Transient behavioral sensitization to nicotine becomes long-lasting with monoamine oxidases inhibitors

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Abstract

Drugs of abuse, such as D-amphetamine or nicotine, are generally considered as acting through an increased release of dopamine in a subcortical structure, the nucleus accumbens, thus inducing locomotor hyperactivity in rats. Following repeated treatments, the same drugs induce a progressive increase in locomotor response called behavioral sensitization. This process has been suggested to play a role in the acquisition and maintenance of addictive behaviors. Here we show that whereas behavioral sensitization to D-amphetamine (0.5 and 0.75 mg/kg) stays constant following three consecutive periods of withdrawal (15, 30 and 30 days), the same experimental conditions completely abolish behavioral sensitization to 0.3 and 0.5 mg/kg nicotine. Indeed, following these periods of withdrawal, locomotor responses to nicotine are identical to those obtained at the first nicotine injection or after repeated saline injections.

However, when a monoamine oxidases inhibitor (MAOI), tranylcypromine (3 mg/kg) or pargyline (30 mg/kg), is co-injected with nicotine, behavioral sensitization is maintained despite submission of the animals to the same withdrawal experimental design. Since tobacco smoke is known to contain many compounds including MAOIs, our data suggest that addictive properties of tobacco may not be limited to nicotine. We propose that MAOIs potentiate effects of nicotine on monoamines release.

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1. Introduction

Tobacco is probably one of the most abused reinforcing agents in humans, and its motivating power is supported by the difficulty encountered when a smoker attempts to quit (Balfour et al., 2000). Although the mechanisms underlying addiction to tobacco are not completely understood, it is generally admitted that nicotine is the major addictive compound contained in tobacco smoke (Balfour et al., 2000; Dani and Heinemann, 1996; Di Chiara, 2000). Nevertheless, clinical studies show that abusers of pure nicotine, i.e., of nicotine isolated from tobacco, do not exist. Furthermore, nicotine administration only incompletely ameliorates withdrawal symptoms and does not prevent a high rate of relapse (Pierce and Gilpin, 2002). In addition, denicotinized cigarettes were found to reduce craving and withdrawal signs, strongly suggesting that agents other than nicotine are involved in tobacco addiction (Pickworth et al., 1999).

Addictive effects of drugs of abuse, such as psychostimulants, opiates and possibly nicotine, have been related to an increased meso-limbic dopaminergic transmission, thus inducing locomotor hyperactivity in rodents (Di Chiara and Imperato, 1988). Moreover, following repeated treatments, these drugs also induce a behavioral sensitization, i.e., an increase in locomotor response, a phenomenon that stays constant even after withdrawal (Robinson and Becker, 1986). This process has been suggested to play a role in the acquisition and maintenance of addictive behaviors (Robinson and Becker, 1986), the long-lasting nature of behavioral sensitization being correlated with relapse and craving observed in humans even after long withdrawal periods (Robinson and Berridge, 1993).

Actually, there is some behavioral and biochemical indications suggesting that nicotine may act differently from other drugs of abuse. For example, although psychostimulants and opiates induce locomotor hyperactivity both in rats and mice, nicotine generally fails to do so in mice (Marks et al., 1983). Moreover, unlike other drugs of abuse, nicotine desensitizes meso-limbic dopaminergic transmission following repeated treatments (Vezina et al., 1992; Pidoplichko et
al., 1997), and the dopaminergic nature of locomotor hyperactivity and behavioral sensitization induced in rats by nicotine is still a matter of debate (Vezina et al., 1994; Boye et al., 2001).

For all these reasons, we have tested more thoroughly the behavioral effects of nicotine and compared these data with those obtained with a major addictive psychostimulant, d-amphetamine. First, kinetics of locomotor responses were analyzed after acute and repeated treatments with each compound. Then, animals were submitted to three consecutive periods of withdrawal to test maintenance of behavioral sensitization. Finally, because tobacco smoke is known to contain a number of compounds among which monoamine oxidases inhibitors (MAOIs) have been the focus of special interest (Poindexter and Carpenter, 1962; Breyer-Pfaff et al., 1996; Rommelspacher et al., 2002), the effects on the maintenance of behavioral sensitization to nicotine of two MAOIs, tranylcypromine and pargyline, were tested.

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats (Iffa-Credo, Lyon, France) weighing 240–260 g on arrival were maintained on a 12 h light/12 h dark cycle (light on between 7 a.m. and 7 p.m.) 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 one week before the experiments. Rats 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.

2.2. Drugs

(−)-Nicotine hydrogen tartrate, tranylcypromine hydrochloride, pargyline hydrochloride and d-amphetamine sulfate were from Sigma Aldrich (France). They were dissolved in saline (NaCl, 0.9%). The solutions’ pHs were adjusted to 7.4 with NaOH. Doses are expressed as salts for tranylcypromine, pargyline and d-amphetamine and as base for nicotine. Nicotine was injected subcutaneously (0.5 ml per injection per rat), whereas tranylcypromine, pargyline, d-amphetamine and saline were injected intraperitoneally (1 ml per injection per rat).

2.3. Estimation of locomotor activity

Locomotor activity was estimated in a bank of eight circular corridors (14 cm wide, 70 cm long) constructed with opaque plastic as previously described (Tassin et al., 1978). Four photocells positioned 3.5 cm above the floor and evenly spaced allowed to estimate horizontal locomotion, each interruption of photocell beam being detected and recorded via an electrical interface to a computer. Data were acquired using Imetronic software (Bordeaux, France).

Rats were habituated to the corridors for a 60-min period and received a pretreatment of saline. Thirty minutes later, they received a treatment with saline or drug, and locomotor activity was measured every 10 min during 60 min.

2.4. Induction of behavioral sensitization

Animals were submitted to five drug injections every other day. Then rats were left for four days in their home cage before receiving a test injection to quantify their level of sensitization. Each rat in each group received the same pretreatment (saline, tranylcypromine or pargyline) and drug treatment (saline or nicotine) during this period and during the three challenges described in the next paragraph.

2.5. Determination of maintenance of behavioral sensitization

Once rats were sensitized, each animal received three challenge injections 15, 45 and 75 days after the test injection, and locomotor activities were recorded, except for behavioral sensitization induced by 0.3 mg/kg nicotine where only 15 and 45 days (C1 and C2) challenge injections were performed.

2.6. Statistics

Results presented are means ± S.E.M. of data obtained with 8–16 animals. Data were subjected to analysis of variance (ANOVA) using GraphPad software, Prism 3.0 (San Diego, CA) for evaluation of main effects and interaction between time and treatment. Where a significant interaction was found (P < .05), subsequent comparisons between means were made using Student’s t test.

3. Results

3.1. Effects of acute and repeated injections of d-amphetamine and nicotine on locomotor activities

As expected, both acute d-amphetamine (0.5 and 0.75 mg/kg) and nicotine (0.3–0.8 mg/kg) injections induce locomotor hyperactivity in rats (Fig. 1A) (P = .019 and P < .001 for 0.5 and 0.75 mg/kg d-amphetamine, respectively, and P = .0098, P < .0001 and P = .0115 for 0.3, 0.5 and 0.8 mg/kg nicotine, respectively, when compared with saline-treated animals, Student’s t test). No difference in locomotor responses was observed between 0.5 and 0.8 mg/kg nicotine injections [F(1,126) = 0.03, P = .86, two-way ANOVA]. Time–response curves indicate that peaks of activity occurred in the first 5 min with nicotine and at 30 min with d-amphetamine (Fig. 1B).
Following repeated injections (see Materials and Methods), both D-amphetamine (0.5 and 0.75 mg/kg) and nicotine (0.3 and 0.5 mg/kg) induced increased locomotor responses on the test day when compared to locomotor hyperactivity obtained at the first injection (Fig. 1B) \[ F(1,72) = 13.63, P = .0004, \] and \[ F(1,336) = 51.65, P < .0001, \] for 0.5 and 0.75 mg/kg D-amphetamine, respectively; and \[ F(1,132) = 31.43, P < .0001, \] and \[ F(1,198) = 135.9, P < .0001, \] for 0.3 and 0.5 mg/kg nicotine, respectively. For 0.8 mg/kg nicotine, there was a significant difference between locomotor responses after acute and repeated injections \[ F(1,60) = 14.93, P = .0003, \] but locomotor responses with 0.8 mg/kg repeated nicotine injections were significantly lower than those after 0.5 mg/kg repeated injections \[ F(1,132) = 7.508, P = .007. \] For each drug, time–response curves of locomotor hyperactivities were similar for acute and repeated injections. However, as previously shown (Drouin et al., 2002), repeated injections of saline induced an enhanced response \[ F(1,108) = 18.90, P < .0001 \] due to an increased reactivity in the first 10 min \( (P < .01, \) post hoc Student’s t test) (Fig. 1B, top left). When 0.8 mg/kg injections of nicotine were repeated, they induced seizures in about 20% of the animals as soon as the third administration. This may explain why locomotor responses following repeated injections were very variable (Fig. 1B, bottom right). Further experiments were therefore only performed with 0.3 and 0.5 mg/kg nicotine.

### 3.2. D-amphetamine and nicotine induce, respectively, long- and short-lasting behavioral sensitization

Once behavioral sensitization developed, animals received three challenge injections (C1, C2 and C3) depending on their experimental group (0.5 or 0.75 mg/kg D-amphetamine or 0.3 or 0.5 mg/kg nicotine) 15, 45 and 75 days after

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**Fig. 1.** Kinetics of rat locomotor responses to acute and repeated D-amphetamine or nicotine injections. Animals were first introduced in the locomotor apparatus for 60 min, then injected with saline and 30 min later injected with different doses of either D-amphetamine or nicotine before locomotor responses were recorded for a further 60 min. Comparisons between locomotor responses following acute or repeated injections of saline, D-amphetamine or nicotine:

- *P < .05; **P < .01; ***P < .001, repeated, significantly different from corresponding acute, Student’s t test.

N = 10 animals per group.
the test injection. Fig. 2 indicates that D-amphetamine-sensitized rats maintained their level of locomotor activity measured on test day at each challenge injection \[ F(1,12) = 0.09, P = .92 \] (C1); \[ F(1,12) = 0.18, P = .85 \] (C2); \[ F(1,12) = 0.499, P = .626 \] (C3); and \[ F(1,64) = 0.07, P = .93 \] (C1); \[ F(1,64) = 0.13, P = .85 \] (C2); \[ F(1,41) = 0.499, P = .626 \] (C3), when compared with test day for 0.5 and 0.75 mg/kg D-amphetamine, respectively. In contrast, nicotine-sensitized rats decreased regularly their locomotor hyperactivity [633.6 ± 114.3 (test day) vs. 431.3 ± 53.6 (C1), \[ F(1,11) = 1.8, P = .09 \] vs. 198.1 ± 13.2 (C2), \[ F(1,11) = 4.8, P = .0005 \] for 0.3 mg/kg nicotine and 769.9 ± 66.2 (test day) vs. 598.7 ± 42.7 (C1), \[ F(1,33) = 2.14, P = .039 \] vs. 275.9 ± 31.1 (C3), \[ F(1,26) = 5.3, P < .0001 \] for 0.5 mg/kg nicotine]. In addition, locomotor activities observed at C2 for 0.3 mg/kg nicotine and at C3 for 0.5 mg/kg nicotine were not significantly different from that obtained at the first corresponding injection of nicotine \[ F(1,14) = 0.75, P = .46 \] and \[ F(1,16) = 0.6737, P = .5101 \] for 0.3 and 0.5 mg/kg nicotine, respectively. Because of the return to basal locomotor response, no C3 was performed for the 0.3 mg/kg nicotine treatment.

Effects of a MAOI, tranylcypromine, were then tested on the duration of behavioral sensitization to 0.5 mg/kg nicotine.

### 3.3. Effects of the co-administration of tranylcypromine with nicotine on behavioral sensitization to nicotine

The co-administration of a MAOI, tranylcypromine (3 mg/kg), with nicotine (0.5 mg/kg) did not modify the development of behavioral sensitization, as shown on Fig. 3 [locomotor activities on day 1 and on test day were not significantly different between nicotine- and nicotine + tranylcypromine-treated animals, \[ F(1,192) = 0.0374, P = .847 \] ]. However, tranylcypromine + nicotine-sensitized rats maintained their level of locomotor activity from test injection to last challenge without significant difference [696.4 ± 62.3 (test day) vs. 560.3 ± 59.3 (C1), \[ F(1,30) = 1.58, P = .12 \] vs. 575.0 ± 68.3 (C2), \[ F(1,30) = 1.31, P = .19 \], vs. 548.7 ± 47.6 (C3), \[ F(1,30) = 1.88, P = .069 \] ]. In addition, it was verified that a significant locomotor difference was obtained in the last challenge (C3) between nicotine and tranylcypromine + nicotine treatments \[ F(1,24) = 4.169, P = .0003 \].

Complementary experiments indicated that repeated injections of tranylcypromine alone did not induce any
Results indicate that about half of the behavioral sensitization to 0.5 mg/kg nicotine and 85% of the behavioral sensitization to 0.3 mg/kg nicotine is lost after 3 weeks. If one considers that the challenge injection of nicotine reactivates behavioral sensitization, this time may even be shorter. In contrast, behavioral sensitization to very low or low doses of d-amphetamine is obviously long-lasting because it is not modified by the same three periods of withdrawal. Long-lasting behavioral sensitization to psychostimulants has been clearly demonstrated (Henry and White, 1995; Robinson and Kolb, 1997). In the case of nicotine, studies however did not analyze behavioral sensitization later than three weeks after withdrawal. They described either a complete extinction (Ksir et al., 1985) or some remaining effects (Miller et al., 2001; Schoffelmeer et al., 2002). These differences may be related to the doses of nicotine or sensitization procedures. In any case, our data indicate that differences between sensitizations to d-amphetamine and nicotine are not linked to the amplitude of the locomotor response to either drug, since locomotor activities after acute or repeated administrations of 0.5 mg/kg d-amphetamine or 0.3 mg/kg nicotine are similar, respectively.

Interestingly, following two or three consecutive periods of withdrawal to 0.3 or 0.5 mg/kg nicotine rat locomotor responses to nicotine are not different from those of animals having received repeated injections of saline. This is also true when animals repeatedly injected with saline receive nicotine for the first time. This latter point indicates that the lack of a response to nicotine is not due to a tolerance to nicotine in nicotine-pretreated rats, but rather suggests that both repeated nicotine and repeated saline injections induce locomotor responses at least partly through a common neurochemical pathway that may implicate noradrenergic neurons as shown for repeated saline injections by Drouin et al. (2002). The striking similarity between curves of locomotor responses obtained with repeated saline injections (Fig. 1B, up-left) and with repeated 0.3 mg/kg nicotine (Fig. 1B, down-left) suggests that the activation by nicotine of noradrenergic neurons (Tung et al., 1989; Mitchell, 1993) amplifies effects of repeated saline injections when behavioral sensitization develops.
4.2. Co-administration of MAOIs with nicotine induces a long-lasting behavioral sensitization

Co-administration of tranylcypromine with 0.5 mg/kg nicotine did not modify the development of behavioral sensitization induced by nicotine alone. However, following three challenges, in contrast to that observed with nicotine alone, locomotor responses did not decrease significantly when compared to the test experiment, although a trend towards a decrease occurred at the first challenge. In addition, it was verified that repeated treatments with tranylcypromine alone did not induce either behavioral sensitization or cross-sensitization to nicotine, indicating that effects observed are due to a synergy between both compounds.

Tranylcypromine is an irreversible mixed inhibitor of monoamine oxidases A and B that was however recently described in in vitro experiments as being also an inhibitor of CYP2A6, the principle enzyme metabolizing nicotine to its inactive metabolite cotinine (Zhang et al., 2001). To verify that effects observed in presence of tranylcypromine were indeed due to MAOI’s effects, we have co-administered with nicotine another MAOI, pargyline, which is a selective MAO B inhibitor and becomes MAOI A and B for higher doses (Johannessen et al., 1989). Data obtained in presence of pargyline are almost superimposable to those observed with tranylcypromine, including the slight decrease in locomotor response at the first challenge injection. Altogether, addition of a MAOI to nicotine does not modify the development of behavioral sensitization but increases the duration of its maintenance.

4.3. Why is behavioral sensitization to nicotine transient?

Behavioral sensitization is thought to be one of the early manifestations of neural plasticity associated with chronic administration of a drug of abuse. Although the biochemical basis of this enhanced responsiveness is still not clear, sensitization is believed to be associated with augmented intensity of subcortical dopaminergic transmission with repeated administrations (White and Kalivas, 1998). This increased dopamine release and its effects on D1 receptors would induce long-lasting changes in synaptic efficacy and structural synaptic modifications possibly responsible for associative learning in addiction (Berke and Hyman, 2000).

Actually, nicotine is known to increase dopamine transmission in the nucleus accumbens when given acutely (Di Chiara and Imperato, 1988; Vezina et al., 1992). However, following repeated injections, nicotine effects appear more controversial; nicotine-induced stimulation of dopamine release in the nucleus accumbens core was found to be increased when compared to acute injection, while the response in the shell was reduced (Cadoni and Di Chiara, 2000). On the other hand, repeating nicotine injections completely suppressed the effect observed with an acute injection on the rate of dopamine utilization in the core of the nucleus accumbens (Vezina et al., 1992). These discrepancies may question the functional significance of increased extracellular dopamine levels or increased electrically evoked release of [(3)H]dopamine from nucleus accumbens slices shown by Schoffelmeer et al. (2002) after repeated nicotine administrations. It must indeed be recalled that bilateral intra-nucleus accumbens nicotine induces an increase in extracellular dopamine levels not associated with an increased locomotor response (Ferrari et al., 2002). This local effect of nicotine is likely due to the stimulation of presynaptic receptors located on glutamatergic (Marchi et al., 2002) and dopaminergic (Giorguieff-Chesselet et al., 1979; Sorenson et al., 1998) nerve terminals. Similar observations were done with d-amphetamine that did not induce locomotor responses when injected bilaterally into the nucleus accumbens despite an important d-amphetamine-induced release of dopamine (Darracq et al., 1998, 2001; Auclair et al., 2002). After that line, to obtain a release of dopamine in the nucleus accumbens associated with a locomotor response, i.e., a functional release of dopamine, an increased activity of VTA dopaminergic neurons may be necessary. In other words, increased extracellular dopamine levels or increased electrically evoked release of [(3)H]dopamine by repeated nicotine may be due to the stimulation of nicotinic presynaptic receptors and not related to functional responses.

Up to now, an increased electrical activity of dopaminergic neurons has been described in anesthetized animals following an acute treatment with nicotine (Erhardt et al., 2002), but nothing is yet known about the development of such responses in awake animals repeatedly injected with nicotine. Altogether, it is far from certain that behavioral sensitization observed with nicotine corresponds to an increased activity of dopaminergic neurons. Indeed, although this finding was made controversial by Louis and Clarke (1998), Vezina et al. (1994) found a behavioral sensitization to nicotine after dopaminergic denervation of the nucleus accumbens. It is therefore tempting to propose that behavioral sensitization to nicotine is short-lasting because repeated nicotine induces only a weak increased activity of meso-limbic dopaminergic neurons, thus hampering the long-term memorization of environmental cues generally attributed to an increased dopaminergic transmission (White and Kalivas, 1998; Berke and Hyman, 2000).

4.4. How can MAOIs increase duration of behavioral sensitization to nicotine?

Monoamine oxidases A and B catalyze the oxidative deamination of exogenous and endogenous bioamines in brain and peripheral tissues. It is considered that reuptake is the main pathway responsible for monoamines clearance in the brain, but experiments performed recently by Wayment et al. (2001) have indicated that monoamine oxidases have a critical role, especially with regards to dopamine clearance in the prefrontal cortex. MAOIs are also likely to decrease
clearance of cortical norepinephrine. As already mentioned, nicotine is a potent activator of noradrenergic neurons located in the locus coeruleus and induces release of norepinephrine in different brain structures (Mitchell, 1993). It is therefore possible that nicotine, in the presence of MAOIs and because of the coupling in prefrontal cortex of noradrenergic and dopaminergic systems (Darracq et al., 1998), increases dopaminergic transmission in subcortical structures. The stimulation by norepinephrine of α1b-adrenergic receptors in the prefrontal cortex was indeed shown to be necessary to obtain a functional release of dopamine in the nucleus accumbens (Darracq et al., 1998; Auclair et al., 2002). Finally, systemic nicotine also increases serotonin release in most of the forebrain structures (see review by Seth et al., 2002). There again, a coupling between serotonergic and dopaminergic systems may also occur (Lucas and Spampinato, 2000) and increase dopamine release in subcortical structures. Altogether, nicotine acutely increases the release of the three monoamines, and the presence of MAOIs is likely to trigger through increased noradrenergic and serotonergic transmissions an increased activity of meso-limbic dopaminergic neurons. This would occur even after repeated injections of nicotine when MAOIs are present and thus allow for a long-lasting behavioral sensitization.

4.5. MAOIs and behavioral sensitization to nicotine in the context of tobacco addiction

Interestingly, many authors have found that tobacco smokers, when compared to nonsmokers, had reduced MAO activities that can reach 40% decreases (Oreland et al., 1981; Yu and Boulton, 1987; Berke et al., 1995; Fowler et al., 1996). This suggests, as mentioned previously, that tobacco smoke contains MAOIs. Inhibition of monoamine oxidases by tobacco smoke was however shown not to be related to nicotine (Carr and Basham, 1991), but to other compounds also present in other psychotropic plants (Uebelhack et al., 1998; Rommelspacher et al., 2002). Our data indicate that behavioral effects of nicotine are transient and insufficient to induce long-term behavioral sensitization in absence of MAOIs. It is therefore possible that the presence of MAOIs is necessary for tobacco to be addictive. If this hypothesis is correct, the extraction of MAOIs would suppress, or at least greatly diminish, addictive properties of tobacco and consequently help individuals quit.

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