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Chronic intermittent nicotine treatment dose-dependently alters serotonergic neurons response to citalopram in the rat

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Abstract

Acetylcholine nicotinic systems and serotonergic systems are known to interact. In rodents, acute and chronic nicotine treatments have consequences on several aspects of the activity of dorsal raphe serotonin (DRN 5-HT) neurons. One hypothesis is that states of functioning of DRN 5-HT neurons (firing rate and sensitivity) vary as a function of nicotine dose and mode of administration during chronic nicotine treatment. In the present study, the firing rate and sensitivity of DRN 5-HT neurons were investigated using single (0.5 and 1 mg/kg) or multiple (3 injections of 0.7 mg/kg) daily injections of nicotine over 10 days. The sensitivity of neurons was tested by the cumulative dose of the selective serotonin reuptake inhibitor citalopram necessary to inhibit their firing. The activity of neurons was tested during treatment, and then 24 and 48 h after nicotine withdrawal. The results show that, on day 10, DRN 5-HT neurons were desensitized (reduced response to citalopram) after chronic single daily injection treatments with the high dose of nicotine (1 mg/kg), while their sensitivity remained unaltered after single daily injections with the low dose (0.5 mg/kg), and after the multiple daily injection paradigm. None of the treatments altered the firing rate of DRN 5-HT neurons. The dose-dependent and time-dependent alterations of serotonergic neurons sensitivity after chronic nicotine treatments are likely the consequences of long-term adaptations of nicotinic receptors. The desensitization of DRN 5-HT neurons after chronic single daily injections of 1 mg/kg of nicotine suggests an antidepressant-like effect of chronic nicotine.

Keywords: Tobacco; Citalopram; Antidepressant; Sensitivity; Firing

1. Introduction

Studies have shown that acetylcholine nicotinic systems interact with serotonergic systems (Seth et al., 2002). Since chronic smoking is characterized by affective disturbances and serotonergic systems have been involved in the affective dimension of smoking (Malone et al., 2003), it is of interest to investigate the interactions between nicotine and serotonergic systems. In rodents, acute and chronic nicotine treatments have consequences on several aspects of the activity of dorsal raphe serotonin (DRN 5-HT) neurons. Acute administration of nicotine increases brain 5-HT release (Ribeiro et al., 1993) and inhibits dorsal raphe nucleus serotonergic neurons (Engberg et al., 2000; Touiki et al., 2005). We have recently shown that the inhibition of DRN 5-HT neurons by nicotine is blocked by the nicotine receptor antagonist mecamylamine, which demonstrates that this inhibition is mediated by nicotine receptors (nAChRs) (Touiki et al., 2007). After nicotine withdrawal, DRN 5-HT neurons become progressively hypersensitized (Rasmussen and Czachura, 1997). According to Rasmussen and Czachura (1997), the sensitivity of DRN 5-HT neurons is not altered during chronic administration of nicotine, but only after withdrawal. Nicotine withdrawal in smokers is associated with an aversive withdrawal syndrome, which includes irritability, anxiety and, sometimes, depressed mood (Hughes and Hatsukami, 1986). While nicotine dependency appears to be related to an effect of nicotine on the activity of brain dopaminergic systems (Corrigall et al., 1994; Pich et al., 1997), symptoms like irritability, anxiety and mood alterations are more likely to be related to alteration of the functioning of serotonergic systems (Lucki, 1998).

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In their study, Rasmussen and Czachura (1997) used osmotic minipumps to administer nicotine. This means that, in their study, nicotine was delivered continuously, failing to reproduce the smoking situation, where peaks and troughs of blood nicotine alternate. It has been shown that both continuous delivery of nicotine (through osmotic pumps) and intermittent delivery (multiple daily injections) upregulate brain nAchRs, with the effects being higher and more widespread with continuous delivery (Ulrich et al., 1997). Successive states of desensitization and resensitization of brain nAchRs in smokers, related to an intermittent action of nicotine, and to nicotine withdrawal may be a key element in understanding the mechanisms of tobacco abuse and of smoking treatment approaches (Zevin and Benowitz, 2000). It is not known whether chronic intermittent administration of nicotine produces successive states of desensitization/resensitization of DRN 5-HT neurons. In electrophysiological studies, states of sensitivity of DRN 5-HT neurons can be evaluated by the response of neurons to an acute challenge by 5-HT reuptake inhibitors (Hyttel, 1982; Maudhuit et al., 1996, 1997) or 5-HT_{1A} agonists (Jolas et al., 1994).

In the present study, we investigated the response of DRN 5-HT neurons to an acute challenge by citalopram, a 5-HT reuptake inhibitor, during chronic intermittent nicotine administration followed by nicotine withdrawal. To achieve this goal, we treated rats with single or multiple daily injections of various doses of nicotine and we studied the sensitivity of DRN 5-HT neurons immediately before nicotine withdrawal, and then 24 h and 48 h after withdrawal.

2. Materials and methods

2.1. Animals

The experiments were performed in Sprague–Dawley rats (IFFA CREDO, France). The animals were housed in groups of four per cage under standard laboratory conditions (temperature 21 °C and lights on from 8AM to 8PM). Animals had free access to standard food pellets (A04 UAR, France) and water. Animals were weighed every day during the experiments, and their behavior roughly assessed. All experiments were undertaken in compliance with the directives of the European Community and French law on animal experimentation.

2.2. Drugs

Nicotine bitartrate and chloral hydrate was purchased from Sigma–Aldrich, France. Citalopram (liquid form) was purchased from Lündbeck, Denmark. Nicotine was dissolved in NaCl 0.9%.

2.3. Treatments

Three experiments were carried out: one chronic experiment using 1 daily injection of nicotine (experiment 1), one chronic experiment using 3 daily injections of nicotine (experiment 2) and one acute experiment (experiment 3). Chronic treatments lasted 10 days. Treatments consisted of intraperitoneal (i.p.) injections of nicotine bitartrate or of the control solution. Nicotine bitartrate contains one third nicotine base, and the doses mentioned for the following experiments are expressed in terms of nicotine base.

2.3.1. Experiment 1

Ten groups of 8 rats were tested using a single daily injection of nicotine (or the control solution) at 9AM. Groups 1–2 received 0.5 mg/kg of nicotine, group 3–5 received 1 mg/kg of nicotine, groups 6–8 received the control solution (sham controls) and group 9–10 received one daily injection of the control solution, except on day 10, when group 9 received an injection of 0.5 mg/kg of nicotine and group 10 received an injection of 1 mg/kg of nicotine. Groups 1, 3, 6, 9 and 10 were tested after the last injection on day 10. Groups 2, 4 and 7 were tested 24 h after the last injection (day 11). Groups 5 and 8 were tested 48 h after the last injection (day 12). Electrophysiological experiments always started at 11AM (in groups 1, 3, 6, 9 and 10 [day 10 recordings], the neurons were, on average, recorded 1 to 2 h after the beginning of the experiment, i.e. approximately 3 to 4 h after the 9AM injection of nicotine or control solution).

2.3.2. Experiment 2

Six groups of 8 animals were tested using 3 daily i.p. injections of 0.7 mg/kg of nicotine (2.1 mg/day) or the control solution (sham controls). Injections were made at 9AM, 2PM and 7PM. Group 1 (nicotine) and group 2 (control) were tested on day 10 (neurons were recorded, on average, 3–4 h after the 9AM injection—last injection). Group 3 (nicotine) and group 4 (control solution) were tested 24 h after the last injection. Group 5 (nicotine) and group 6 (control solution) were tested 48 h after the last injection.

2.3.3. Experiment 3

Three additional groups of 8 animals were maintained for 10 days under the same experimental conditions as in the previous experiments, except that the animals received no chronic i.p. injections (naïve controls). These rats were tested on the 10th day, 3 to 4 h after a single injection of 0.5 or 1 mg/kg of nicotine or the control solution. Injections were made at 9AM.

2.4. Electrophysiology

The methods used were the same as those used in a previous publication (Touiki et al., 2005). In summary: The rats were anaesthetized with chloral hydrate (400 mg/kg i.p.). A catheter (Biotrol) was implanted into the left jugular vein for intraveinous injections. Drugs were injected with a microsyringe (Hamilton) through the catheter. The animals were fixed into a stereotaxic frame (typeM, Unimecanique, France). The scalp was opened and a hole was drilled into the skull. A vertical descent was performed with enamelled 3 MΩ tungsten electrodes (Frederick Haer & Co) to the dorsal raphe nucleus, coordinates (according to the Paxinos and Watson, 1986 Atlas): 1–1.5 mm anterior to the interaural line, 0 mm lateral and 4.8–5.5 mm below the surface of the dura.

The serotonergic neurons were identified according to standard criteria (Aghajanian et al., 1972): biphasic action potentials of 2–3 ms duration, slow firing rates (0.5–2.5 spikes/s) and regular rhythm. Electrical signals were amplified using an AC amplifier Neurolog NL104. The signal was digitalized by a CED1401

(Cambridge Electronic Design), recorded on the hard disk of a PC computer using Spike2 data capture programs and then analyzed with programs we developed with the Spike2 analysis program.

The baseline activity of each DRN 5-HT neuron was recorded for at least 5 min prior to intraveinous injections of citalopram. Citalopram was injected at a dose of 0.08 mg/kg at the rate of one injection every 2 min for the four first injections, after which the doses were doubled. Injections were repeated until complete cessation of neuron firing.

After the recordings, a small current (approximately 15 μA for 10 s) was applied through the electrode in order to label the point of injection. The animals were then perfused intracardially with 10% formalin. 60 μm frozen serial sections were cut and stained by the Nissl method, and the localization of the tip of the electrode was verified under a microscope.

2.5. Statistics

Data are expressed as the cumulative dose (mg/kg) of citalopram inhibiting 50% of the basal firing of neurons, or ID50 (means± SEM). Results were analysed by ANOVA (one-way analysis of variance) and, in case of significance, Student's *t*-tests were used to compare the treated groups with their control groups. Differences were considered as significant if $p<0.05$. For more than two groups we used ANOVA and, in case of significance, Fisher's PLSD test for *post hoc* analysis (Statview V4.57 Abacus Software, USA), $p<0.05$ was recognized as statistically significant.

3. Results

3.1. Experiment 1

A single daily injection of 1 mg/kg of nicotine for 10 days produced a significant decrease in the response of DRN 5-HT neurons to citalopram on the last injection day (the cumulative dose of citalopram inhibiting the firing of 5-HT neurons of nicotine-treated rats was significantly greater than that inhibiting the firing of neurons of sham control rats) (Fig. 1). The difference in neuronal response was significant between controls (ID50= 0.053±0.006) and rats treated with 1 mg/kg nicotine (ID50= 0.378±0.10) ($p<0.005$) (Fig. 2a) or with 0.5 mg/kg nicotine (ID50=0.138±0.015) ($p<0.05$).

There was a significant difference between treatment with 1 mg/kg nicotine (ID50=0.378±0.10) and treatment with 0.5 mg/

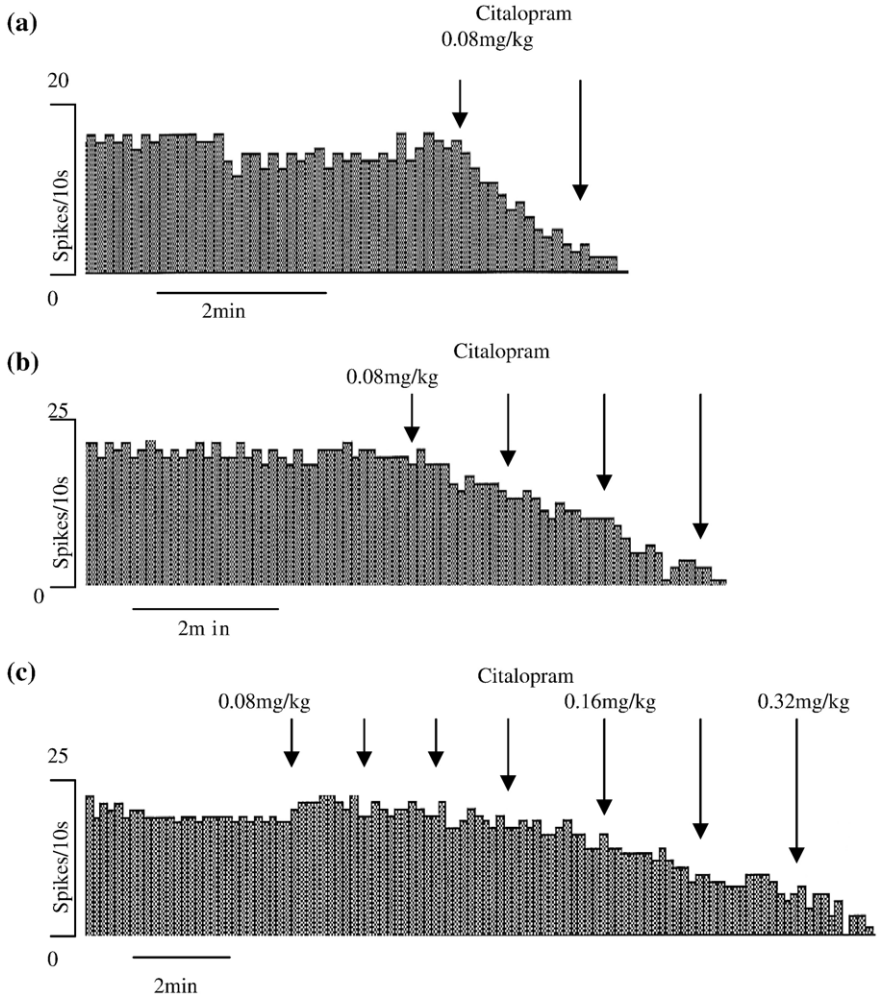

Fig. 1. Examples of integrated firing rate histograms of single DRN 5HT neurons before and during citalopram cumulative administration the last injection day following a 10-day treatment (1 i.p./day) by (a) saline, (b) nicotine 0.5 mg/kg, (c) nicotine 1 mg/kg.

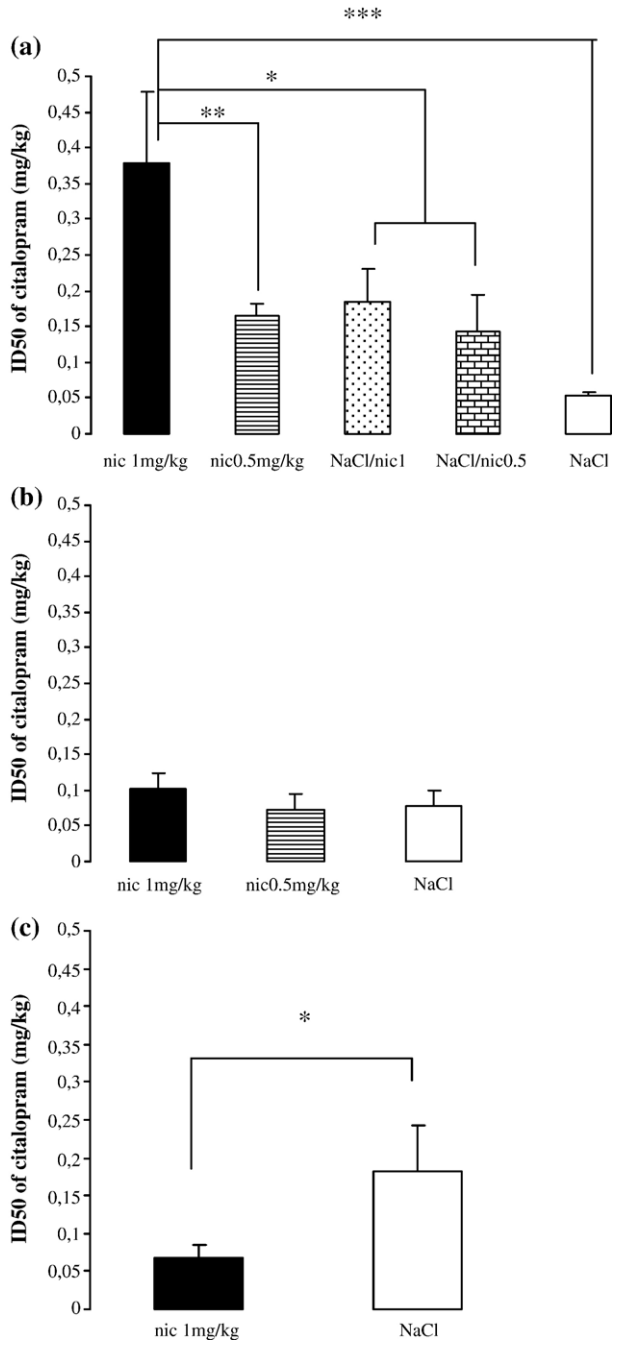

Fig. 2. ID50 (mg/kg) of cumulative citalopram necessary to inhibit completely DRN 5HT neurons the last injection day following a 10-day treatment (1i.p./day): (a) nicotine 1 mg/kg (nic 1 mg/kg); nicotine 0.5 mg/kg (nic 0.5 mg/kg); saline and nicotine 1 mg/kg the last injection day (NaCl/nic1); saline and nicotine 0.5 mg/kg the last injection day (NaCl/nic0.5); chronic saline injections (NaCl). Comparisons betwween the five groups show significant differences between (nic 1 mg/kg) and (nic 0.5 mg/kg), (NaCl/nic0.5), (NaCl/nic1) or (NaCl). F-ratio=4.827 with $k1=4$ and $k2=39$, $\alpha=0.05$. (b) 24 h after withdrawal; F-ratio=0.558 with $k1=2$ and $k2=16$, $\alpha=0.05$. (c) 48 h after withdrawal. F-ratio=7.641 with $k1=1$ and $k2=8$ $\alpha=0.05$. *$p<0.05$; **$p<0.01$; ***$p<0.005$.

kg (ID50=0.138±0.015) ($p<0.01$), and with a single 1 mg/kg or 0.5 mg/kg nicotine injection on day 10 in rats in rats previously chronically treated with the control solution (ID50=0.185±0.046 and ID50=0.133±0.051, respectively) ($p<0.05$) (Fig. 2a).

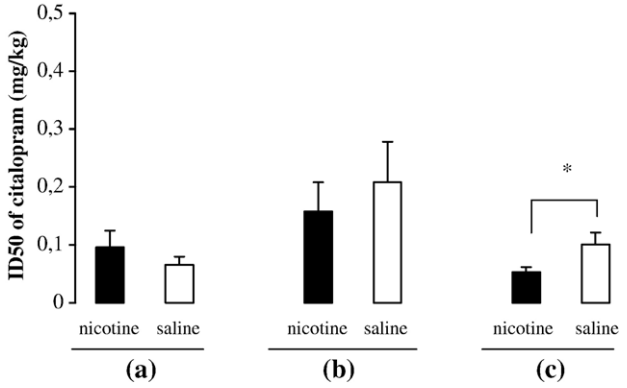

Fig. 3. ID50 (mg/kg) of cumulative citalopram necessary to inhibit completely DRN 5HT neurons in rats following 10-day nicotine treatment using 3 i.p./day (2.1 mg/kg/day) or 10-day chronic saline injection using 3 i.p./day: (a) 3 to 4 h after the last injection (day 10); (b) 24 h after withdrawal (day 11); (c) 48 h after withdrawal (day 12). *$p<0.05$.

A single injection of nicotine (0.5 mg/kg or 1 mg/kg) on day 10 in rats previously chronically injected with the control solution desensitized neurons significantly compared to sham control animals, but significantly less than the chronic treatment with 1 mg/kg nicotine ($p<0.05$) (Fig. 2a: F-ratio=4.642 with $k1=4$ and $k2=39$, $\alpha=0.05$).

On day 11 (24 h after the last injection), no significant difference existed between chronically treated (0.5 or 1 mg/kg) and sham control rats (Fig. 2b: F-ratio=2.165 with $k1=2$ and $k2=15$, $\alpha=0.05$).

On day 12 (48 h after the last injection), rats previously treated with 1 mg/kg of nicotine showed their serotonergic neurons to be significantly sensitized (ID50=0.05±0.005) compared to sham controls (ID50=0.182±0.06) ($p<0.05$) (rats treated with 0.5 mg/kg nicotine were not tested) (Fig. 2c: F-ratio=7.641 with $k1=1$ and $k2=7$, $\alpha=0.05$).

3.2. Experiment 2

After a 10-day treatment with 3 injections per day of 0.7 mg/kg of nicotine, the sensitivity of treated rats' neurons did not significantly differ from that of the controls (Fig. 3a: F-ratio=0.787 with

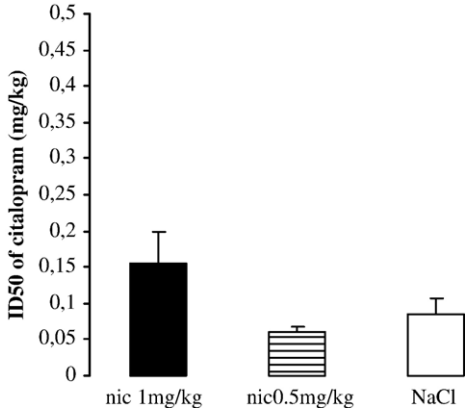

Fig. 4. ID50 (mg/kg) of cumulative citalopram necessary to inhibit completely DRN 5HT neurons in rats following a single i.p. injection of nicotine 1 mg/kg (nic 1 mg/kg), nicotine 0.5 mg/kg (nic 0.5 mg/kg) or saline (NaCl) (no chronic treatment). F-ratio=2.140 with $k1=2$ and $k2=15$, $\alpha=0.05$.

Table 1
Basal firing frequencies (Hz) of DRN 5HT neurons

Treatment	*n*	Basal frequency (Hz) as mean±SEM	*P*	
Control	6	1.23±0.13		
Single IP nicotine 1 mg/kg	6	1.36±0.18	0.6959	*F*-ratio=2.046
Single IP nicotine 0.5 mg/kg	6	1.41±0.29	0.588	*k*1=2; *k*2=15
Control	9	1.39±0.17		
Chronic nicotine 1 mg/kg (1 IP/day)	11	1.43±0.14	0.8342	*F*-ratio=1.109
Chronic nicotine 0.5 mg/kg (1IP/day)	12	1.65±0.12	0.2275	*k*1=4;
Chronic NaCl+last IP nicotine 1 mg/kg	6	1.68±0.20	0.3156	*k*2=39
Chronic NaCl+last IP nicotine 0.5 mg/kg	6	1.46±0.26	0.8482	
Control	6	1.21±0.21		
24 h after withdrawal 1 mg/kg(1 IP/day)	8	1.05±0.14	0.5197	*F*-ratio=1.351
24 h after withdrawal 0.5 mg/kg (1 IP/day)	6	0.99±0.18	0.4395	*k*1=2; *k*2=17
Control	4	1.12±0.18		
48 h after withdrawal 1 mg/kg (1 IP/day)	7	1.11±0.11	0.9629	
Control	6	1.45±0.19		
Chronic nicotine 2.1 mg/kg/day (3 IP/day) (3 to 4 hours after the last injection)	7	1.34±0.19	0.7015	
Control	9	1.01±0.14		
24 h after withdrawal 2.1 mg/kg/day (3 IP/day)	9	0.96±0.13	0.8756	
Control	7	1.05±0.09		
48 h after withdrawal 2.1 mg/kg/day (3 IP/day)	8	1.04±0.10	0.9254	

$k1=1$ and $k2=11$, $\alpha=0.05$). Twenty-four hours later, the sensitivity of the neurons did not differ from that of controls (Fig. 3b: *F*-ratio=0.342 with $k1=1$ and $k2=16$, $\alpha=0.05$). 48 h later, the neurons were significantly sensitized compared to controls ($p<0.05$) (Fig. 3c: *F*-ratio=4.392 with $k1=1$ and $k2=13$, $\alpha=0.05$).

3.3. Experiment 3

None of the acute nicotine injections produced any alteration in the sensitivity of DRN 5-HT neurons (Fig. 4: *F*-ratio=2.140 with $k1=2$ and $k2=15$, $\alpha=0.05$). Therefore, the desensitization of DRN 5-HT neurons in nicotine-treated rats observed in the previous experiments cannot be explained by the last injection made on day 10 because a single injection of nicotine without chronic previous treatment (naïve rats) does not desensitize neurons.

In all experiments, the basal firing rate of DRN 5-HT neurons was measured and no alteration was observed (Table 1). The weight of treated animals remained similar to the controls (data not shown), and none of the treated animal showed any sign of toxicity.

4. Discussion

The results of the present study show that chronic intermittent nicotine treatment in the rat has different effects on DRN 5-HT neurons depending on the dose and on the frequency of the daily injections of nicotine. The sensitivity of 5-HT neurons to inhibition of activity by citalopram was reduced after single daily injections of the high dose of nicotine, while the sensitivity of neurons was not reduced when a single injection of the low dose of nicotine was made (compared to animals injected with saline during 9 days and with nicotine 0.05 or 1 mg/kg on day 10), or when three daily injections were made. The results also show that the neurons of treated animals have no alteration of sensitivity 24 h after nicotine withdrawal, but have an apparent increase in sensitivity 48 h after withdrawal.

The choice of the nicotine doses used in the present study was made in the context of previously published studies dealing with the effects of chronic nicotine treatments on serotonergic systems (Rasmussen and Czachura, 1997 [6 mg/kg/day through osmotic pumps]) and nACh receptors (Ulrich et al., 1997 [0.7 mg/kg twice daily]), and with brain pharmacokinetics of nicotine (Ghosheh et al., 2001 [five consecutive injections of 0.3 mg/kg]). We have not tested injection doses greater than 1 mg/kg because acute injections of doses greater than 1 mg/kg are often considered as acutely toxic or sub-toxic. In our study, injections of 1 mg/kg were called a high dose, as well as 3 injections of 0.7 mg/kg (2.1 mg/kg cumulative daily dose), while single injections of 0.5 mg/kg were called a low dose. Chronic single or multiple daily injections produced no toxic effects (in particular, animals presented no weight loss).

The results show that in naïve rats (Experiment 3), an acute nicotine treatment, even with a high dose (1 mg/kg), does not alter the sensitivity of neurons. Since we observed alterations of neuronal activity after chronic single daily injections of 1 mg/kg, the absence of effect in naïve rats suggests that, under our experimental conditions, a chronic pre-treatment is necessary to change the sensitivity of DRN 5-HT neurons. The results also show (Experiment 1) that rats chronically treated with single daily injections of a low dose of nicotine have no alteration of the sensitivity of neurons when compared to animals chronically treated with saline and administered 0.5 or 1 mg/kg of nicotine on the last day. However, neurons of sham controls (animals chronically injected with saline, no nicotine injection) were significantly more sensitized than neurons of these 3 groups of animals (chronically treated with a low dose of nicotine, or chronically treated with saline and administered 0.5 or 1 mg/kg of nicotine on the last day). The technique of chronic daily i.p. injections, which is known to be stressful to animals (Egger et al., 1986), may have altered the sensitivity of neurons; therefore, a chronic single daily injection of nicotine (in the 3 groups of animals previously described) may have, by itself, produced a moderate but significant

alteration in the sensitivity of neurons. Alternatively, the stress associated with 10 daily injections of vehicle may have predisposed the animals to the effects of acute nicotine. Nevertheless, in Experiment 1, a significant difference in sensitivity was observed between animals chronically treated with 1 and 0.5 mg/kg. Therefore, the desensitization of DRN 5-HT neurons observed after single daily injections is dose-dependent.

A decrease in rats' DRN 5-HT neuron sensitivity in response to citalopram has been previously reported after chronic treatments with 5-HT reuptake inhibitors antidepressants (Jolas et al., 1994; Maudhuit et al., 1997), and after REM sleep deprivation (Maudhuit et al., 1996), but never after chronic nicotine treatment. Therefore, the desensitization of DRN 5-HT neurons observed in our experiments may represent a mechanism by which nicotine has antidepressant-like effects. Antidepressant effects of chronic nicotine have been shown in humans (Salín-Pascual et al., 1995), and in the learned helplessness paradigm in the rat, the dose of 1.5 mg/kg/day (not very different from the effective dose in our experiments) being the effective dose (Semba et al., 1998). 5-HT reuptake inhibitors are thought to decrease the sensitivity of DRN 5-HT neurons through an enhancement of 5-HT synaptic release. The increase in 5-HT release would reduce the function of terminal 5-HT_{1A} autoreceptors in the DRN, thereby decreasing the sensitivity of neurons (Le Poul et al., 1995). Besides an antidepressant-like effect, it has also been shown that a chronic nicotine treatment can alter the density of 5-HT transporters (Xu et al., 2001), which may consequently alter sensitivity of DRN 5-HT neurons. Further work is necessary to determine to which extent chronic nicotine treatments can alter 5-HT function in a way similar to that of antidepressants, in particular if chronic nicotine can enhance 5-HT transmission as 5-HT reuptake inhibitors do.

Our results show that when 0.7 mg/kg of nicotine are administered 3 times a day (2.1 mg/day) over 10 days, there is no change in the sensitivity of DRN 5-HT neurons on day 10. These results are similar to those of Rasmussen and Czachura (1997), who have shown that a chronic administration of nicotine delivered through minipumps (6 mg/kg of nicotine base per day for 12 days) does not alter the sensitivity of DRN 5-HT neurons on day 12. Therefore, three daily injections of 0.7 mg/kg of nicotine result in an effect similar to constant delivery of a high dose of nicotine (6 mg/day), and significantly differ from the effect of a single daily injection of a high dose of nicotine. Single daily injections of different doses of nicotine and repeated daily injections are likely to induce different adaptation processes of nAChRs in the central nervous system. We propose that in the single injection paradigm using 1 mg/kg, a durable adaptation process of nAChRs have occur (we cannot speculate on the type of adaptation), associated with a desensitization of DRN 5-HT neurons. In the single daily injections paradigm using 0.5 mg/kg of nicotine, injections would occur too far apart to induce durable adaptation processes of nAChRs (a change in nAChRs sensitivity may have occurred, but did not persist sufficiently to have consequences on the sensitivity of DRN 5-HT neurons 3 to 4 h after the injection). In the three daily injection paradigm, injections would be sufficiently frequent and quantitatively important to induce a long-term adaptation of nAChRs. This adaptation process would be different from that produced by the daily injections of 1 mg/kg of nicotine since the response of the response of the DRN 5-HT neurons were different, but we cannot speculate on these differences. However, brain nAChRs were likely upregulated in a widespread manner in the three daily injection paradigm since it has been shown that a chronic treatment with a similar daily dose of nicotine (2.4 mg/kg/day) upregulates nAChRs (Besson et al., 2007). A restricted regional upregulation of nAChRs likely occurred in the 1 mg/kg single daily injection paradigm since Ulrich et al. have shown that the chronic intermittent injection of 1.4 mg/kg/day of nicotine produces an upregulation of nAChRs only in the frontal cortex and hippocampus. The effects of nicotine on DRN 5-HT neurons clearly depend upon the activation of nAChRs by nicotine (Touiki et al., 2007), but the mechanisms by which a chronic stimulation of nAchRs dose-dependently modulates the sensitivity of DRN 5-HT neurons remain to be investigated.

According to Ghosheh et al. (2001), the half-life of brain nicotine is 52 min in the rat. Five injections of 0.3 mg/kg of nicotine made every 30 min are necessary to obtain a brain nicotine steady state (Ghosheh et al., 2001), but the duration of this steady-state is not known. Our injections were quantitatively in the range of those used in the Ghosheh et al. study. Nicotine has three biotransformation products in the brain, nornicotine, cotinine and norcotinine (Crooks et al., 1997). The brain half-lives of norcotinine, cotinine and nornicotine, are 3, 6, and 4 times longer, respectively, than that of nicotine itself (Ghosheh et al., 1999). Therefore, nicotine metabolites may also be involved in the adaptation processes of nAchRs following chronic stimulation. We have previously shown that, similarly to nicotine, nornicotine inhibits DRN 5-HT neurons by acting on nicotine receptors (Touiki et al., 2007). Cotinine is not active on DRN 5-HT neurons (Touiki et al., 2005). Therefore, the nicotine metabolite nornicotine may be a compound which participates in the long-term effects of chronic nicotine treatment on the sensitivity of DRN 5-HT neurons.

Our results show that 24 h after nicotine withdrawal the sensitivity of DRN 5-HT neurons is similar to that of controls, and that 48 h after nicotine withdrawal the sensitivity of neurons is significantly increased. The 48-h delayed hypersensitivity occurs in all groups of chronically treated animals tested (single daily injections of 1 mg/kg, as well as repeated daily injections). However, the increase in sensitivity of neurons of treated animals is concomitant with a decrease in sensitivity of neurons in the sham control group. Further work is therefore necessary to determine whether the increase in sensitivity observed in our experiments 48 h after nicotine withdrawal is related to the progressive decrease in sensitivity in the control group or to an increase in sensitivity of neurons after withdrawal. A hypersensitivity of DRN 5-HT neurons 48 h after nicotine withdrawal has been reported by Rasmussen and Czachura (1997), who observed a recovery from the state of hypersensitivity only 7 days after nicotine withdrawal. Hypersensitivity of DRN 5-HT neurons has been associated with states of stress, anxiety and depression (Maudhuit et al., 1997; Lucki, 1998). Our results may therefore be in accordance with the hypothesis that the increase in 5-HT neuron sensitivity observed in rats 48 h after nicotine withdrawal constitutes a part of the neural basis of the "affective" symptomatology observed after nicotine withdrawal in smokers (irritability, anxiety and depressed mood). Such relationships between the activity of serotonergic systems and

nicotine withdrawal symptoms have been previously suggested by other authors (Cheeta et al., 2001; Harrison et al., 2001).

In all the experiments, the basal firing rate was unaffected by chronic nicotine administration or nicotine withdrawal despite the alterations of sensitivity of neurons to citalopram. This observation is in accordance with the results of Rasmussen and Czachura (1997). This absence of effect may be in part related to the relatively small number of neurons in each group, or to the use of anesthesia. Previously, 5-HT_{1A} antagonists have been shown to have little effect on the firing rate of serotonergic cells in anesthetized animals (Fletcher et al., 1996). However, in unanesthetized cats, 5-HT_{1A} antagonists have been shown to increase the activity of serotonergic neurons, especially during periods of behavioral activation (Fornal et al., 1996).

In conclusion, our results show that chronic repeated intermittent nicotine treatments can change the sensitivity of serotonergic neurons, depending on the frequency of daily nicotine administration and on the dose of nicotine administered. In particular, chronic single daily injections of 1 mg/kg of nicotine produces a state of desensitization of DRN 5-HT neurons which is similar to that observed after chronic antidepressant treatments. These dose-dependent and time-dependent alterations of serotonergic neuron sensitivity are likely related to different long-term adaptations of nAChRs.

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