotine), $P=0.007$; $t=200$: 443 ± 46 (saline) *vs* 207 ± 55 (nicotine), $P<0.001$) and disappeared at later time points.

Acquisition of Nicotine Self-Administration after Tranylcypromine

As has been reported previously (Belluzzi *et al*, 2005), in our rigorous experimental conditions in which there is no training or food deprivation, naïve rats did not acquire self-administration of nicotine when compared with saline (absence of significant nicotine dose × time interaction $F(4,32)=0.031$ $P=0.998$) (Figure 5a). However, rats pretreated with tranylcypromine (3 mg/kg) quickly learned to self-administer nicotine (Figure 5a), and displayed sustained self-administration throughout the 5-day test period at rates substantially higher than those working for saline with tranylcypromine pretreatment ($F(1,14)=8.871$, $P=0.01$), or those working for nicotine ($F(1,13)=12.613$, $P=0.004$) or saline ($F(1,17)=28.935$, $P<0.001$) with saline pretreatment. Tranylcypromine increased the level of saline self-administration ($F(1,11)=10.572$, $P=0.008$), but there also was a significant MAOI treatment × nicotine dose × time interaction: $F(3,219)=3.219$ $P=0.016$), indicating that rats pretreated with tranylcypromine worked for injections of nicotine at significantly higher rates than for saline. Analysis across all five daily sessions showed a significant difference between nicotine reinforced and nonreinforced responding in rats pretreated with tranylcypromine (reinforced/nonreinforced responses × time interaction $F(4,40)=10.344$, $P<0.001$), as well as a significant nicotine

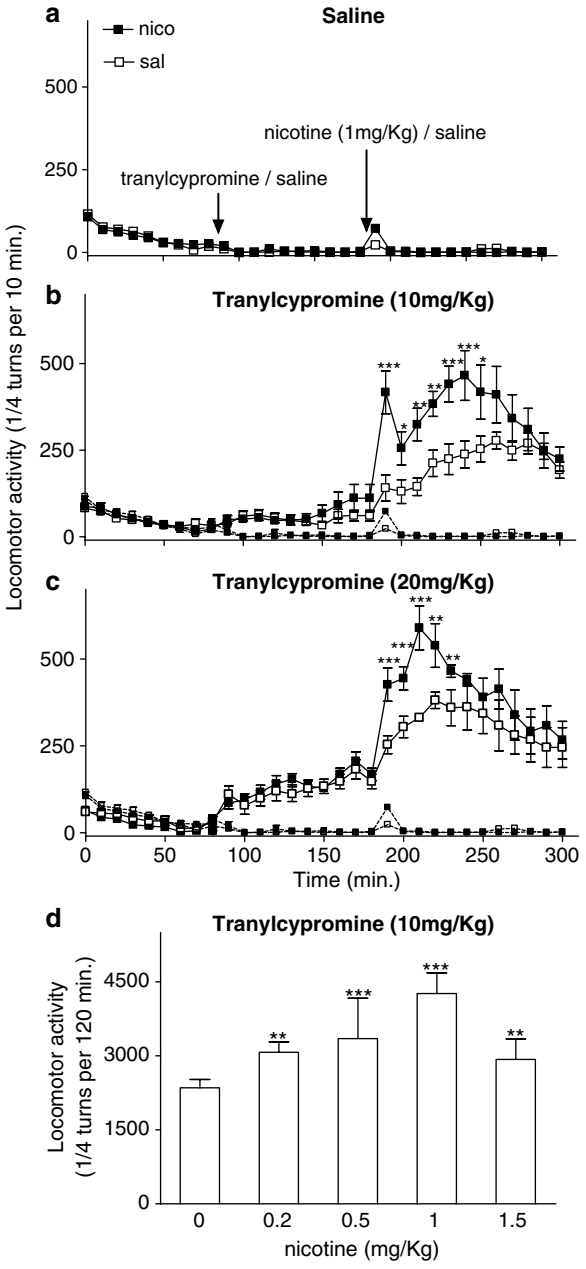

Figure 3 Locomotor response to nicotine in mice pretreated with tranylcypromine or saline: Animals were pretreated with (a) saline, tranylcypromine (10 mg/kg) (b) or tranylcypromine (20 mg/kg) (c) at $t = 90$ min and received an injection of either saline (sal) or nicotine (1 mg/kg) (nico) at $t = 190$ min. ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$ when compared with tranylcypromine/saline-treated animals. (d) Dose–response histograms of locomotor response to different doses of nicotine in 10 mg/kg tranylcypromine pretreated animals. ***$P < 0.001$ and **$P < 0.01$ when compared to saline-treated animals; $N = 8$–10 animals per group.

dose × reinforced/non reinforced responses × time interaction ($F(4,56) = 5.488$, $P = 0.001$) (Figure 5b).

DISCUSSION

The first finding of this study is that nicotine can induce a long-lasting locomotor hyperactivity in mice, but only if they are pre-treated with MAOIs. However, only irreversible blockade of both MAO-A and -B can induce this phenomenon. This effect of nicotine on locomotor response is most likely related to the stimulation of nicotinic receptors since it did not occur in mice KO for the $\beta 2$ nAChR subunit.

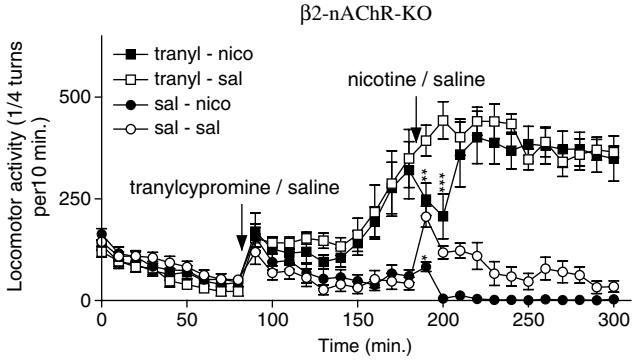

Figure 4 Locomotor response to nicotine in $\beta 2$-nAChR-KO animals pretreated with tranylcypromine: Animals received an injection of saline or tranylcypromine (10 mg/kg) at $t = 90$ min and an injection of saline or nicotine (1 mg/kg) at $t = 190$ min. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, tranyl–nico *versus* tranyl–sal and sal–nico *versus* sal−sal; $N = 8$–9 animals per group.

The second finding of this study is that pretreatment with tranylcypromine, a mixed irreversible MAOI, sustains nicotine self-administration in rats which, under the same experimental conditions but with saline pretreatment, do not self-administer nicotine.

These findings suggest that the psychoactive effects of smoking, including rewarding and locomotor effects, may result from an interaction of nicotine with MAOIs that occur naturally in tobacco.

Mixed and Irreversible MAOIs Increase Locomotor Activity in Mice and Trigger Nicotine-Induced Locomotor Response

The data obtained with 15 individual or combined MAOIs indicate that only tranylcypromine, phenelzine, and the combination of clorgyline and selegiline induce locomotor activity. Tranylcypromine and phenelzine are irreversible mixed MAOIs, whereas clorgyline and selegiline are both irreversible and specific for MAO-A and -B, respectively. These findings indicate that only the irreversible blockade of both MAO-A and -B initiates a locomotor response in mice. Interestingly, this effect needs at least 80 min to develop, suggesting that an accumulation of extracellular monoamines is necessary for the effect to appear.

Whereas each MAO isozyme exhibits some substrate specificity for noradrenaline, dopamine, or serotonin, there is sufficient overlap that both types of MAO must be inhibited to influence brain monoamine levels greatly (Shih, 2004). Thus, concomitant blockade of both MAO-A and -B may be necessary to induce locomotor activation. Furthermore, the finding that only irreversible MAOIs induce a locomotor response suggests that a sufficient accumulation of extracellular monoamines can only occur following sustained MAO inhibition. When nicotine was tested following MAOI pretreatment, the same three combinations of MAOI that induced locomotor activity also triggered a

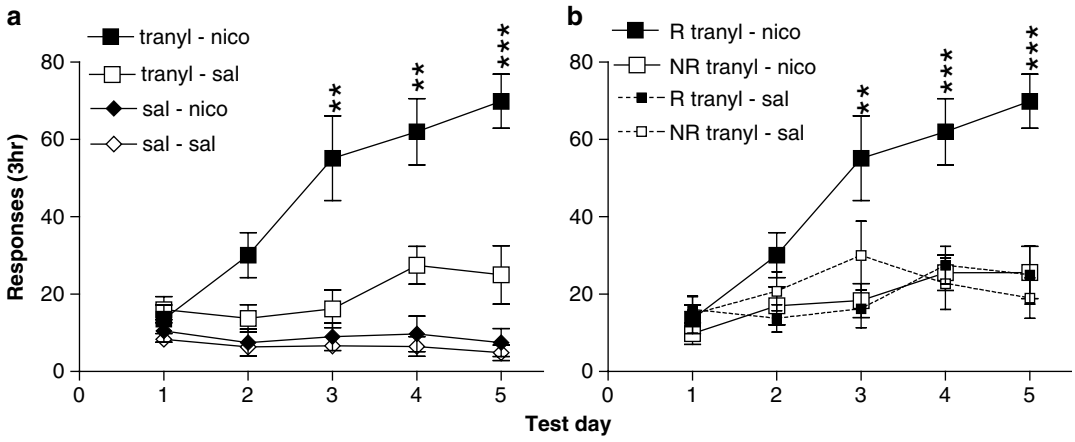

Figure 5 Effect of tranylcypromine on nicotine self-administration: naïve animals were tested in daily 3-h self-administration sessions on an FR1 schedule. The mean (±SEM) total responses are plotted daily for each treatment group. (a) Reinforced responding for either nicotine (10 μg/kg/injection) or saline, in rats pretreated with saline or tranylcypromine (3 mg/kg, i.p.). Rats pretreated with tranylcypromine and having access to i.v. injection of nicotine (10 μg/kg/injection) self-injected significantly more than rats pretreated with tranylcypromine having access to intravenous injection of saline or those pretreated with saline $^{**}P<0.05$, $^{***}P<0.001$ *vs* tranyl–sal; $N=7$–15 per group. (b) Nonreinforced and reinforced responding for nicotine in rats pretreated with tranylcypromine. Groups of rats shown in (a), that self-administered nicotine or saline after a pretreatment by tranylcypromine are included for comparison (R line). The NR line shows nose-pokes at the nonreinforced hole for the animals selfadministering nicotine (NR tranyl–nico) or saline (NR tranyl–sal). Reinforced responding for nicotine was significantly greater than nonreinforced responding. $^{**}P<0.05$, $^{***}<0.001$ *vs* NR tranyl–nico; $N=7$–15 per group.

locomotor response to nicotine. This strongly suggests that the locomotor response to nicotine also necessitates an increase in the extracellular levels of monoamines, an increase that nicotine alone cannot sustain. Interestingly, when MAO activities in the brains of tobacco smokers were analyzed, both MAO-A and -B were found to be decreased (Fowler *et al* 1996a, b).

Further experiments performed in our laboratory have indicated that pretreatment with D-amphetamine which, as mentioned earlier, also stimulates locomotor activity but is 5000-fold less potent than tranylcypromine as a MAOI (Zirkle and Kaiser, 1964), does not induce any locomotor response to nicotine (AS Villégier *et al*, unpublished data). This indicates, first that the stimulatory locomotor effect of MAOIs cannot explain their induction of a locomotor response to nicotine and, second, that increases in extracellular levels of noradrenaline or dopamine (Darracq *et al*, 1998) induced by D-amphetamine are not sufficient to produce a nicotine locomotor response. Indeed, microdialysis experiments have shown that it is specifically the facilitation of serotonergic transmission by MAOIs which is related to the increase in locomotor activity observed with nicotine (AS Villégier *et al*, unpublished data).

It is interesting to note that harmane and norharmane, two MAOIs known to be present in tobacco (Poindexter and Carpenter, 1962), have no effect on nicotine locomotor response under our experimental conditions. This can be explained if one considers that they are both reversible MAOIs and block either MAO-A or -B (see Figure 1a). This suggests that, among the 3000 compounds contained in tobacco, harmane and norharmane probably do not contribute to the higher addictive effects of tobacco when compared with nicotine alone. Another MAOI, acetaldehyde, is very abundant in tobacco and tobacco smoke and was recently shown to enhance nicotine self-administration in adolescent rats (Lefèvre, 1989; Bates *et al*, 1999; Belluzzi *et al*, 2005). This compound may be a good candidate as a MAOI which facilitates the rewarding effects of tobacco.

Why Does Nicotine Induce Locomotor Hyperactivity in Rats and not in Mice ?

Although weak, nicotine exhibits a locomotor stimulant effect in rats and also, following repeated administration, behavioral sensitization (Vezina *et al*, 1994; Nisell *et al*, 1996; Villégier *et al*, 2003). We show here that, in mice, MAOI pretreatment is necessary to obtain an acute locomotor response to nicotine. One reason for this difference could be that MAO activity differs between rats and mice.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin that induces a neurodegeneration of nigrostriatal dopaminergic neurons in mice and Parkinson's disease in man (Gerlach and Riederer, 1996). It has been shown that MPTP induces neurodegeneration through one of its toxic metabolites, 1-methyl-4-phenylpyridine (MPP+). It is MAO-B which converts MPTP to MPP+ and the inhibition of MAO-B by deprenyl prevents the neurotoxic effects of MPTP (Heikkila *et al*, 1984; Langston *et al*, 1984; Fuller *et al*, 1988). Moreover, mice deficient in MAO-B do not sustain damage to striatal dopaminergic terminals after MPTP injection (Grimsby *et al*, 1997). Interestingly, rats have been reported to be at least partly resistant to the neurotoxic effects of MPTP (Mokry, 1995). This suggests that MAOs are less efficient in rats than in mice, thus allowing nicotine effects on monoamine extracellular levels, such as those on DA utilization or release (Tassin *et al*, 1992; Vezina *et al*, 1992; Di Chiara, 2000), to be maintained longer. MAO inhibition by irreversible MAOIs would induce a long-lasting increase in extracellular monoamines levels in mice.

Implication of β2 nAChR in Locomotor Effects Induced by Nicotine

To date, 12 neuronal nAChR subunits have been cloned in mammals, eight of which (α3-7, β2-4) are expressed in rat dopaminergic neurons (Le Novere *et al*, 1996; Charpantier

et al, 1998; Klink *et al*, 2001; Champtiaux *et al*, 2002). Three main types of heteromeric nAChRs ($\alpha4\beta2$, $\alpha6\beta2$, and $\alpha4\alpha6\beta2$) have been identified in dopaminergic terminal fields, whereas (non$\alpha6$)$\alpha4\beta2$ nAChRs represent the majority of functional heteromeric nAChRs on dopaminergic neuronal somata and dendrites (Champtiaux *et al*, 2003). Homomeric $\alpha7$ and heteromeric $\alpha4\beta2$ nAChRs, located in the ventral tegmental area (VTA) on glutamatergic and GABAergic neurons, respectively, have also been proposed to control the electrophysiological response of mesencephalic dopaminergic neurons to nicotine (Mansvelder and McGehee, 2000; Mansvelder *et al*, 2002).

$\beta2$-nAChR-KO mice exhibited an enhanced locomotor response to saline and tranylcypromine when compared with WT mice, thus confirming the hyper-reactivity to stress and drugs of abuse previously described in this strain (Villégier *et al*, 2004). However, nicotine-induced locomotor stimulation was not observed in $\beta2$-nAChR-KO mice pretreated with tranylcypromine. This was not due to a ceiling effect. Although comparable locomotor responses were obtained with tranylcypromine (10 mg/kg) in $\beta2$-nAChR-KO mice and tranylcypromine (20 mg/kg) in WT mice, nicotine (1 mg/kg) only evoked locomotor activation in the WT mice pretreated with 20 mg/kg tranylcypromine and not in $\beta2$-nAChR-KO mice. Moreover, nicotine induced a locomotor inhibition in $\beta2$-nAChR-KO mice pretreated with tranylcypromine. Altogether, this suggests that the nicotine-evoked locomotor response in WT animals pretreated with tranylcypromine is related to the stimulation of nAChRs containing the $\beta2$ subunit, such as the $\alpha4\beta2$ nAChRs. The inhibition observed in $\beta2$-nAChR-KO mice may be due to a hyperdepolarization block of VTA-dopaminergic neurons caused by the glutamate released by the stimulation of the homomeric $\alpha7$ nAChRs located in the VTA (Mansvelder *et al*, 2002).

Nicotine Self-Administration in Rats Pretreated with Tranylcypromine

To determine whether our data obtained on locomotor responses to nicotine in mice may be related to an eventual rewarding effect of nicotine, we tested the effect of MAOI pretreatment on nicotine self-administration in rats. As previously described, nicotine self-administration is weaker than for other drugs of abuse and often needs facilitation by the use of food restriction or cocaine pretreatment. Since our experiments did not include any of these conditions, nicotine was not self-administered in saline-pretreated rats, as has been shown previously (Belluzzi *et al*, 2005). However, when pretreated with tranylcypromine, rats exhibited a robust self-administration, strongly suggesting that MAOIs facilitate the effects of nicotine on the reward system. Tranylcypromine also increased the global activity of the animals as seen by increased saline self-injections. However, the significant difference observed between the reinforced and the nonreinforced hole indicates that MAOIs specifically stimulate nicotine-seeking behaviour.

CONCLUSION

Pretreatment of mice with irreversible and mixed MAOI(s) facilitates nicotine-induced locomotor activation. The absence of a locomotor response to nicotine in $\beta2$-nAChR-KO mice confirms the involvement of nAChRs containing $\beta2$ subunits in the locomotor effects observed in the presence of MAOIs in WT mice. Finally, the facilitation of nicotine self-administration by MAOI pretreatment strongly indicates that the MAOIs present in tobacco and tobacco smoke act in synergy with nicotine to induce addiction. The utilization of MAOIs in experimental models of reward may therefore provide a more complete scheme of the addictive properties of tobacco.

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