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Chronic Nicotine Enhances Basal and Nicotine-Induced Fos Immunoreactivity Preferentially in the Medial Prefrontal Cortex of the Rat

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In the present study, expression of the immediate early gene protein products Fos and Jun-B within the dorsolateral striatum, the core and shell of the nucleus accumbens (NAC), the medial prefrontal cortex (mPFC), and the ventrolateral orbital cortex was examined. Rats were injected SC with either saline or nicotine (0.5 mg/kg) once daily for 12 days. On day 13, animals received a challenge injection of either saline or nicotine (0.5 or 1.0 mg/kg, SC) and 2 h later their brains were examined for Fos-like (FLI) and Jun-B-like (JLI) immunoreactivity. Chronic nicotine significantly increased basal expression of FLI selectively in the mPFC. Nicotine challenge significantly increased FLI in the mPFC of saline-treated animals and even further increased FLI in the mPFC of nicotine-treated animals. In the shell of the NAC, nicotine challenge also increased FLI in nicotine-treated animals, whereas it increased JLI only in saline-treated animals. After chronic nicotine treatment, injection of D_1 receptor antagonist SCH 23390 (0.1 mg/kg, IP) 10 min before a nicotine challenge (0.5 mg/kg, SC), significantly attenuated the nicotine-induced FLI in the mPFC and the shell of the NAC. These results suggest that the regionally selective effect of nicotine challenge on FLI is due to enhanced dopaminergic transmission, mediated via stimulation of D_1 receptors.
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The rewarding and dependence-producing actions of nicotine appear to be due, at least in part, to stimulation of the mesolimbic dopamine (DA) system (see Grenhoff and Svensson 1989; Clark 1990; Svensson et al. 1990; Corrigall 1991; Nisell et al. 1995). Thus, nicotinic receptors have been found to be located on both the cell bodies and the terminals of mesolimbic DA neurons (Clarke and Pert 1985; Schwartz et al. 1984), and lesions of the mesolimbic DA system attenuate nicotine self-administration and nicotine-induced locomotor stimulation in rats (Clarke et al. 1988; Corrigall et al. 1992). Furthermore, nicotine has been shown to increase neuronal activity, particularly burst activity, of midbrain DA cells (Clarke et al. 1985; Grenhoff et al. 1986; Lichtensteiger et al. 1982; Mereu et al. 1987, Nisell et al. 1996). Systemically administered nicotine also increases release (Imperato et al. 1986; Nisell et al. 1994), synthesis, and metabolism of DA (Andersson et al. 1981; Grenhoff and Svensson 1988) preferentially in the nucleus accumbens (NAC) as compared to the dorsolateral striatum (STR).

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The nicotine-induced locomotion is significantly enhanced with chronic pretreatment with the drug (Morrison and Stephenson 1972; Clarke and Kumar 1983; Benwell and Balfour 1992; Nisell et al. 1996). Although this behavioral activation is to a considerable extent dependent on mesolimbic dopaminergic transmission (see above), previous reports utilizing in vivo microdialysis have demonstrated not only increased (Benwell and Balfour 1992) but also unchanged (Damsma et al. 1989; Nisell et al. 1996) nicotine-induced DA release in the NAC after chronic nicotine treatment. Moreover, the behavioral sensitization observed after chronic, intermittent nicotine treatment for 12 days has been shown to be parallelled by an increase in nicotine-induced DA release in the medial prefrontal cortex (mPFC), whereas the response in the NAC was unaffected (Nisell et al. 1996). Recently, by means of in vivo voltammetry, systemic administration of nicotine was shown to increase DA release to a greater extent in the NAC_{shell} than in the NAC_{core} of the rat, both when nicotine was administered acutely and after chronic nicotine treatment (Nisell et al. 1997).

Although the effects of nicotine on midbrain DA neurons have attracted considerable attention, the postsynaptic consequences of nicotine-induced DA release are less well characterized. As shown by Hakan et al. (1993), nicotine inhibits fimbriae-driven activity in NAC neurons, and this response was affected by haloperidol in a minority of recorded cells; in the frontal cortex, iontophoretically applied nicotine has been shown to affect the firing rate in about one-third of the neurons recorded, causing an excitation in the vast majority of cases (Abdulla et al. 1995).

Another way to evaluate the postsynaptic consequences of DA is to examine the expression of immediate early genes, such as *c-fos* and *jun-B*, by means of in situ hybridization of their mRNAs or by immunohistochemistry of the protein they encode in dopaminergic projection regions. Several immediate early genes can apparently serve as markers for neuronal activity and may, accordingly, be activated by various stimuli, as well as by drugs (for review, see Morgan and Curran 1991). Recently, we have demonstrated a D_1-receptor mediated increase in Fos-like immunoreactivity (FLI) in several limbic regions of the brain after burst-like electrical stimulation of the median forebrain bundle (Chergui et al. 1996). Furthermore, the expression of *c-fos* and other immediate early genes has been found to be increased in DA target areas by several drugs that increase the extracellular concentration of DA, such as cocaine, amphetamine, and morphine (Graybiel et al. 1990; Young et al. 1991; Moratalla et al. 1993; Liu et al. 1994). Indeed, systemic administration of nicotine has also been shown to increase Fos-like immunoreactivity (FLI) in several brain regions, including some which receive their dopaminergic innervation from the ventral tegmental area (VTA) (Ren and Sagar 1992; Matta et al. 1993; Pang et al. 1993; Kiba and Jayaraman 1994). Moreover, local administration of nicotine into the VTA of drug-naive rats has recently been shown to increase FLI in the NAC (Panagis et al. 1996).

To assess the postsynaptic effects of nicotine in target areas of the midbrain DA system, we examined the effects of systemically administered nicotine on FLI and Jun-B-like immunoreactivity (JLI) in the mPFC, the core and shell of the NAC and the STR in rats treated chronically with nicotine or saline. Immunoreactivity was also assessed in the ventrolateral orbital cortex (VLO), a brain region which does not appear to receive dopaminergic innervation from the VTA (Simon et al. 1979), to test whether the response in this area would be different from that in the other regions. Furthermore, in animals treated chronically with nicotine the challenge injections were preceded by pretreatment with the D_1-receptor antagonist SCH 23390 to examine whether the nicotine-induced effects in the regions studied are dependent on dopaminergic transmission via D_1 receptors.

MATERIALS AND METHODS

Animals, Drugs, and Experimental Protocol

Male albino Wistar rats (BK Universal, Sollentuna, Sweden) weighing 250–300 g at the beginning of experiments were used. The animals were housed in groups, three to four animals per cage, under standard laboratory conditions and maintained on a 12-h light/dark cycle (lights on at 6:00 A.M.), with ad libitum access to food and water (-)-Nicotine di-(+)-tartrate salt (Sigma) was dissolved in saline, and pH was adjusted to 7.2–7.4 with sodium hydroxide.

In the first experiment, one group ($n = 22$) of animals received one daily subcutaneous (SC) injection of nicotine (0.5 mg/kg; expressed as free base) for 12 consecutive days. A second group ($n = 21$) was treated for the same period with daily injections of saline (1 ml/kg; SC). Injections were given between 12:00 P.M. and 1:00 P.M. in a counterbalanced manner among animals within each cage, i.e., one or two received saline and the rest received nicotine, and after injections the animals were immediately placed in their home cage. Approximately 24 h after the twelfth injection animals were allocated to different groups of challenge injections: 0.5 mg/kg of nicotine (in saline-or nicotine-treated animals, $n = 7$ in each group), 1.0 mg/kg of nicotine (in saline- or nicotine-treated animals, $n = 7$ in each group) or 1 ml/kg of saline (in saline- or nicotine-treated animals, $n = 7$ and $n = 8$ in each group, respectively) was administered SC, and 2 h later animals were killed and immunohistochemical procedures begun. This regimen of chronic nicotine administration has previously been shown to result in sensitization to nicotine-induced behavioral, electrophysiological, and biochemical actions (Nisell et al. 1996).

In the second experiment, all animals (n = 29) received 12 daily injections of nicotine (0.5 mg/kg; SC), as described above. On the day of experiments, animals received an intraperitoneal (IP) injection of either 0.1 mg/kg of the D_1-receptor antagonist SCH 23390 or 1.0 ml/kg of saline 10 min before an SC injection of either 0.5 mg/kg of nicotine or 1.0 ml/kg of saline (n = 7 for each combination, except for SCH 23390 plus nicotine, where n = 8). The challenge dose of nicotine used in the second experiment (0.5 mg/kg; SC) was chosen based on results from the first experiment. Two hours after the second injection, animals were killed and immunohistochemical procedures were commenced.

Immunohistochemistry

Immunohistochemistry was performed as described by Robertson and Fibiger (1992) and Chergui et al. (1996). Brains from seven to eight animals, each time counterbalanced across treatment groups were processed at a time, i.e., one or two animals from each group were included in every run. Two hours after drug administration, the animals were deeply anesthetized with pentobarbital (100 mg/kg, IP) and transcardially perfused with saline (150 ml) followed by 300 ml of 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline (PBS). Subsequently, the brains were removed, postfixed for 2 h in paraformaldehyde, and placed overnight in 15% sucrose in PBS. The brains were then cut in 30 μm sections on a microtome.

Sections were washed in PBS (2 × 15 min) and placed in 0.3% H_2O_2 in PBS and then washed in PBS (3 × 20 min) before being incubated for 48 h in PBS containing 0.3% Triton X, 0.02 Na Azide, and either 2% Normal Rabbit Serum and Fos primary antibody diluted 1:3000 (sheep polyclonal antibody, Cambridge Research Biochemicals OA-11-824) or 2% Normal Swine Serum and Jun-B primary antibody diluted 1:750 (rabbit ployclonal antibody, Santa Cruz Biotechnology, Inc., cat# sc-46). The sections were then washed in PBS (3 × 20 min), and incubated for 1 h with a dilution, 1:200, of either biotinylated rabbit anti-sheep secondary antibody (anti-Fos incubated sections) or goat anti-rabbit secondary antibody (anti-Jun-B incubated sections: Vector Laboratories). After washing in PBS (3 × 20 min) and then being incubated for 1 h in PBS containing avidin biotinylated horseradish peroxidase complex (1:100, Vector Laboratories), the sections were washed again in PBS and then rinsed for 10 min in 0.2 mol/L Na acetate buffer. The reaction was visualized using a glucose oxidase -3,3-diaminobenzidine nickel method (Shu et al. 1988) terminated by washing in PBS. The dilutions of both primary and secondary antibodies, as well as of the peroxidase complex, used in the present study, were selected on the basis of preliminary experiments in which the background staining was minimal. In addition, omission of the primary antibodies from the immunohistochemical procedures depleted Fos- or Jun-B-immunoreactivity.

Sections were mounted on gelatin-coated slides, dehydrated, and coverslipped for microscopic observation. Immunostained nuclei were counted in sections cut: +3.5 mm for the mPFC and the VLO, +1.7 mm for the core and shell of the NAC, and +1.2 mm for the STR, relative to bregma (Paxinos and Watson 1986). The number of Fos- or Jun-B-positive nuclei was manually counted by two examiners, who were blind to treatment conditions, at 250× magnification within a grid covering 500 × 500 μm of the mPFC, the VLO, and the STR and 300 × 300 μm of the shell and core of the NAC (Figure 1). Cells were counted bilaterally in three sections from each region in all brains, and an average value of all measurements was calculated for each region. Given that immunostaining was performed in sections from animals from all treatment groups within each run, possible differences in staining sensitivity between different runs were controlled for.

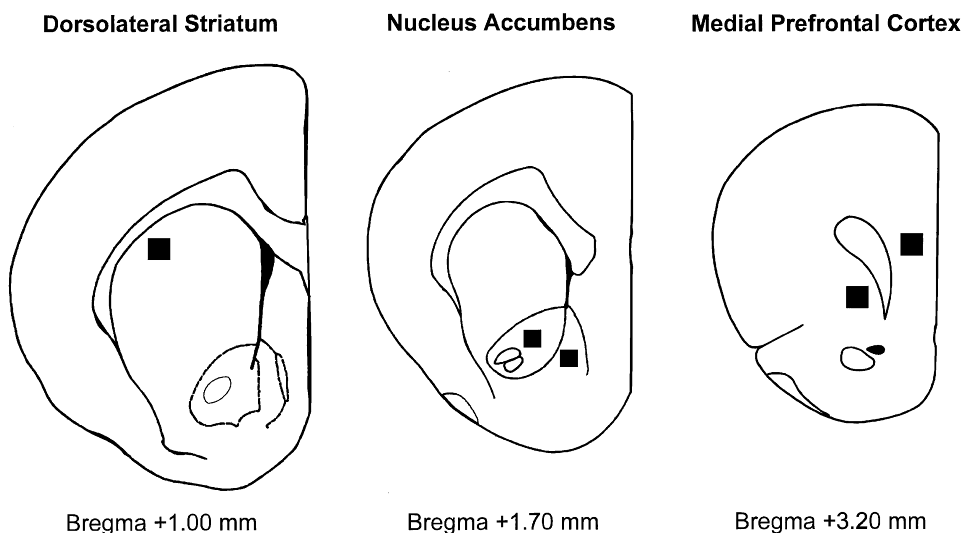

Figure 1. Schematic drawings indicating areas (according to Paxinos and Watson 1986) over which immunoreactive nuclei were counted. *Middle panel*: left and right square indicates the core and shell of the nucleus accumbens, respectively. *Right panel*: left and right square indicates the ventrolateral orbital cortex and the medial prefrontal cortex, respectively.

Statistical Analysis

The mean (± SEM) value for the number of Fos or Jun-B immunostained nuclei in each region was calculated and presented in Figure 2 (Fos, first experiment), Figure 3 (Fos, second experiment), and Table 1 (Jun-B, first experiment). Representative photomicrographs illustrating Fos-like immunoreactive nuclei in the mPFC after various treatments (first experiment) are included in Figure 4. The data from the first experiment were statistically evaluated with two-way ANOVA, with one factor being the chronic treatment, i.e., saline or nicotine, and the second factor being the challenge injection, i.e., saline or nicotine in two doses. If a significant interaction effect was indicated by the two-way ANOVA, multiple comparisons between the various groups were performed using the Tukey honest significant difference (HSD) test. On the other hand, if only a significant challenge effect was indicated by the two-way ANOVA, the effect of various challenge injections in animals treated with either saline or nicotine were evaluated with one-way ANOVA followed by the Tukey HSD test. Also, if only a significant treatment effect was indicated by the two-way ANOVA, differences in the number of immunostained nuclei after a particular challenge injection between saline- and nicotine-treated animals were evaluated with Student's *t*-test. The data from the second experiment were also evaluated with two-way ANOVA, with one factor being the first challenge injection, i.e., saline or SCH 23390, and the second factor being the second challenge injection, i.e., saline or nicotine. As in the first experiment, when a significant interaction effect was indicated by the two-way ANOVA, multiple comparisons between the various groups were performed using the Tukey HSD test. On the other hand, if only significant challenge effects were indicated by the two-way ANOVA, the effect of various challenge injections were evaluated with Student's *t*-test. In all cases a two-tailed $p < .05$ was considered significant; data were statistically evaluated using the CSS:Statistica (Statsoft) program.

RESULTS

In the mPFC, nicotine challenge dose-dependently increased the number of Fos-positive nuclei in both saline- and nicotine-treated animals (Figure 2 and 4). Also, the animals treated chronically with nicotine showed a higher number of Fos-positive nuclei after both saline and nicotine challenge (Figure 2 and 4). An overall two-way ANOVA revealed a significant treatment ($F_{1,37} = 24.76$, $p < .001$) and challenge ($F_{2,37} = 16.38$, $p < .001$) effect, whereas there was no significant interaction effect. Subsequently, one-way ANOVAs revealed significant effects of the nicotine challenge injections vs. saline challenge in both saline-treated and nicotine-treated animals ($F_{2,18} = 19.63$, $p < .001$ and $F_{2,19} = 6.62$, $p < .01$, respectively). In saline-treated animals, nicotine challenge (0.5 or 1.0 mg/kg; $n = 7$, both groups) significantly increased FLI in comparison to saline ($n = 7$) challenge ($p = .0006$ and $p = .0002$, respectively; Figure 2). In nicotine-treated animals, nicotine challenge (0.5 or 1.0 mg/kg; $n = 7$, both groups) also increased FLI as compared to the effect of saline ($n = 8$) challenge ($p = .0007$ and $p = .042$, respectively; Figure 2 and 4). In addition, the number of Fos-positive nuclei observed after a saline challenge was significantly higher in nicotine-treated ($n = 8$), as compared to saline-treated ($n = 7$) animals ($p = .017$; Figure 2). Furthermore, the number of Fos-positive nuclei observed after nicotine challenge (0.5 mg/kg) was significantly higher in nicotine-treated than in saline-treated animals ($p = .0016$; Figure 2).

In the VLO, statistical analysis of the data showed that neither chronic nicotine administration nor nicotine challenge (0.5 or 1.0 mg/kg), in saline- or nicotine-treated animals, significantly affected the number of Fos-positive nuclei, as compared to the numbers observed in the respective control groups (Figure 2).

In the shell of the NAC, the overall two-way ANOVA revealed a significant challenge effect ($F_{2,37} = 9.84$, $p < .001$), whereas neither the treatment nor the interaction effects reached statistical significance. Subsequent analysis with one-way ANOVAs showed significant effects of nicotine challenge injections vs. saline challenge in both saline-treated and nicotine-treated animals ($F_{2,18} = 6.12$, $p < .01$ and $F_{2,19} = 6.00$, $p < .01$, respectively). In saline-treated animals, challenge injection with the 1.0 mg/kg, but not the 0.5 mg/kg, dose of nicotine significantly increased FLI as compared to saline challenge ($p = .007$; Figure 2). In nicotine-treated animals, a challenge injection with the 0.5 mg/kg, but not the 1.0 mg/kg, dose of nicotine significantly increased the number of Fos-positive nuclei compared to saline challenge ($p = .008$; Figure 2).

An overall two-way ANOVA on data from the core of the NAC indicated a significant challenge effect ($F_{2,37} = 6.34$, $p < .01$), whereas neither the treatment nor the interaction effects were statistically significant. When the data were analyzed with one-way ANOVAs, a significant effect of nicotine challenge injections vs. saline challenge was shown only in saline-treated animals ($F_{2,18} = 9.34$, $p < .01$). Challenge with nicotine (0.5 and 1.0 mg/kg) significantly increased FLI compared to the effect of saline challenge in saline-treated animals ($p = .027$ and $p = .001$, respectively; Figure 2).

In the dorsolateral STR, an overall two-way ANOVA did not reveal any significant effects of nicotine challenge vs. saline challenge in either saline-treated or nicotine-treated animals on FLI (Figure 2); also, chronic nicotine administration did not significantly affect FLI

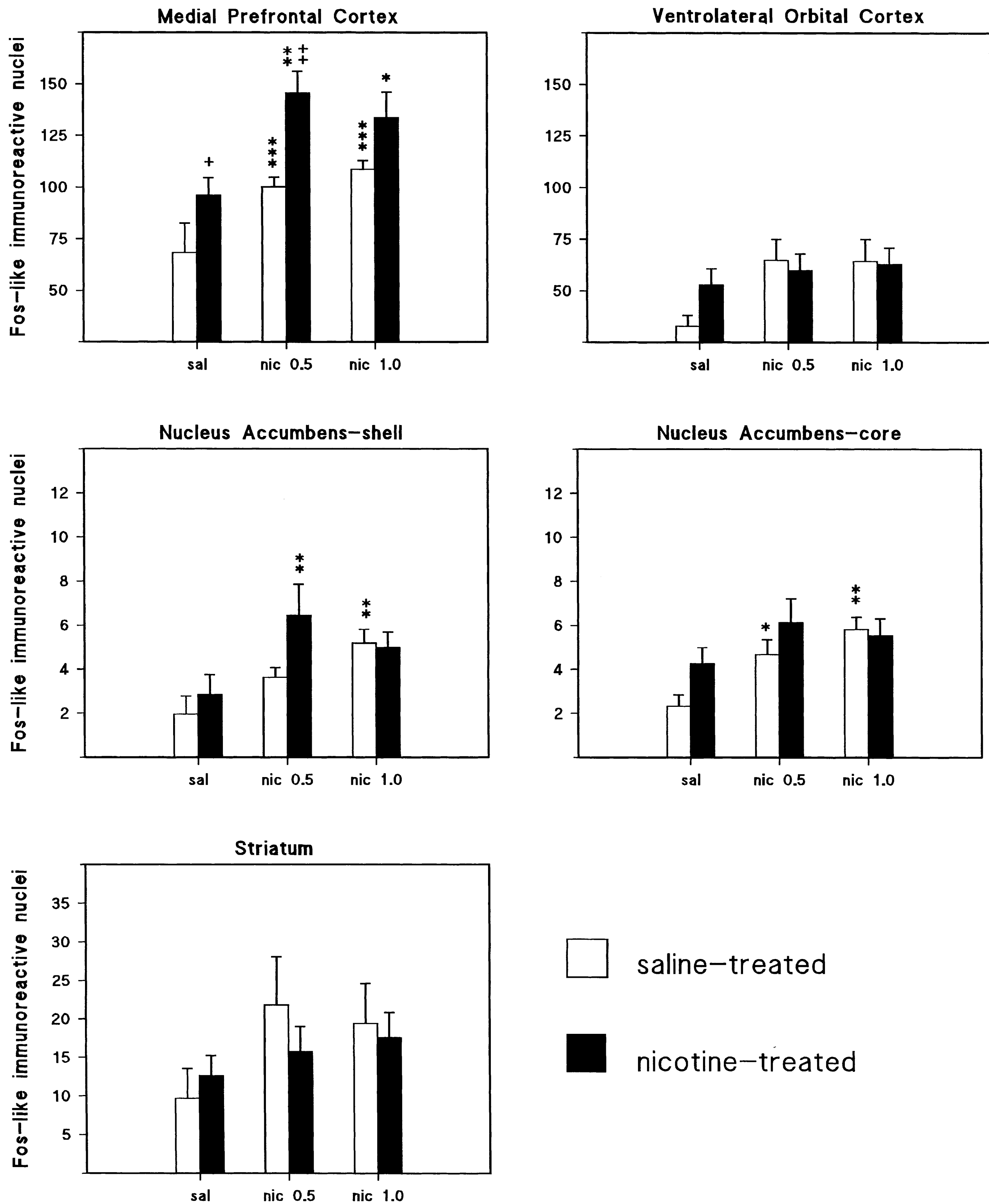

Figure 2. Effects of a challenge injection of nicotine (nic) or saline (sal) on Fos-like immunoreactivity in animals treated chronically with either saline or nicotine, as indicated in the figure, in the examined regions of the brain. Data are expressed as mean (+SEM) number of Fos-like immunoreactive nuclei counted in 30-μm thick sections. * $p < .05$, ** $p < .01$ nicotine vs. saline challenge; $^{+}p < .05$; $^{++}p < .01$, chronic saline vs. chronic nicotine for each challenge dose of nicotine.

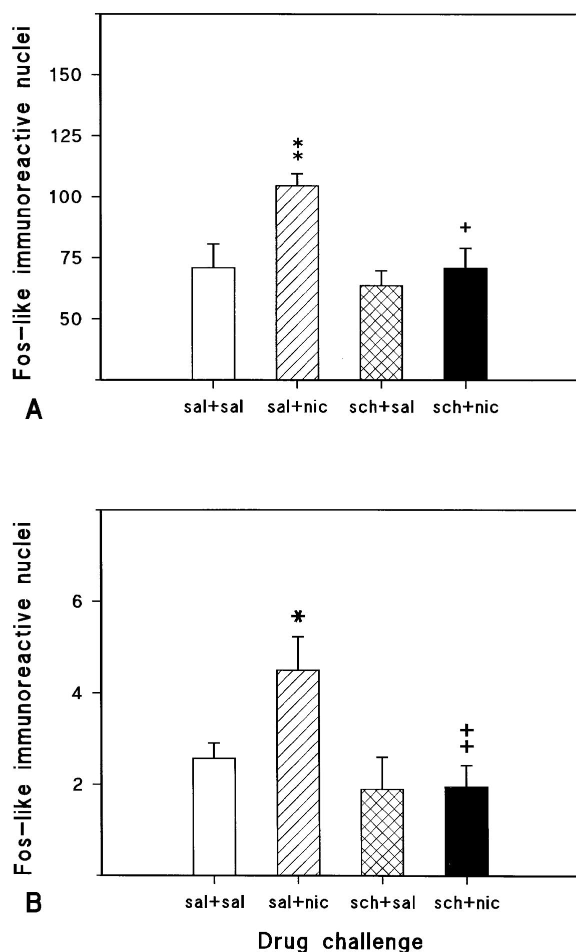

Figure 3. Effects of two injections, 10 min apart, of saline (sal), saline and nicotine (nic), SCH 23390 and saline or SCH 23390 (sch) and nicotine on Fos-like immunoreactivity in (**A**) medial prefrontal cortex and (**B**) shell of the nucleus accumbens, in animals chronically treated with nicotine. Data are expressed as mean (+SEM) number of Fos-like immunoreactive nuclei counted in 30-μm thick sections. * $p < .05$, ** $p < .01$ p, sal+nic vs. sal+sal; $^{+}$ $p < .05$, $^{++}$ $p < .01$, sch-nic vs. sal+nic.

in animals challenged with either saline or nicotine (Figure 2).

In the mPFC, pretreatment with SCH 23390 prevented the increase in FLI induced by nicotine (0.5 mg/kg) in nicotine-treated animals. An overall two-way ANOVA revealed significant challenge effects (saline or SCH 23390; $F_{1,25} = 20.96$, $p < .001$; saline or nicotine; $F_{1,25} = 15.88$, $p < .001$), as well as a significant interaction effect ($F_{1,25} = 9.24$, $p < .01$). Multiple comparisons indicated that animals receiving nicotine showed significantly ($p = .00024$) more FLI than the respective controls ($n = 7$ in both groups; Figure 3*A*). SCH 23390 by itself ($n = 7$) did not affect the number of Fos-positive nuclei in the PFC, whereas it significantly ($p = .00032$) attenuated the NIC-induced FLI ($n = 8$; Figure 3*A*). Similar results were obtained in the shell of the NAC, where pretreatment with SCH 23390 blocked the nicotine-induced increase in the number of Fos-positive nuclei ($p = .006$; Figure 3*B*) in nicotine-treated animals without affecting FLI by itself. Also, similarly to the results of the first experiment, nicotine challenge did not significantly affect FLI in the VLO, the core of the NAC or the striatum in nicotine-treated animals compared to the effects of saline challenge (data not shown). SCH 23390, by itself, did not affect the number of Fos-positive nuclei in the VLO, the core of the NAC or the STR in nicotine-treated animals (data not shown).

With the exception of the shell of the NAC, the number of Jun-B-positive nuclei was not significantly affected by either chronic nicotine treatment or by nicotine challenge (0.5 or 1.0 mg/kg) in either saline- or nicotine-treated animals in any of the examined brain regions (Table 1). Specifically, overall two-way ANOVAs did not reveal any significant treatment, challenge, or interaction effects, except for the shell of the NAC, where a significant challenge effect was indicated ($F_{2,37} = 4.28$, p .05). Subsequently, one-way ANOVAs showed a significant effect of challenge nicotine injections only in the saline-treated rats ($F_{2,18} = 7.41$, $p < .01$), which was due to the fact that nicotine (0.5 and 1.0 mg/kg) significantly increased JLI as compared to saline challenge ($p = .007$ and $p = .014$, respectively; Table 1).

DISCUSSION

The major finding of the present study is that chronic, intermittent administration of nicotine results in an enhancement of basal as well as nicotine-induced increases in FLI, specifically in the mPFC. Furthermore, administration of the D_1 receptor antagonist SCH 23390 before nicotine challenge in rats treated chronically with nicotine blocks the nicotine-induced increase in FLI in the mPFC.

The nicotine-induced increase in FLI in the mPFC of saline-treated, i.e., drug-naive, animals is in accordance with the results of Sharp et al. (1993), who reported an increase in *c-fos* mRNA in the cingulate gyrus in response to systemic nicotine. Moreover, these authors observed that the stimulatory effect of nicotine in the cingulate gyrus, in contrast to several other brain regions, is not dose-dependent, similarly to the present findings. The mechanism by which nicotine exerts its stimulatory effect on FLI in the mPFC remains to be established. Thus, activation of frontal cortical neurons is observed after local application of nicotine (Abdulla et al. 1995). Nicotine also induces release of acetylcholine and glutamate, which could excite cortical neurons (Lapchak et al. 1989; Vidal 1994). Moreover, systemic nicotine increases extracellular levels of DA in the

Table 1. Effects of a Saline (sal) or Nicotine (nic) Challenge on the Number of Jun-B-like Immunoreactive Nuclei in the Examined Regions of the Brain in Rats Treated Chronically with Either Saline or Nicotine

				Nucleus Accumbens		
	n	Medial Prefrontal Cortex	Ventrolateral Orbital Cortex	Shell	Core	Striatum
sal-sal	7	14.9 ± 3.0	3.6 ± 1.2	2.1 ± 0.6	8.5 ± 2.3	4.4 ± 1.1
sal-nic 0.5	7	16.5 ± 2.1	4.5 ± 0.9	4.8 ± 0.5[b]	13.8 ± 1.6	5.2 ± 0.5
sal-nic 1.0	7	18.3 ± 2.0	3.4 ± 0.5	4.6 ± 0.5[a]	12.3 ± 1.0	3.5 ± 0.7
nic-sal	8	24.4 ± 3.4	3.6 ± 0.3	3.6 ± 0.9	8.7 ± 0.9	3.0 ± 0.2
nic-nic 0.5	7	29.6 ± 4.7	6.0 ± 0.9	4.9 ± 1.0	13.5 ± 2.4	3.9 ± 0.8
nic-nic 1.0	7	21.7 ± 3.8	5.6 ± 0.9	4.2 ± 0.7	13.4 ± 3.0	5.9 ± 2.0

Data are expressed as mean (± SEM) number of Jun-B-like immunoreactive nuclei counted in 30-μm thick sections; n = the number of animals in each group.
[a] $p < .05$.
[b] $p < .01$ nicotine vs. saline challenge.

mPFC (Nisell et al. 1996) and, since both direct and indirect DA receptor stimulation in the brain has been shown to increase FLI (see Huges and Dragunow 1995), the nicotine-induced increase in FLI may also, at least in part, be due to increased DA release. Also, the fact that nicotine failed to significantly affect FLI in the VLO may indirectly support the importance of DA for nicotinic effects in the cortex.

The enhanced nicotine-induced prefrontal cortical FLI after chronic nicotine-treatment may reflect a functional activation within this region, since chronic nicotine treatment has previously been shown to augment nicotine-induced excitation of cortical neurons (Abdulla et al. 1995). In fact, even the increased FLI observed in the mPFC after a challenge injection with saline in nicotine-treated animals may reflect such a functional activation within this region after chronic nicotine treatment. Alternatively, the increase in basal FLI levels could be related to conditional neuronal activation in response to repeated nicotine injections, i.e., FLI was increased after a saline challenge in animals expecting to receive nicotine. In this regard, it is noteworthy that exposure of chronically cocaine-treated rats to a cocaine-paired environment has been shown to induce FLI

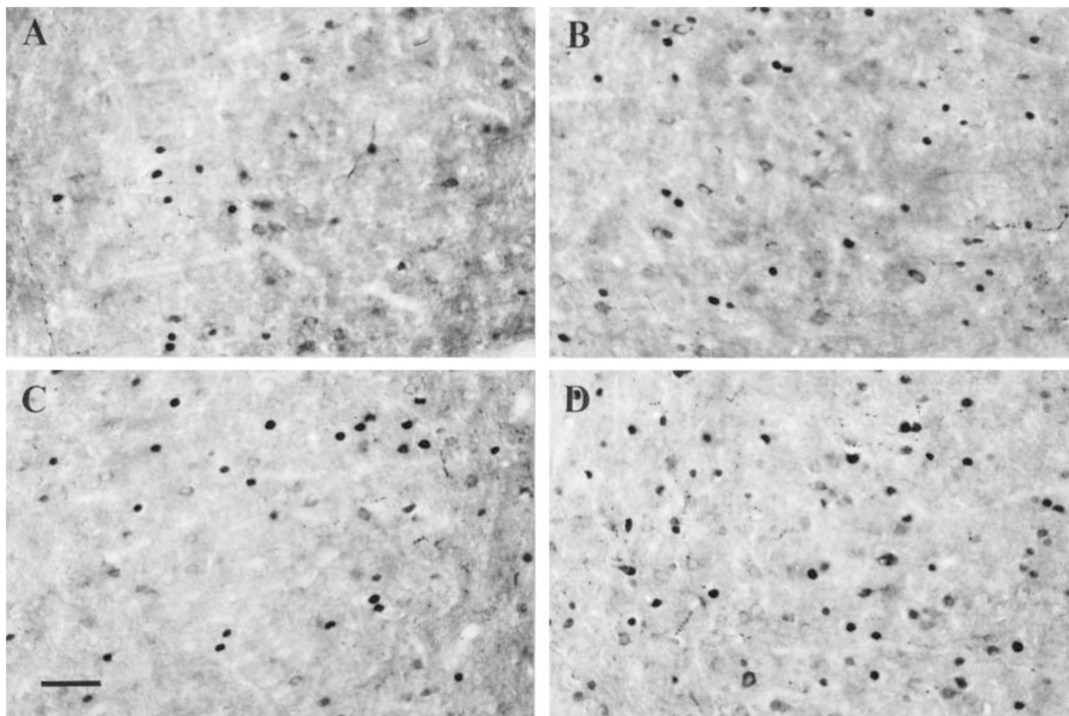

Figure 4. Representative photomicrographs illustrating Fos-like immunoreactive nuclei in the medial prefrontal cortex at 250 × magnification after different treatments. **A**: saline challenge in drug-naive animals; **B**: nicotine challenge in drug-naive animals; **C**: saline challenge after nicotine treatment; and **D**: nicotine challenge after nicotine treatment. Bar indicates 50 μm.

within the cingulate cortex, but not within the NAC, although FLI within both regions was induced in response to injections of cocaine (Brown et al. 1992). On the other hand, since our animals received a saline challenge approximately 24 h after the last nicotine injection, the high levels of FLI observed after saline may also be related to a stress reaction caused by nicotine abstinence. Such an interpretation is compatible with previous studies in which ethanol and morphine withdrawal have been shown to result in an increase in cortical FLI (Beckmann et al. 1995; Matsumoto et al. 1993).

The increase in prefrontal cortical FLI after the 0.5 mg/kg dose of nicotine was significantly higher than the response to a saline challenge in the chronically nicotine-treated rats, although the underlying mechanisms remain presently unclear. Nicotine pretreatment in rodents does not affect the disposition of a challenge injection of NIC (Pekonen et al. 1993), a finding that argues against an altered metabolic rate of nicotine as a mechanism by which the effect of the drug is enhanced. Repeated administration of nicotine is associated with an increase in brain nicotinic binding (Ksir et al. 1985; Lapchak et al. 1989), a phenomenon that could be of importance for the sensitization to both behavioral and biochemical effects of nicotine. However, Lapchak et al. (1989) demonstrated an increased binding of nicotine both in mPFC and in the striatum. Thus, regional differences in receptor binding after chronic nicotine could not explain the increased responsivity to nicotine that occurs selectively in the mPFC. On the other hand, a differential distribution of nicotinic receptor subunits has been demonstrated in cortical compared with subcortical areas (Wada et al. 1989). Consequently, there may be regional differences in the functional properties of receptors and, accordingly, also in their adaptation to chronic agonist exposure, which could help to explain the regional selectivity observed.

Since SCH 23390 blocked nicotine-induced FLI in the mPFC of nicotine-treated rats, it appears that DA-D_1 receptor stimulation is of crucial importance for mediating this effect of nicotine. Chronic, intermittent nicotine treatment has previously been shown to increase nicotine-induced release of DA in the mPFC (Nisell et al. 1996). Thus, the enhanced effect of nicotine on FLI in the mPFC can clearly be related to increased DA release, specifically to an enhanced dopaminergic transmission via D_1 receptors. Changes in the sensitivity of DA receptors might, theoretically, also contribute to the enhanced nicotine-induced FLI within the mPFC after nicotine treatment. However, studies examining binding to DA receptors located subcortically after chronic NIC administration have not yielded conclusive results (Janson et al. 1992; Kirch et al. 1992; Reilly et al. 1987). Thus, the notion that altered DA receptor sensitivity may contribute to the enhanced effects of nicotine on cortical FLI remains to be corroborated.

The pattern of FLI after a nicotine challenge in drug-naive animals was somewhat similar in the NAC and in the STR, although significant effects were observed only in the shell of the NAC after the high dose (1.0 mg/kg) of nicotine and in the core of the NAC after either dose of nicotine (0.5 and 1.0 mg/kg). The present finding that nicotine challenge does not significantly affect FLI in the STR of drug-naive animals contrasts the results of Kiba and Jayaraman (1994). These authors found that acute injections of nicotine, in doses similar to those used in the present study, induced FLI in the STR, mainly in its central and dorsomedial parts, although no statistical analysis appears to have been performed. In the present study, nevertheless, a pattern of response very similar to that reported by Kiba and Jayaraman was observed. Furthermore, in the present experiments drug-naive animals received 12 daily injections of saline, which may influence the effect of nicotine on FLI, similarly to that of amphetamine. Thus, the ability of systemically administered amphetamine to increase *c-fos* expression in the brain is substantially reduced in animals that have been subjected to daily saline injections before the amphetamine challenge (Persico et al. 1993).

In nicotine-treated animals, the pattern of response was clearly altered, i.e., a statistically significant increase in FLI was observed within the shell of the NAC compared to saline challenge only with the 0.5 mg/kg dose of nicotine, whereas no significant effect was observed in the core of the NAC. Interestingly, as recently shown by in vivo voltammetry (Nisell et al. 1997), the nicotine-induced DA release is more pronounced in the shell than in the core of the NAC, both in drug-naive as well as in nicotine-pretreated rats. Thus, the enhanced effect of nicotine in the NAC_{shell} of nicotine-treated animals may be causally related to the nicotine-induced DA release within this region. Similarly, the finding that nicotine failed to significantly elevate FLI in the core of the NAC in nicotine-treated rats may be due to the previously reported decrease in DA release in this region after chronic nicotine administration (Nisell et al. 1997). As is the case in the mPFC, the ability of SCH 23390 to block the nicotine induced FLI in the NAC_{shell} of nicotine-treated rats suggests that DA D_1 receptor stimulation is of critical importance for this effect.

The fact that nicotine increased JLI, in the present study, only in the shell of the NAC of drug-naive animals, is interesting in view of previous reports, where several dependence-producing drugs have been found to affect levels of both *jun-B* mRNA as well as JLI in brain neurons. Thus, cocaine has been shown to increase *jun-B*, as well as *c-fos*, mRNAs in rat striatal neurons (Moratalla et al. 1993). Moreover, cocaine has been shown to increase *jun-B* mRNA levels in the NAC, an effect which wanes after chronic treatment with the drug (Hope et al. 1992). Also, acute administration of

morphine increases levels of *jun-B* in the STR but not in the NAC (Garcia et al. 1995), and nicotine has been reported to induce expression of *jun-B* mRNA in the rat superior cervical ganglion (Koistinaho et al. 1993).

Previous reports have suggested that the adaptive response to nicotine in some dopaminergic projection areas may differ from those of other psychostimulant drugs (e.g., Nisell et al. 1996). In general, repeated (1–2 weeks) administration of other psychostimulant drugs, such as cocaine and amphetamine, results in a reduced expression of *c-fos* or *jun-B* compared with the acute effects of these drugs in several brain regions including the mPFC, the NAC, and the STR (Hope et al. 1992; Steiner and Gerfen 1993; Daunais and McGinty 1994; Persico et al. 1993). This profile of response was also observed in the present study in the core of the NAC, as far as the effects of chronic nicotine on FLI concerns, and in the shell of the NAC regarding the effects of nicotine on JLI. In contrast, the stimulatory effects of nicotine on FLI in the mPFC were retained after chronic nicotine treatment.

In summary, the present results demonstrate a preferential enhancement of basal as well as nicotine-induced FLI in brain, mainly within the mPFC, after chronic, intermittent nicotine treatment, an effect that may be due to the enhanced nicotine-induced DA release. Such effects of chronic nicotine treatment on DA-related activity in the mPFC may be significant both for nicotine-induced behavioral sensitization in experimental animals and for the appearance of nicotine dependence in several species, including humans.

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