Condition-Independent Sensitization of Locomotor Stimulation and Mesocortical Dopamine Release Following Chronic Nicotine Treatment in the Rat

MAGNUS NISELL, GEORGE G. NOMIKOS, PETER HERTEL, GEORGE PANAGIS, AND TORGYNY H. SVENSSON

Department of Physiology and Pharmacology, Division of Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden

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ABSTRACT Chronic nicotine (NIC) pretreatment has been shown to enhance NIC-induced locomotor stimulation, an effect that seems critically dependent on activation of brain dopamine (DA) systems. In the present study the effects of chronic, intermittent NIC treatment were examined in the rat to establish whether such behavioral sensitization is associated with specific, regional changes in brain dopaminergic activity. Male rats received daily injections in their home cage with either saline (SAL) or NIC (0.5 mg/kg, s.c.) for 12 days. Twenty-four hours later, the locomotor activity of the animals subjected to NIC challenge as well as the functional responsiveness of the mesolimbocortical dopaminergic system were assessed. To this end, microdialysis experiments were performed in awake animals, measuring extracellular concentrations of DA and its metabolites in the prefrontal cortex (PFC) and the nucleus accumbens (NAC). Extracellular single cell recordings from DA neurons in the ventral tegmental area (VTA) were also performed in anesthetized animals. NIC (0.5 mg/kg, s.c.) increased all measured parameters of locomotor activity, with the exception of rearing, in SAL-pretreated animals; these effects were substantially enhanced after pretreatment with NIC. Nicotine (0.5 mg/kg, s.c.) increased DA release in both the PFC and the NAC in SAL-treated animals. Nicotine pretreatment significantly enhanced this effect in the PFC, whereas it did not affect the response in the NAC. Low doses of intravenously administered NIC dose-dependently increased burst activity, starting at 12 µg/kg in the SAL pretreated animals and at 6 µg/kg in the NIC-pretreated animals, and also dose-dependently increased firing rate in SAL as well as NIC-pretreated animals, although starting at a higher dose level, i.e., 25 µg/kg. These results demonstrate that behavioral sensitization after chronic NIC treatment is accompanied by an enhanced dopamine release specifically within the PFC. This phenomenon may be highly significant for the dependence-producing effects of NIC, particularly in association with major psychiatric disorder, such as schizophrenia.

INTRODUCTION

Several lines of experimental evidence indicate that stimulation of brain dopamine (DA) systems is of major significance for the reinforcing and dependence producing properties of nicotine (NIC; see Clarke, 1990; Corrigall, 1991; Grenhoff and Svensson, 1989; Svensson et al., 1990). Accordingly, nicotinic receptors seem to be located both on cell bodies and terminals of mesolimbic and nigrostriatal DA neurons (Clarke and Pert, 1985; Schwartz et al., 1984) and synthesis, as well as metabolism of DA is increased mainly in mesolimbic, as compared to nigrostriatal, DA neurons after systemic NIC administration (Andersson et al., 1981; Grenhoff and Svensson, 1988). Furthermore, NIC administration has been shown to increase neuronal DA release in vitro (Giorgiueff-Chesselet et al., 1979; Rapier et al., 1988; Rowell et al., 1987), as well as in vivo, utilizing microdi-

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analysis, both when NIC is administered locally into the ventral tegmental area (VTA), i.e., the cell-body region of mesolimbocortical DA neurons, and into the nucleus accumbens (NAC; Mifsud et al., 1989; Nisell et al., 1994). Systemic administration of NIC has also been shown to elevate extracellular DA concentrations in the NAC (Damsma et al., 1989; Imperato et al., 1986; Nisell et al., 1994), although this effect appears to be due to stimulation of nicotinic receptors located in the VTA rather than those located in the NAC (Nisell et al., 1994). Moreover, electrophysiological experiments have shown that acute NIC treatment increases both the burst activity and the firing rate of midbrain DA neurons (Grenhoff et al., 1986; Mereu et al., 1987) and, as shown by Calabresi et al. (1989), also this stimulatory action of NIC on the mesolimbic DA system may, at least in part, be due to activation of somatodendritic nicotinic receptors located in the VTA.

The biochemical and electrophysiological findings described above are consonant with behavioral studies which indicate that the stimulatory action of NIC on locomotor activity, as well as the self-administration of NIC, can be prevented by DA depletion in the NAC (Clarke et al., 1988; Clarke, 1990; but see Vezina et al., 1994). Also, the hyperlocomotion induced by systemic NIC appears to be mediated predominantly by nicotinic receptors in the VTA, since NIC applied locally in this area produces a more pronounced effect than that of intra-accumbal administration (Leikola-Pelho and Jackson, 1992; Reavill and Stolerman, 1990). Furthermore, blockade of nicotinic receptors in the VTA, but not in the NAC, abolishes NIC self-administration in rats (Corrigall et al., 1994). These results emphasize the importance of nicotinic receptors located in the VTA, rather than those in the NAC, for mediating the DA related behavioral effects of NIC.

In contrast to the effects of NIC on subcortical DA release, little is known about its action on cortical release of DA. Clearly, nicotinic receptors are present in the cerebral cortex of rats (Clarke et al., 1984; Härfstrand et al., 1988; Larsson et al., 1987). However, lesioning of catecholamine axons does not decrease the binding of radiolabeled acetylcholine in the rat cortex (Schwartz et al., 1984), indicating that the nicotinic receptors are not present on dopaminergic terminals in the prefrontal cortex (PFC). Thus, effects of NIC on mesocortical DA release may instead be effectuated at the somatodendritic level within the VTA.

Interestingly, the locomotor stimulation caused by NIC administration is enhanced by chronic pretreatment with the drug (Benwell and Balfour, 1992; Clarke and Kumar, 1983; Morrison and Stephensson, 1972; Stolerman et al., 1973). This behavioural sensitization, which has been found to be associated with an increase in brain nicotinic receptors (Ksir et al., 1985; Lapchak et al., 1989), is strongly affected by conditioning to the environment associated with drug administration (Reid et al., 1994; Walter and Kuschinsky, 1989). However, according to some studies repeated NIC treatment does not seem to influence the effects of a NIC challenge on DA utilization or release in the NAC, as assessed by both ex vivo (Mitchell et al., 1989; Vezina et al., 1992) and in vivo studies (Damsma et al., 1989). In contrast, Vezina et al. (1992) provided some evidence ex vivo that repeated injections of NIC may increase DA utilization in the medial PFC, i.e., another major terminal field for VTA-DA neurons. In general, sensitization to the locomotor stimulating action of several psychostimulant and dependence-producing drugs other than NIC has been found critically dependent on augmented DA transmission in the NAC (Kalivas and Stewart, 1991). However, blockade of N-Methyl-D-Aspartate (NMDA) receptors concurrent with chronic NIC treatment has recently been shown to somewhat attenuate NIC-induced enhancement of locomotor activity (Shoaib and Stolerman, 1992; 1994). Although the observed behavioral sensitization was accompanied by enhanced accumbal DA release, the latter effect appeared to be totally abolished by NMDA receptor blockade. These results indicate, in fact, that sensitization to the locomotor stimulant effect of NIC may not require accumbal dopaminergic activation. Tentatively, the enhanced behavioral responsivity to NIC after chronic pretreatment may, instead, be due to a rather unique pattern of neuronal adaptation, involving the mesocortical DA system.

In the present study, therefore, rats were chronically treated with NIC and motor activity measurements were performed in order to assess the change in responsivity to a NIC challenge in NIC-treated compared to drug naive animals. In addition, in vivo microdialysis measurements of DA and its metabolites in the PFC and the NAC were performed in freely moving animals as well as in vivo electrophysiological recordings from VTA-DA neurons in order to investigate further whether chronic NIC treatment causes sensitization of mesocortical or mesolimbic DA activity, or both.

**MATERIALS AND METHODS**

**Animals, drugs, and experimental protocol**

Male albino rats (BK Universal, Sollentuna, Sweden), Wistar for microdialysis and behavioral experiments and Sprague-Dawley for electrophysiological experiments, weighing 250-300 g at the beginning of experiments were used. The animals were housed under standard laboratory conditions and maintained on a 12 h light/dark cycle (lights on at 06.00 h), with ad libitum access to food and water. (-)-Nicotine di-(+)-tartrate salt (Sigma Chemical Co., St. Louis, MO) was dissolved in saline and pH was adjusted to 7.2-7.4 with sodium hydroxide. One group of animals received one daily subcutaneous (s.c.) injection of NIC (0.5 mg/kg; expressed as free base) for 12 consecutive days. A second group was treated for the same period of time with one daily injection of saline (SAL). NIC or SAL was injected subcutaneously (1 ml/kg) between 12:00 and 13:00 h. After
injection the animals were immediately placed in their home cage. This regimen did not influence significantly the weight gained by rats that were treated chronically with NIC as compared to those treated with SAL.

**Behavioral experiments**

Motor activity was measured in two computer-assisted photocell equipment boxes described in detail by Ericson et al. (1991). The motor activity experiments were conducted in a square open-field arena (68 × 68 × 45 cm) equipped with two rows of photocells, sensitive to infrared light, placed 4.0 and 12.5 cm above the floor. The open-field was enclosed in a ventilated, sound-attenuating box which was kept dark during experimental sessions. Movement of the animal resulted in interruptions of photobeams, which were collected and counted by a Commodore PC20-III microcomputer. The following variables were calculated: horizontal activity (all photobeams interruptions in the lower rows); peripheral activity (all interruptions of photobeams spaced next to the wall in the lower rows); forward locomotion (successive interruptions of photocells in the lower rows when the animal was moving in the same direction); and rearing (all photobeam interruptions in upper rows). The percentages of peripheral activity and forward locomotion counts to horizontal activity counts were also calculated to assess the pattern and the quality of locomotion. Thus, the percentages of peripheral to horizontal activity counts and forward locomotion to horizontal activity counts are indicative of the spatial distribution of movements and perseverance of forward locomotion, respectively, in an open-field (Ericson et al., 1991).

On the day of experiments, i.e., 24 h after the last injection of SAL or NIC (see treatment schedule above), rats were brought to the behavioral testing room in carrying cages and placed in the open-field arena for a 1 h habituation period. Then both groups of rats (NIC or SAL pretreated) were first injected with SAL (1 ml/kg; s.c.) and immediately placed in the open-field arena for a 1 h recording session. Thereafter, animals were injected with NIC (0.5 mg/kg expressed as free base, s.c.), and immediately placed in the open-field for an additional 1 h recording session. The open-field arena was wiped clean after each session. Behavioral experiments were conducted during the day between 08:00 and 17:30 h.

**Microdialysis experiments**

On the 11th day of injections animals were anesthetized with barbiturates (Mebumal, 60 mg/kg, i.p.) and placed in a stereotaxic frame (Kopf, Tujunga, CA). Vertical probes of the concentric type were stereotaxically implanted in either the PFC or the NAC. Coordinates (in mm; with the incisor bar set at −3.3) measured from bregma were: AP + 3.0, ML ± 0.6, DV-5.2 and AP + 1.6, ML ± 1.4, DV-8.2 for the PFC and NAC, respectively (Paxinos and Watson, 1986).

Dialysis experiments were conducted approximately 48 h after surgery during the daylight period, in freely moving rats. Dialysis occurred through a semipermeable membrane (copolymer of acrylonitrile and sodium methallyl sulfonate, I.D. = 0.24 mm, 40,000 Da, AN 69 Hospal), having an active surface length of 4.0 mm and 2.25 mm for the PFC and the NAC probes respectively. Microdialysis was performed using automated on-line sampling (Nisell et al., 1994; Nomikos et al., 1989). The dialysis probes were perfused with a physiological solution containing 147 mM NaCl, 3.0 mM KCl, 1.3 mM CaCl₂, 1.0 mM MgCl₂, and 1.0 mM sodium phosphate (pH 7.4) at a rate of 2.5 μl/min set by a microperfusion pump (Harvard Apparatus, South Natick, MA). The dialysate was loaded directly into the sample loop of the injector (Valco Instruments Co., Houston, TX) and automatically injected into the analytical system every 40 min or 20 min for the animals implanted with probes in the PFC or the NAC, respectively. An adjustable timer (DVSP, Valco) controlled the loading and injection modes of the injector. Within a week after the experiments, animals were sacrificed and brains preserved in 5% formalin. Each brain was sliced on a microtome (50 μM), stained with neutral red, and examined under microscope for probe placement. Only rats with probes verified to be located in the PFC or NAC (see Fig. 1A,B) were included in this study.

Concentrations of DA, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ED) as previously described (Nisell et al., 1994; Nomikos et al., 1989). Separation of DA and the acid metabolites was achieved by reverse-phase liquid chromatography (150 × 4.6 mm, Nucleosil 5 μM, C18) with mobile phase consisting of 0.055 M sodium acetate with 0.7 mM octanesulfonic acid, 0.01 mM Na₂EDTA and 11% methanol (pH 4.1, adjusted with glacial acetic acid). The mobile phase was delivered by an HPLC pump (LKB 2150) at 0.8 ml/min. Electrochemical detection was accomplished using a coulometric detector (Coulchem II, model 5200, ESA) with a high sensitivity analytical cell (5011) in which amine detection was achieved by the sequential oxidation and reduction of the eluent (coulometric electrode = +0.4 V; amperometric electrode = −0.2 V). Chromatograms were recorded on a two-pen chart recorder (Kipp and Zonnen, Delft, Holland). After a stable (<10% variation) outflow of DA and metabolites were established, SAL (1 ml/kg; s.c.) followed 120 min later by NIC (0.5 mg/kg, expressed as free base; s.c.) was administered to all animals.

**Single unit recordings**

Animals were chronically (12 days) treated with SAL or NIC according to the schedule described above. Approximately 24 h after the last injection, animals were anesthetized with chloral hydrate (400 mg/kg, i.p.), with additional doses given when needed to maintain surgi-
cal anesthesia throughout the experiment. Rectal temperature was maintained at 37–38°C by means of an electric heating pad. A tracheal cannula and a jugular vein catheter were inserted before the animal was mounted in a David Kopf stereotaxic instrument. A hole was drilled above the recording area, i.e., AP + 3.0, measured from the interaural line, and ML ± 0.7 mm (Paxinos and Watson, 1986). Electrodes were pulled from Omegadot glass capillaries and filled with 2% Pontamine Sky Blue in 2 M NaCl. The tips were broken under microscope, yielding an impedance of 2.0–4.0 MΩ at 135 Hz. Presumed DA neurons were found 7.5–8.5 mm from the brain surface and displayed characteristics of histochemically identified DA neurons (Grace and Bunney, 1983; Wang, 1981), i.e., typical triphasic spike waveforms of more than 2.0 ms duration and basal firing rates of 1–10 Hz. Extracellular action potentials were amplified, discriminated and monitored on an oscilloscope and an audiomonitor. Discriminated spikes were fed, via a Cambridge Electronics Design 1401 interface, into an AST Bravo LC 466d computer with Spike 2 software. Analysis of the temporal pattern of firing of the DA neurons was performed off-line with Spike 2 software. Burst firing, firing rate, and variation coefficient were calculated over a period of 500 consecutive inter-spike intervals. The onset of a burst was defined by an interval shorter than 80 ms and burst termination at the next interval exceeding 160 ms (Grace and Bunney, 1984). Burst firing was quantified as the percentage ratio between spikes in bursts and the total number of spikes. Variation coefficient was defined as the percentage ratio between the standard deviation and the mean value of the inter-spike intervals (Werner and Mountcastle, 1963).

Nicotine was administered in the dose range 6–100 μg/kg (expressed as free base; i.v.), cumulative dose, with doubling of the dose for each step yielding a total of five consecutive intravenous injections 3–5 min apart. At the end of each experiment a negative current of 5 μA was passed for 8 min through the electrode to mark the recording site with dye (Lodge et al., 1974). The animals were killed by an overdose of chloral hydrate and the brains were preserved in 5% formaldehyde and later sliced on a microtome in 50 μm thick sections and stained with neutral red for histological verification of recording sites. All recording sites included in this study were located within the VTA. Furthermore, the anatomical localization of recording sites in either the nucleus paranigralis (PN; Fig. 1C) or the nucleus parabrachialis pigmentosus (PBP; Fig. 1D), two
major anatomical subdivisions of the VTA in the rat (Bayer and Pickel, 1990; Murase et al., 1993b; Oades and Halliday, 1987; Paxinos and Watson, 1986; Phillipson, 1979), was determined.

Data analysis

Behavioral raw values of 10 min recordings of the four variables (horizontal and peripheral activity, forward locomotion, and rearing) were subjected to a square root transformation or expressed as the percentages of peripheral activity and forward locomotion to horizontal activity counts and statistically analyzed by two-way ANOVA with repeated measures followed by the Newman-Keuls test with a criterion of P < 0.05 to be considered significant.

Biochemical data were calculated as percent changes of dialysate basal concentrations, 100% being defined as the average of the last three values before SAL injection. All subsequent measures were related to these values, and the mean percentages were calculated for each sample across rats in all groups. The percent changes (last baseline plus all postinjection samples) were then used for statistical evaluation. Data were analyzed by one- and two-way (treatment × time) analysis of variance (ANOVA) with repeated measures, followed by the Newman-Keuls test for multiple comparisons with a criterion of P < 0.05 to be considered significant.

Electrophysiological data were presented as means ± S.E.M. with the exception of burst firing values which were presented as means only, since they deviate from a normal distribution. Five hundred spikes preceding the first drug injection and five hundred spikes after injection of each dose of NIC were used for calculating baseline and postinjection values. The effects of NIC on firing rate and variation coefficient were analyzed by Student's paired t-test, whereas burst firing values were analyzed by Wilcoxon matched-pairs signed ranks test. Comparisons between groups were made by using either a Student's unpaired t-test for firing rate and variation coefficient data, or a Mann-Whitney U test for burst firing data. In all statistical analyses a two-tailed P < 0.05 was considered significant.

RESULTS

Effects of nicotine on locomotor activity

During the 1 h habituation period and after SAL injection, no difference in various parameters of locomotor activity was detected between animals treated chronically with SAL (n = 9; Fig. 2) or NIC (n = 9; Fig. 2). A challenge injection of NIC, however, produced substantially higher effects on the measured parameters of locomotor activity in animals treated chronically with NIC compared to the SAL-treated animals (Fig. 2). Specifically, NIC increased horizontal and peripheral activity as well as forward locomotion more in animals treated chronically with NIC than in those treated with SAL (Fig. 2A,B,C). This difference was statistically significant (P = 0.001–0.05) during the first 10 min period (Fig. 2A,B,C). In contrast to the other measures of locomotor activity, NIC challenge in the SAL treated rats transiently decreased rearing (Fig. 2D). On the other hand, NIC administered in the animals treated chronically with NIC produced a pronounced increase in rearing that was significantly (P = 0.001–0.05) different from controls during the first 30 min postinjection period (Fig. 2D). Chronic NIC treatment did not influence significantly the spatial distribution of movements or the pattern of locomotion after NIC challenge as reflected in the ratios of peripheral to horizontal activity and forward locomotion to horizontal activity counts, respectively (data not shown).

Effects of nicotine on extracellular concentrations of DA and its metabolites in the PFC

Basal values of extracellular DA and its metabolites in the PFC did not differ significantly between the two groups of animals (NIC- or SAL-pretreated). The overall mean (±S.E.M.) basal values of DA, DOPAC, and HVA in the SAL pretreated group (n = 6) were 0.57 ± 0.07, 33.6 ± 6.2, and 55.2 ± 8.5 fmol/min, respectively, and in the NIC pretreated group (n = 7) 0.58 ± 0.10, 41.1 ± 4.3, and 76.5 ± 9.3 fmol/min, respectively.

Saline did not significantly affect extracellular DA concentrations in SAL- or NIC-pretreated animals (Fig. 3A). Nicotine significantly increased dialysate DA concentrations for 80 min (P = 0.001–0.002) compared to baseline in SAL-pretreated animals (n = 6, Fig. 3A). The peak effect (+40%) appeared within the latter 40 min of this period. In NIC pretreated animals (n = 7), NIC produced a significant increase in extracellular DA levels compared to baseline (P = 0.0002–0.02; Fig. 3A) with the peak effect (+67%) appearing within 40 min after the injection of NIC; DA levels then remained significantly elevated throughout the rest of the sampling period. Between groups comparisons revealed that the effect of NIC challenge on cortical DA release in rats chronically treated with NIC was significantly larger than in SAL-pretreated animals (P = 0.002-0.04) during the first 80 min after the injection of NIC (Fig. 3A).

A challenge injection of NIC significantly elevated cortical concentrations of DOPAC compared to baseline levels (+57%, 80–200 min post injection; Fig. 4A) in SAL pretreated animals as well as in NIC-pretreated animals (+45%, 40–200 min postinjection; Fig. 4A). Similarly, extracellular concentrations of HVA were significantly elevated in animals pretreated with SAL (+71%, 80–200 min postinjection; Fig. 4C) and in animals pretreated with NIC (+62%, 80–200 min postinjection; Fig. 4C). No difference was found in the effects of NIC on extracellular concentrations of DA metabolites...
between SAL- and NIC-pretreated animals at any time-point.

Effects of nicotine on extracellular concentrations of DA and its metabolites in the NAC

Basal values of extracellular DA and its metabolites in the NAC did not differ significantly between the two groups of animals (NIC- or SAL-pretreated). The overall mean (±S.E.M.) basal values of DA, DOPAC, and HVA in the SAL pretreated group (n = 5) were 4.82 ± 1.43, 902 ± 83 and 482 ± 47 fmol/min, respectively, and in the NIC-pretreated group (n = 6) 3.85 ± 0.78, 702 ± 106, and 348 ± 47 fmol/min, respectively.

Saline did not significantly affect extracellular DA concentrations in SAL- or NIC-pretreated animals (Fig. 3B). Nicotine significantly increased (P = 0.00002–0.003) dialysate DA concentrations, compared to baseline, 20–140 min postinjection in SAL-pretreated animals (n = 5, Fig. 3B). The peak effect (+72%) appeared within 80 min after the injection of NIC. In NIC-pretreated animals (n = 6), NIC produced a significant increase in extracellular DA levels compared to baseline (P = 0.0002–0.0015; Fig. 3B) 40–140 min postinjection, with the peak effect (+60%) appearing within 80 min after the injection of NIC. Between group comparisons did not reveal any significant difference in accumbal DA concentrations between SAL- or NIC-pretreated animals at any time-point. Given that basal DA levels were approximately 25% higher in the SAL-pretreated animals, data were also expressed and analyzed as absolute values. Still, no significant difference was found between SAL- or NIC-pretreated animals in their response to a NIC challenge.

A challenge injection of NIC significantly elevated accumbal concentrations of DOPAC compared to base-
line levels in rats pretreated with SAL (+46%, 40–120 min postinjection; Fig. 4B) as well as in rats pretreated with NIC (+41%, 40–140 min postinjection respectively; Fig. 4B). Also, accumbal concentrations of HVA were significantly increased by a NIC challenge in both SAL (+58%, 40–180 min postinjection; Fig. 4D) and NIC-pretreated animals (+58%, 40–180 min postinjection; Fig. 4D). No difference was found in the effects of NIC on extracellular concentrations of DA metabolites between SAL- and NIC-pretreated animals at any time-point.

**Effects of nicotine on neuronal activity of ventral tegmental area DA cells**

Baseline values of the three parameters of neuronal activity did not significantly differ between SAL- or NIC-pretreated animals. In general, NIC (6–100 μg/kg, i.v.) administered to SAL- as well as NIC-pretreated rats markedly increased burst activity without, or only modestly, affecting firing rate (Fig. 5).

In rats chronically treated with SAL, NIC significantly increased burst firing compared to baseline (n = 17-5) when administered in the dose range 12–100 μg/kg (P = 0.002–0.043; Fig. 6A). In contrast, NIC administered to rats pretreated with NIC (n = 20-9) significantly increased burst activity in the dose range 6–100 μg/kg (P = 0.003–0.049; Fig. 6A). Between group comparisons did not reveal any significant difference in response magnitude to various challenge doses of NIC between SAL- and NIC-pretreated animals. Statistical analysis did not reveal any significant difference in DA neuron responsivity to any dose of NIC between cells located in the PN or PBP nuclei (data not shown).

However, given that cells which were not positively assigned to either the PN or the PBP were excluded, it was not possible to assess the effects of high doses of NIC.

In both SAL- and NIC-pretreated animals challenge injections of NIC caused a discrete but significant increase in the firing rate of VTA-DA neurons (Fig. 6B). Furthermore, in both groups of rats, this effect of NIC was significant in the dose range 25–100 μg/kg and histological inspection did not reveal any significant difference in responsivity between DA neurons located in the PN or PBP nuclei (data not shown).

The variation coefficient was not significantly affected by any dose of NIC challenge in either SAL- or NIC-pretreated animals (Fig. 6C), nor did the histological examination reveal any significant difference in NIC's effect on the variation coefficient after SAL or NIC pretreatment between DA neurons located in the PN or PBP nuclei (data not shown).

**DISCUSSION**

The major finding of the present study in the rat is that sensitization of behavioral responses to a NIC challenge after chronic, intermittent treatment with the drug is paralleled by a selective enhancement of DA release in the PFC. In contrast, the stimulatory effect of NIC on accumbal DA release was not altered by chronic, intermittent NIC pretreatment.

The presently observed behavioral sensitization to NIC during chronic administration is consonant with previous studies (c.f. Introduction), although in some of these an initial behavioral depression was observed with subsequent tolerance development (Clarke and...
Kumar, 1983; Morrison and Stephenson, 1972; Stolerman et al., 1973). However, other studies report an immediate, stimulatory effect of NIC on locomotor activity in drug-naive animals, similar to our results, using roughly the same dose range (Ksir et al., 1985). Interestingly, also in the present study one, specific behavioral parameter, rearing, showed an initial decrease to acutely administered NIC in agreement with other results (Jerome and Sanberg, 1987). Moreover, this behaviorally depressant effect of NIC displayed tolerance during our chronic, condition-independent treatment. Similar results on rearing have been obtained also in condition-dependent experiments with NIC (Walter and Kuschinsky, 1989). Thus, the overall behavioral sensitization to NIC in our as well as other behavioral studies appears to involve both sensitization to the central stimulant effect of the drug as well as a concomitant desensitization to some of its behaviorally depressant actions. The overall sensitization seems quite robust, since it has been observed both in condition-dependent (e.g., Clarke and Kumar, 1983) and in several condition-independent studies (Benwell and Balfour, 1992; Morrison and Stephenson, 1972; Stolerman et al., 1973), including the present study.

The NIC-induced DA release in the PFC in drug-naive rats has, to our knowledge, not been previously reported. Furthermore, this effect of NIC on cortical DA release was enhanced after chronic NIC administration, which is in accordance with some previous observations (Vezina et al., 1992) seen after similar pretreatment with NIC. However, our finding that the increased effect on cortical DA release after chronic NIC was not accompanied by an enhanced effect also on extracellular levels of DOPAC and HVA in the PFC suggests that the sensitization after chronic NIC is restricted to DA release, but may not include DA synthesis.
Fig. 5. Lower panel: Rate meter histogram recorded from a typical ventral tegmental area neuron showing the effect of four consecutive doses of nicotine (6, 12, 25, and 50 μg/kg, i.v.) on the firing rate. Upper panel: Interspike time-interval histograms (ISHs) were made during periods indicated by the braces, before nicotine administration and after 12 and 50 μg/kg of nicotine. Burst firing is indicated by the solid black bars.

In accordance with previous reports (Damsma et al., 1989; Imperato et al., 1986; Nisell et al., 1994), also this study found that systemic injections of NIC increased extracellular concentrations of DA in the NAC in drug-naive (i.e., SAL-pretreated) animals. The finding that pretreatment with NIC did not alter this response is in agreement with some previous results (Damsma et al., 1989). On the other hand, our results are in apparent contrast to those of Benwell and Balfour (1992), who found that pretreatment with NIC produced an apparently increased effect of NIC on accumbal DA release. The latter study, however, failed to show an increase in DA release in the NAC in response to acutely administered NIC, in contrast to most other studies (c.f. Introduction). These discrepant results may, at least in part, be explained by different experimental procedures that make direct comparisons between studies difficult. Studies in which ex vivo tissue measurements of DA metabolism were used to assess the effect of NIC after chronic, intermittent treatment have been interpreted to support both enhanced (Clarke et al., 1988), attenuated (Lapin et al., 1989; Vezina et al., 1992) as well as unchanged (Lapin et al., 1987, Mitchell et al., 1989) effects. Taken together, the results of both ex vivo and in vivo biochemical studies do not provide unequivocal evidence for consistent changes in accumbal dopaminergic transmission following chronic NIC treatment, in consonance with our results.

The stimulatory effect of NIC on the neuronal activity of VTA-DA cells in SAL-pretreated animals confirms previous data (Grenhoff et al., 1986), which showed a particularly pronounced effect of acute NIC on burst activity in VTA-DA neurons. Accordingly, in the present study, NIC significantly increased burst activity in control animals at a lower dose (12 μg/kg) than that needed to produce the modest, but significant increase in firing rate (25 μg/kg). In another electrophysiological study, by Mereu et al. (1987), intravenous administration of NIC to awake, tubocurarine-paralyzed rats produced a substantial increase in the firing rate of VTA-DA neurons, in a similar dose-range to that used in our experiments. However, in similar experiments in chloral hydrate anesthetized rats these authors found that NIC induced a transient decrease in firing rate. Although a very short-lasting inhibition of neuronal activity, often followed by a long-lasting increase, was observed also in some cells in our experiments, excitation remains as the major electrophysiological effect of NIC on VTA-DA cells.

In the present experiments, the stimulatory effect of NIC on burst activity was more pronounced in cells of animals pretreated with NIC than in those pretreated...
VTA-DA neuronal activity

![Graph showing the effects of nicotine on burst firing, firing rate, and variation coefficient in VTA-DA neurons.](image)

**A** Burst firing, **B** Firing rate, and **C** Variation coefficient as a function of nicotine dose (µg/kg) and treatment group (Chronic saline vs. Chronic nicotine).

**Fig. 6.** Effects of nicotine (6, 12, 25, 50, and 100 µg/kg, i.v.) on single neurons of the ventral tegmental area with regard to three parameters of neuronal firing: (A) burst firing, (B) firing rate, and (C) regularity of firing, measured by the variation coefficient. Animals received daily injections of saline (n = 17-5; open bars) or nicotine (0.5 mg/kg, s.c.; n = 20-9; solid bars) for 12 consecutive days. Stars indicate level of significance for the effect of each dose of nicotine compared to its respective control. *, P < 0.05; **, P < 0.01.

with SAL. Thus, a challenge injection of NIC already at the lowest dose (6 µg/kg) significantly enhanced burst activity of VTA-DA neurons in NIC, but not SAL pre-treated rats. As previously suggested (Nisell et al., 1995), the preferential action of NIC on burst activity, i.e., the physiological response of these cells to attentional and motivational sensory stimuli (Schultz, 1986; Schultz et al., 1993), may be highly relevant to the process of learning the habit of tobacco smoking. In addition, an enhanced burst stimulatory effect of NIC during chronic treatment may represent an important mechanism for maintenance of the acquired smoking behavior.

The mechanisms behind the enhanced effect of NIC on behavior as well as cortical DA release after chronic, intermittent treatment remain presently unclear. The fact that chronic NIC pretreatment in rodents does not affect the disposition of a challenge injection of NIC (Pekonen et al., 1993) argues against an altered metabolic rate as a mechanism, whereby the effect of NIC is enhanced. As previously shown, behavioral sensitization to NIC is associated with an increase in brain nicotinic binding (Ksir et al., 1985; Lapchak et al., 1989) and NIC binding sites are also increased after tobacco smoking in human cortical and subcortical regions (Benwell et al., 1988). This phenomenon may be of importance for the sensitization to both behavioral and biochemical effects of NIC. However, Lapchak et al. (1989) demonstrated an increased binding of NIC both in the PFC and in the striatum, a finding that argues against the notion that regional differences in receptor binding after chronic NIC could explain the selectively increased responsivity to NIC in the mesocortical dopaminergic projection. On the other hand, a differential distribution of nicotinic receptor subunits has been demonstrated in cortical as compared to subcortical areas (Wada et al., 1989). Consequently, there may be regional differences in receptor function and, accordingly, also in their adaptation to chronic agonist exposure which would help to explain the regional selectivity observed.

Changes in the sensitivity of DA receptors may also be involved in the development of sensitization to the response to NIC. It is now well established that DA neurons projecting to the PFC are equipped with release modulating autoreceptors, but seem to lack autoreceptors modulating synthesis (see Roth and Elsworth, 1994). Thus, one tentative explanation to our finding that chronic pretreatment with NIC selectively enhances NIC-induced DA release within the PFC without affecting DA synthesis, may be desensitization of release modulating DA autoreceptors. Furthermore, a change in sensitivity of postsynaptic DA receptors in the NAC after chronic NIC treatment could, conceivably, contribute to the behavioral sensitization to NIC, in spite of the fact that accumbal DA release was not altered. However, studies examining binding to subcortical DA receptors after chronic NIC administration have not yielded conclusive results (Jansson et al., 1992; Kirch et al., 1992; Reilly et al., 1987). Thus, the notion that altered DA receptor sensitivity may contribute to the observed sensitization to the behavioral and biochemical effects of NIC remain to be verified.

Although chronic NIC treatment selectively enhanced the NIC induced DA release in the PFC, the
magnitude of the response in the PFC after chronic NIC was still similar to that seen in the NAC both before and after NIC pretreatment. Thus, the increased DA release in the NAC may still contribute to the overall behavioral stimulation in NIC-pretreated animals, as suggested previously (Clarke et al., 1988). In addition, different doses of administered NIC as well as differences in the length of the NIC treatment period may account for discrepancies between the present results and those of Benwell and Balfour (1992) or Reid et al. (1994), which both show behavioral sensitization accompanied by increased release of accumbal DA after NIC pretreatment. Specifically, in the latter studies rats were treated with NIC for 5 days and it is possible that the sensitized increase in DA release in the NAC observed after pretreatment with NIC wanes when NIC is administered for a longer period which was used in the present study. The shorter duration of NIC treatment utilized by Reid et al. (1994) may also explain why these authors only observed condition-dependent sensitization to the behavioral effects of NIC.

Several experiments have suggested that the dopaminergic innervation of the PFC in particular, and PFC activity in general, influences subcortical dopaminergic activity. For example, 6-hydroxydopamine lesions of the PFC have been reported to increase various parameters of subcortical dopaminergic functioning (Carter and Pycock, 1980; Pycock et al., 1980a,b). Although some aspects of these original observations have not been replicated (Joyce et al., 1983; Oades et al., 1990), it has recently been shown by Deutch and co-workers in a series of experiments that anatomically specific prefrontal cortical DA lesions augment the responsiveness of the mesolimbic DA system (for reviews, see Deutch, 1992, 1993). However, experimentally induced, general inhibition of the PFC region has been found to attenuate accumbal DA release (Moghaddam and Karrenman, 1994; Murase et al., 1993a,b). Moreover, Murase et al. (1993b) as well as Taber and Fibiger (1993) demonstrated increased subcortical DA release after regional stimulation of the PFC. Consequently, a successive augmentation of cortical DA release during chronic NIC administration, as indicated by the present study, might per se serve to attenuate an initially enhanced accumbal dopaminergic response, as observed after the short-lasting NIC pretreatments.

As previously suggested (Nisell et al., 1995; Svensson et al., 1990), the observed enhancement of mesocortical dopaminergic activity may be of considerable interest in view of the extremely high prevalence of tobacco smoking among schizophrenic patients (Lohr and Flynn, 1992). Substantial clinical information exists, which demonstrates reduced functional activity in the frontal cortex, so-called hypofrontality, in schizophrenics, a phenomenon which has been shown to correlate with negative symptoms (Ingvar, 1987; Weinberger, 1993). Interestingly, acute NIC can partly restore the reduced burst firing in VTA-DA neurons, which has been found associated with experimental hypofrontality (Tung et al., 1990). Thus, the present findings suggest that NIC may be even more effective in reversing functional consequences of hypofrontality, at the VTA-DA neuronal level, during chronic treatment. Although behavioral sensitization has been observed also after chronic treatment with cocaine, this phenomenon, in contrast to the sensitization to NIC, is accompanied by a decreased dopaminergic activity in the PFC (Sorg and Kalivas, 1993). Similarly, chronic pretreatment with morphine attenuates the initial, stimulatory effect on prefrontal DA utilization seen after a single injection (Vezina et al., 1992). The differential adaptive patterns of the mesocortical DA system to these, other types of dependence-producing drugs might help to explain why NIC dependence, in contrast to the dependence to cocaine or morphine, does not distort mental functioning which involves the prefrontal cortex.

In summary, the present study demonstrates a condition-independent sensitization of NIC-induced locomotor activity after chronic, intermittent treatment with NIC which is paralleled by an enhanced NIC-induced DA release in the PFC but with an apparently unaltered DA response in the NAC. Moreover, NIC pretreatment was found to augment the functional responsiveness of VTA-DA neurons to the stimulatory actions of a low dose of NIC, in particular as regards burst activity. The selective enhancement of dopaminergic transmission in the PFC may be unique for NIC, as compared to other drugs of abuse. Interestingly, a preferential, stimulatory effect on prefrontal cortical dopamine release has also been observed following acute administration of several atypical antipsychotics and potent 5-HT2-receptor antagonists such as clozapine and amperozide, as well as selective 5-HT2-receptor agonists, such as (R)-8-OH-DPAT (Arborelius et al., 1993; Moghaddam and Bunney, 1990; Nomikos et al., 1994; Svensson et al., 1995). This may, in turn, suggest a tentative role for such agents as pharmacological aids in smoking-cessation programs. Our findings support the notion that the high prevalence of NIC dependence in schizophrenia may, in part, represent an attempted self medication.

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