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Enhanced motor activity and brain dopamine turnover in mice during long-term nicotine administration in the drinking water

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Abstract

Nicotine was administered chronically to NMRI mice in their drinking water in gradually increasing concentrations to measure gross motor activity and brain nicotine concentrations over 24 h on the 50th day of nicotine administration. Also, the striatal postmortem tissue concentrations and accumbal extracellular concentrations of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured to study the role of dopaminergic systems in nicotine-induced hyperactivity in mice. The cerebral nicotine concentration was at its highest at the end of the dark period. The activity of nicotine-treated mice and their striatal DA metabolism were parallelly increased at 2 to 3 h after midnight and in the forenoon. Microdialysis experiments carried out in the forenoon showed that the extracellular levels of DA and DOPAC were elevated in the nucleus accumbens of these mice. Nicotine did not alter the circadian rhythmicity of activity in the mice. Rather, our findings suggest that the mice consume more nicotine when active and this might lead to enhanced release and metabolism of DA and further, to enhanced motor behavior. These findings support the suggestions that nicotine's effects on limbic and striatal DA are critical for its stimulating effects.

Keywords: Chronic nicotine; Motor activity; Dopamine; Striatum; Nucleus accumbens

1. Introduction

The habit of tobacco-smoking, a major public health problem that increases morbidity and mortality (Peto et al., 1992), is evidently a form of drug addiction to nicotine (Stolerman and Jarvis, 1995). Like other addictive drugs nicotine reinforces self-administration and place preference in animal studies (Corrigall and Coen, 1989; Henningfield and Goldberg, 1983; Stolerman and Shoaib, 1991). The reinforcing properties of nicotine are thought to be mediated through central dopaminergic systems, especially by the mesolimbic system (Corrigall et al., 1992; Stolerman and Shoaib, 1991). In addition to their role in reinforcement, the brain dopaminergic pathways are crucial in the locomotor stimulatory effects of psychomotor stimulants as well as of several other abused drugs (Wise and Bozarth, 1987; Di Chiara and Imperato, 1988). In rats, nicotine stimulates locomotor activity, and a more pronounced stimulatory effect (reverse tolerance or behavioral sensitization) has been demonstrated after repeated nicotine administration (Benwell and Balfour, 1992; Clarke and Kumar, 1983; Morrison and Stephenson, 1972; Stolerman et al., 1973). Furthermore, there is evidence that increased dopaminergic activity in the nucleus accumbens is responsible for the nicotine-induced enhancement of locomotor activity in rats (Benwell and Balfour, 1992; Clarke, 1990; Leikola-Pelho and Jackson, 1990). In mice, acute nicotine usually decreases activity (Hatchell and Collins, 1977; Marks et al., 1985; Pietilä et al., 1998), and a locomotor stimulating effect of nicotine in mice is described in only a few reports (Freeman et al., 1987; Sershen et al., 1987). Sensitized locomotor response to nicotine has not been demonstrated in mice.

We have earlier shown that nicotine can be administered in the drinking water to NMRI mice in doses resulting in plasma nicotine concentrations similar to and above those reported in smokers (Pekonen et al., 1993). Increased ^{3}H-nicotine binding in cortex and midbrain as well as tolerance to the locomotor depressant effects of a nicotine challenge were found after withdrawal from 7-week oral nicotine administration, whereas after 4-week oral nicotine

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no tolerance to the locomotor depressant effect of nicotine was found (Pietilä et al., 1998). The plasma nicotine and cotinine concentrations of these mice were at their highest during the night showing that the mice as nocturnal animals consumed most of their daily fluid intake during the night (Pietilä et al., 1995) and thus received most of their daily nicotine dose during their active period as the humans smoking do (Benowitz et al., 1990). Also, we found that the circadian rhythm of dopamine (DA) metabolism was significantly altered so that the tissue concentrations of DA and its main metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the nicotine-treated mice were elevated in the forenoon but did not differ from controls at three other time points (15:00, 21:00 or 05:00) (Pietilä et al., 1995). Furthermore, recently we found that the locomotor activity was increased in the forenoon in mice still drinking nicotine (Gäddnäs et al., 2000). In the present experiments we have further studied the circadian rhythmicity of the stimulatory effects of nicotine during chronic administration and the relationship of nicotine-induced stimulation to cerebral DA turnover. We measured gross motor activity of mice continuously over 24 h on the 50th day of chronic nicotine administration in the drinking water. We used in vivo microdialysis to measure extracellular levels of DA and its metabolites in the nucleus accumbens of freely moving mice in the forenoon on the 50th day of nicotine administration. Further, we measured the postmortem striatal concentrations of DA and its metabolites at that time in the dark period when differences were found in activity between nicotine-treated and control mice. Also, we estimated brain concentrations of nicotine at four different times of day in mice during the chronic nicotine treatment.

2. Methods

2.1. Administration of nicotine

Five-week-old male NMRI mice (body weight 20–25 g at the beginning of experiments) bred locally in the Laboratory Animal Center, University of Helsinki, were divided randomly into nicotine-receiving and control mice. Experimental animals were maintained in accordance with internationally accepted principles, and the experimental setup was approved by the Committee for Animal Experiments of the Faculty of Science at the University of Helsinki. Groups of six mice were housed in a cage and had free access to mouse chow. The ambient temperature was held at 20–22 °C. The mice were maintained on a 12:12-h light/dark (LD) cycle (12 h light, 12 h dark) with lights on from 06:00 to 18:00. (−)-Nicotine (Fluka, Buchs, Switzerland) was administered chronically in the drinking water (the sole source of fluid) as described earlier by Pekonen et al. (1993). The concentration of nicotine in the drinking water was gradually increased at 3–4-day intervals from 50 to 350 μg/ml at 3 weeks, and after this at 7-day intervals up to 500 μg/ml at 7 weeks to coax the mice to drink as steadily as possible. The control mice drank tap water during the entire treatment. Body weights and fluid intake were recorded once a week. After 2–3 weeks the fluid intake and the weight gain of the nicotine-treated mice started to diminish, so that at 7 weeks the mice offered the 500 μg/ml nicotine solution drank about 47% and weighed 9% less than the corresponding control mice, as has also been found previously (Pekonen et al., 1993). Due to the decreased consumption of water the amount of nicotine consumed daily remained at about 60 mg/kg from the third week onward up to the end of the 7-week study. Nicotine is known to be a powerful stimulant of antidiuretic hormone release and thus reduces the output of urine (Burn et al., 1945). Since small rodents maintain their fluid balance very effectively, it appears that the mice reduced their fluid intake to compensate for the reduced fluid output. In addition, the hypodipsia produced by the higher nicotine concentrations could be partially due to the bitter taste of the solution offered.

2.2. Activity experiments

The mice were brought in their home cages (six mice per cage) to the experimental room 3–4 days before the experiments and were housed in a Scantainer (Scanbur, Denmark). On the test day the animals were placed in groups of three in transparent plastic cages (22 × 38 × 15 cm) with perforated plastic lids. New sawdust covered the bottom of the cage and food was scattered on the floor. The cages were placed in activity monitors (MED Associates' open field activity monitor, St. Albans, USA) and the animals were allowed to habituate for 2–3 h before the activity monitoring began at 10:30. Infrared photobeam interruptions were registered and analyzed by the software of the activity monitor. The activity was monitored continuously over 24 h. The counting intervals were 30 min. Nicotine solution or tap water (control mice) was continuously available during the acclimation period and during the whole experiment.

2.3. Measurements of striatal DA and metabolites

The mice were decapitated and the brain was removed from the skull and placed on a glass plate over ice. The optic nerves and olfactory bulbs were removed and discarded. The cortical hemispheres were spread apart and separated by cutting the corpus callosum, and the hippocampi were peeled away. The medial borders of the striata were freed, and the striata (caudate putamen + nucleus accumbens) removed without the underlying cortex. The striata were frozen on dry ice, weighed (mean weight 25 mg) and stored at −80° until assayed. The concentrations of DA, DOPAC and HVA were measured by high-performance liquid chromatography (HPLC) with electrochemical detection after Sephadex G-10 gel chromatographic cleanup of samples as described by Haikala (1987). The system for determining DA consisted of an ESA Coulochem 5100A detector

equipped with a model 5014 analytical cell and a Pharmacia LKB, model 2150, pump with an SSI model 20-0225 pulse damper. The column (Spherisorb ODS2 5 μm, 25 cm, 4.6 mm ID) was kept at 40 °C with a Bio-rad column heater. The system for determining DOPAC and HVA consisted of an ESA Coulochem II detector equipped with model 5014 analytical cell, Beckman, model 110B, pump with SSI model LP-21 pulse damper and Spherisorb ODS2 5 μm (25 cm, 4.6 mm ID) column. Monoamine and metabolite values were calculated as micrograms per gram (μg/g) wet weight of tissue.

2.4. Microdialysis

2.4.1. Surgery

After 6 weeks chronic nicotine treatment the mice were implanted with guide cannulae (CMA/7, CMA/Microdialysis, Stockholm, Sweden) under chloral hydrate anesthesia (450 mg/kg ip). The mice were also treated with buprenorphine (0.6 mg/kg sc) to relieve pain during surgery. The coordinates for guide cannulae were calculated relative to bregma and were aimed at the point above the nucleus accumbens (A/P= + 1.4, L/M= + 0.9, D/V = − 3.8) according to the atlas by Franklin and Watson (1997). The cannula was fastened to the skull with dental cement (Aqualox, Voco, Germany) and two stainless steel screws. After the surgery the mice were placed into individual test cages ($30 \times 30 \times 40$ cm) and allowed to recover for at least 5 days before the experiment. The mice were kept individually in the same cages throughout the experiments. At about 4 p.m. on the day before the experiment, a microdialysis probe (CMA/7, CMA/Microdialysis, Stockholm, Sweden; 1.0 mm membrane, OD 0.24 mm) was inserted into the guide cannula, and the probe was infused with a modified Ringer solution (147 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 1.0 mM $MgCl_2$ and 0.04 mM ascorbic acid) at a flow rate of 0.5 μl/min. In the morning of the experimental day (about 8 a.m.) the flow rate of the infusion was increased to 2 μl/min. After a stabilization period of 2 h the collection of actual microdialysis samples (every 20 min, 40 μl/sample) was started. The average concentration of the first four stable samples (differing not more than ±15% from average) was determined. Nicotine solution or tap water (control mice) was continuously available during the 5-day recovery period and during the experiment.

2.4.2. Determination of DA and its metabolites

The system used for determination of the extracellular concentrations of DA, DOPAC and HVA consisted of an ESA Coulochem II detector (ESA, Massachusetts, USA) equipped with a model 5014B microdialysis cell, a Pharmacia LKB model 2248 HPLC pump (Pharmacia LKB, Sweden) and a SSI model LP-21 pulse damper (Scientific Systems, Pennsylvania, USA). The column (Spherisorb ODS2, 3 μm, 4.6×100 mm) was kept at 40 °C with a column heater (Croco-Cil, France). The mobile phase used consisted of 0.5 M NaH_2PO_4 buffer, pH 4.0 (adjusted with 1.0 mM citric acid), 0.6–0.8 mM octane sulfonic acid, 16% methanol and 1.2 mM EDTA. The flow rate of the mobile phase was 1.0 ml/min. Thirty-five microliters of the dialysis sample was injected onto the column with a CMA/200 autoinjector (CMA, Stockholm, Sweden). DA was reduced with an amperometric detector (potential − 100 mV) after being oxidized with a coulometric detector (+300 mV); DOPAC and HVA were oxidized with the coulometric detector. The chromatogram was processed with a Hitachi D-2000 chromato-integrator (E. Merck, Darmstadt, Germany).

2.5. Measurement of cerebral nicotine concentrations

For the measurements of brain nicotine concentrations some of the mice were from Day 28 of nicotine administration onwards maintained on a reversed LD cycle with lights on from midnight to noon. On the 50th day of nicotine administration mice were killed by decapitation at 5 or 9 h after light onset or at 3 or 11 h after light offset. Thus, the mice kept with lights on from 06:00 to 18:00 were killed at 11:00 or 21:00 and the mice kept with lights on from midnight to noon were killed at 09:00 or 23:00. After removing the cerebellum the brain tissue was weighed and homogenized in 3.0 ml/g 0.1 N HCl. The homogenates were stored at − 80 °C until assayed. Nicotine concentrations were measured by a gas chromatographic–mass spectrometric (GC–MS) method using selected ion monitoring as described by Leikola-Pelho et al. (1990) with minor modifications (Seppä et al., 2000). Analyses were performed on a Hewlett-Packard 5970 quadropole MS coupled to a Hewlett-Packard 5890 GC using a NB-54 fused silica column (15 m, 0.22 mm ID). Fragment ions of *m/z* 84 (nicotine) and 129 (quinoline) were used for single ion monitoring. The concentrations of nicotine were calculated as nanograms per gram (ng/g) brain tissue.

2.6. Statistics

Results are given as means ± S.E.M. Activity data were calculated over 1.5-h intervals and the statistical analysis was performed by one-way analysis of variance (ANOVA) for repeated measures followed by Tukey–Kramer post hoc test. The concentrations of DA and its metabolites were analyzed using Student's *t* test. Results were considered significant at $P < .05$.

3. Results

3.1. Activity

The activity of groups of three mice over 24 h on the 50th day of nicotine administration is shown in Fig. 1.

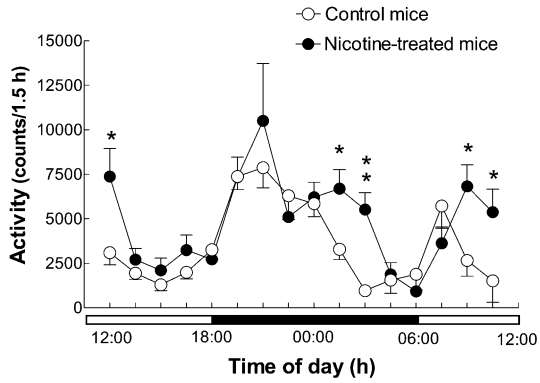

Fig. 1. Activity (counts/1.5 h) of groups of three mice over 24 h on the 50th day of chronic nicotine administration in the drinking water. Mice were maintained on a 12:12-h LD cycle with lights on from 06:00 to 18:00. Means ± S.E.M. from seven groups of three mice are given. * $P < .05$, ** $P < .01$ versus control mice drinking tap water.

ANOVA revealed significant treatment [$F(1,180) = 9.5$, $P < .01$] and time [$F(15,180) = 9.3$, $P < .001$] effects and a significant Treatment × Time interaction [$F(15,180) = 2.3$, $P < .01$]. The diurnal patterns of activity in control mice and nicotine-treated mice were bimodal with two activity peaks, one starting immediately after switching off the lights and a second one following the light onset. The nicotine-drinking mice were significantly more active than the control mice drinking tap water in the three consecutive 1.5-h intervals between 07:30 and 12:00 during the light period ($P < .05$, Tukey–Kramer post hoc test). Also, in the consecutive 1.5-h intervals between midnight and 03:00 during the dark period the nicotine-treated mice were more active than the controls ($P < .05$ and $P < .01$, respectively; Tukey–Kramer post hoc test).

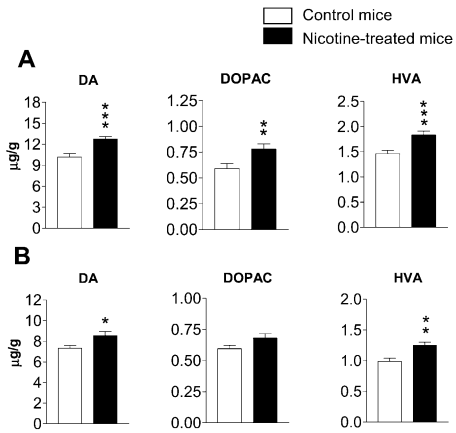

Fig. 2. The striatal concentrations of DA, DOPAC and HVA in mice at 11:00 (A) and 02:00 (B) on the 50th day of chronic nicotine administration in the drinking water. Control mice were drinking tap water. The values given (µg/g) are the mean concentrations ± S.E.M. from 11–17 mice maintained on a 12:12-h LD cycle with lights on from 06:00 to 18:00. * $P < .05$, ** $P < .01$, *** $P < .001$ versus control mice. (Panel A modified from Pietilä et al., 1995 with permission).

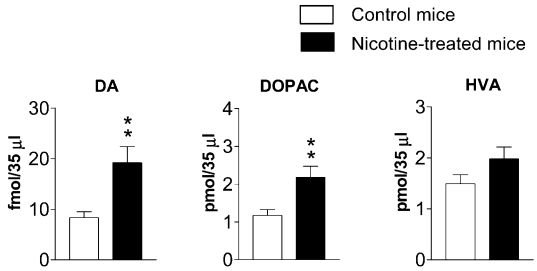

Fig. 3. The concentrations of DA, DOPAC and HVA in the nucleus accumbens dialysates of freely moving mice in the forenoon (10:00 to 12:00) on the 50th day of chronic nicotine administration in the drinking water. Four consecutive 40 µl dialysate samples were collected at 20-min intervals from each animal. The concentrations as pmol (DA) or fmol (DOPAC, HVA)/35 µl sample were averaged. The columns show the means ± S.E.M. of the data obtained from 12 mice maintained on a 12:12-h LD cycle with lights on from 06:00 to 18:00. ** $P < .01$ versus control mice drinking tap water.

3.2. Striatal and accumbal DA

We have earlier found increased concentrations of DA ($P < .001$), DOPAC ($P < .01$) and HVA ($P < .001$) in striatal tissue of mice at 11:00 o'clock on the 50th day with nicotine in the drinking water (Fig. 2A, reproduced from Pietilä et al., 1995). In the present experiment we found significantly elevated striatal concentrations of DA and HVA ($P < .05$ and $P < .01$, respectively) at 02:00 o'clock. Also, the striatal concentration of DOPAC tended to be elevated ($P = .06$) (Fig. 2B). The concentrations of DA and DOPAC were significantly elevated in the nucleus accumbens dialysates of the nicotine-treated mice ($P < .01$, respectively) (Fig. 3). The concentration of HVA in the dialysates was not significantly elevated ($P = .11$).

3.3. Brain concentration of nicotine

The mean brain concentrations of nicotine over 24 h on the 50th day with nicotine in the drinking water are shown in Fig. 4. The highest concentration (329 ± 53 ng/g) was

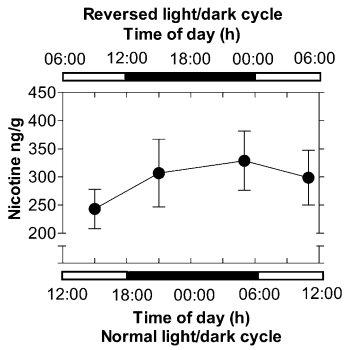

Fig. 4. Mean brain concentrations of nicotine over 24 h in mice on the 50th day of chronic administration of nicotine in the drinking water. The values given (ng/g) are means ± S.E.M. from 11–13 mice maintained on a 12:12-h LD cycle with lights on from 06:00 to 18:00 or from 00:00 to 12:00 (reversed LD cycle), for details see Methods.

found in the dark period at 11 h after light offset (05:00) and the lowest concentration (243 ± 35 ng/g) at 9 h after light onset (15:00).

4. Discussion

The main finding of this study is that on the 50th day of chronic nicotine administration in the drinking water the motor activity of mice was enhanced together with increased DA release and metabolism. Differences in activity levels between nicotine-treated and control mice were found for short periods (3–5 h) both in the light and in the dark phase of the circadian cycle. However, the circadian rhythmicity of activity was not altered. Interestingly, the periods of higher activity in nicotine-treated mice occurred immediately following the activity peaks in the circadian cycle. In fact, the increased activity occurred as prolongation of activity peaks. In laboratory animals the drinking behavior has been found to correlate with the ambulatory activity of the animals (Kita et al., 1985). As discussed in the Introduction, our previous results suggest that mice consume most of the drinking fluid, and thus nicotine, during their active phases and we propose that this intake of nicotine leads to stimulation of motor activity.

The daily pattern of activity found in the present experiment confirms the bimodal circadian pattern obtained in former studies on laboratory mice (Weinert and Waterhouse, 1999). The first, longer-lasting activity peak (in the control mice from about 18:00 to 03:00) occurred after lights were switched off, and the second peak (in the control mice from about 06:00 to 09:00) occurred after the lights were switched on. In the beginning of the active periods the control mice and the nicotine-treated mice were about as active, but in both periods the nicotine-treated mice remained active for about 2 h longer than the control mice. The increased motor activity in nicotine-drinking mice might reflect behavioral sensitization similar to that which has been shown in rats (for references see Introduction). However, it is to be noted that at 24 h after withdrawal from 7-week treatment a subcutaneous injection of a small dose of nicotine (0.5 mg/kg) induced no stimulation of locomotor activity in these mice although the mice were tolerant to the locomotor depressant effect of a nicotine challenge (Pietilä et al., 1998). At this time point Benwell et al. (1995) found clear sensitization of locomotion in rats treated with continuous nicotine infusion (4 mg/kg/day) for 14 days. Sparks and Pauly (1999) showed that consumption of nicotine-containing drinking solution resulted in locomotor activation in C57Bl/6 mice during the dark phase of the circadian cycle, but the activity levels were not altered during the light phase. However, the measurements were only done up to the third day of nicotine administration. In rat experiments, nicotine infusion by subcutaneously implanted minipumps was shown to increase the activity during the light period but not during the night. Tolerance to the stimulant effects of nicotine occurred in 5–6 days (Morley and Garner, 1990). Our findings show that in mice the increased activity level is maintained after extended periods of continuous nicotine treatment in the drinking water and, further, that the activity of mice is stimulated by nicotine both in the light and in the dark phase of the circadian cycle during long-term treatment.

Previously, we reported that on the 50th day with nicotine in the drinking water the striatal DA metabolism in mice was increased in the forenoon at 11:00 (Pietilä et al., 1995). At this time point in the present experiments as well as in our previous experiment (Gäddnäs et al., 2000) the motor activity was increased in nicotine-treated mice. Furthermore, in the previous experiment (Pietilä et al., 1995) no alterations were found in the striatal DA metabolism in the afternoon (at 15:00) or at 21:00 or 05:00 in the dark period, and interestingly in the present experiments no enhanced activity was found at these time points. Because we found increased activity in nicotine-treated mice at 2 to 3 h after midnight in this experiment, we measured the striatal concentrations of DA and its metabolites at this time point (02:00) and found that they were increased. Thus, the DA metabolism and the motor activity were parallelly increased in nicotine-treated mice both in the light and dark phase of the circadian cycle. These findings suggest that the cerebral dopaminergic systems are important in the mediation of nicotine-induced hyperactivity in mice as they have been shown to be of importance in rats (Clarke, 1990; Louis and Clarke, 1998).

The brain area dissected as striatum for the postmortem determinations of DA and its metabolites contained the dorsal (caudate–putamen) as well as the ventral striatum (nucleus accumbens). As the stimulant action of nicotine on motor activity has been linked to enhanced dopaminergic transmission in the nucleus accumbens, we estimated the extracellular concentrations of DA and its metabolites in this area by microdialysis. These experiments were carried out in the forenoon during the activity peak of the light period, when it is technically possible to carry out microdialysis experiments. Indeed, concentrations of DA and DOPAC were increased in nicotine-treated mice as compared to control mice. To our knowledge the effects of chronic continuous nicotine administration on DA output in nucleus accumbens has not been studied previously in mice. Our result is, however, consistent with rat experiments in which nicotine was given by continuous infusion by osmotic minipumps (3 mg/kg/day) for 9 days (Carboni et al., 2000). In contrast, Hildebrand et al. (1998) and Benwell et al. (1995) failed to obtain a significant increase of DA output in nucleus accumbens dialysates after 9 or 14 days of continuous infusion (3–4 mg/kg/day) by osmotic minipumps to rats, suggesting that tolerance had developed to this response. Also, we previously found that mice are clearly tolerant to the DA metabolism enhancing effect of a small dose of nicotine at 24 h after withdrawal from 7-week chronic administration (Pietilä et al., 1996). How-

ever, the results of the present study show that even during long-term continuous nicotine administration the nicotine-induced DA release stimulates activity.

We have earlier shown that in mice treated with nicotine in the drinking water the highest plasma concentration of nicotine was found at nighttime (114 ng/ml at 05:00) when it was more than double the concentrations found at other time points studied (54 ng/ml at 11:00, 48 ng/ml at 15:00, 33 ng/ml at 21:00) (Pietilä et al., 1995). In agreement with previous studies (Benowitz et al., 1990; Mansner and Mattila, 1977; Rowell and Li, 1997) we found that the brain nicotine concentrations exceeded several-fold the plasma nicotine concentrations. In the present study the cerebral concentration of nicotine was at its highest at nighttime at 11 h after lights off. However, the fluctuations in cerebral nicotine concentrations during the day were rather small as compared to fluctuations in plasma concentrations (Pietilä et al., 1995). This can be due to the longer half-life of nicotine in the brain (Sastry et al., 1995).

The cerebral nicotine concentration, found in the nicotine-drinking mice in this experiment, is high enough to induce desensitization of nicotinic acetylcholine receptors (Grady et al., 1994; Pidoplichko et al., 1997; Rowell and Li, 1997). However, our results indicate that even in the presence of these high levels of nicotine, chronic exposure to nicotine does not abolish completely the nicotine-induced stimulation of activity or its effect on DA release and metabolism. Thus, it is possible that desensitization of nicotinic acetylcholine receptors is not complete even in a chronic smoker. Human smoking is characterized by intermittent peak concentrations of nicotine with each cigarette smoked, superimposed on a low, steady state baseline level. As the day progresses baseline levels rise and the influence of peak levels becomes less important. Blood or plasma nicotine concentrations sampled in the afternoon in smokers range from 10 to 50 ng/ml. The increment in venous blood nicotine concentration after smoking a single cigarette ranges from 5 to 30 ng/ml depending on how the cigarette is smoked (Benowitz et al., 1990). If a smoker smokes until bedtime, significant nicotine levels persist all night. It has been shown that nicotinic acetylcholine receptors containing certain subunits (alfa3/alfa6) are relatively resistant to inactivation (Olale et al., 1997). This allows DA release in response to nicotine to take place also during chronic exposure. Furthermore, elevated levels of DA have been found in striatal tissue from smokers as compared with nonsmokers (Court et al., 1998). Recently, increased DA activity in the human basal ganglia of cigarette smokers was found in experiments using [^{18}F]DOPA positron emission tomography (PET) technique (Salokangas et al., 2000).

In conclusion, our findings show that the amounts of nicotine consumed by the mice in the drinking water during their active periods is sufficient to stimulate mesolimbic DA release and to induce enhanced motor activity. Thus, complete tolerance does not develop to these effects even after long-term continuous nicotine exposure. These findings are consistent with the view that limbic and striatal DA are critical for the stimulatory effects, and do not exclude their role in the reinforcing properties of nicotine.

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