Pharmacology, Biochemistry and Behavior 100 (2011) 109–118

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Repeated pre-exposure to tobacco smoke potentiates subsequent locomotor responses to nicotine and tobacco smoke but not amphetamine in adult rats

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ARTICLE INFO

Article history:
Received 18 March 2011
Received in revised form 21 July 2011
Accepted 9 August 2011
Available online 17 August 2011

Keywords:
Tobacco smoke
Nicotine
Amphetamine
Locomotor response
Rats

ABSTRACT

These studies investigated if pre-exposure to tobacco smoke affects the locomotor response to tobacco smoke, nicotine, and amphetamine in adult rats. The rats were habituated to an open field for 3–4 days and then exposed to tobacco smoke for 2 h/day for 13–14 days. The effect of exposure to tobacco smoke on locomotor activity was investigated after 1, 7, and 14 days of smoke exposure and after one 2-hour exposure session that followed a 3-week off period. The effects of tobacco smoke on the locomotor responses to nicotine (0.04 and 0.4 mg/kg, base) and amphetamine (0.1 and 0.5 mg/kg) were investigated on day 14, one day after the last smoke exposure session. The locomotor response to tobacco smoke was increased after 7 and 14 days of smoke exposure and after one exposure session after the 3-week off-period. The acute administration of the high dose of nicotine (0.4 mg/kg) led to a brief period of hypoactivity that was followed by a period of hyperactivity. Pre-exposure to tobacco smoke attenuated the nicotine-induced hypoactivity and potentiated the nicotine-induced hyperactivity. The low dose of nicotine (0.04 mg/kg) did not affect locomotor activity in the control rats but increased the total distance traveled in the tobacco smoke exposed rats. Exposure to tobacco smoke did not affect the locomotor response to amphetamine. These findings indicate that exposure to tobacco smoke leads to tolerance to the depressant effects of nicotine and potentiates the stimulant effects of nicotine and tobacco smoke.

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1. Introduction

Tobacco addiction is a chronic brain disorder that is characterized by loss of control over smoking, withdrawal symptoms upon smoking cessation, and relapse after periods of abstinence (American Psychiatric Association, 2000). It has been estimated that smoking leads to the premature death of 435,000 people/year in the United States and 3–5 million people worldwide (Ezzati and Lopez, 2003; Mokdad et al., 2004). Nicotine is one of the main components of tobacco smoke that leads to and maintains smoking (Bardo et al., 1999; Crooks and Dwoskin, 1997; Stolerman and Jarvis, 1995). Experimental studies indicate that nicotine is self-administered by rats, mice, and non-human primates (Corrigall and Coen, 1989; Goldberg and Spealman, 1982; Martellotta et al., 1995). In addition, nicotine induces conditioned place preference in rats and mice (Le Foll and Goldberg, 2005; Risinger and Oakes, 1995; Yamada et al., 2010). The rewarding and stimulating effects of nicotine have been suggested to play an important role in the initiation of smoking in humans (Koob et al., 1998; Picciotto, 2003).

Repeated exposure to a drug of abuse can lead to a potentiation of locomotor and neurochemical responses to a challenge dose of the same drug, other drugs of abuse, and stressors (i.e., drug sensitization)(Pierce and Kalivas, 1997; Stam et al., 2000; Vanderschuren and Kalivas, 2000). It has been hypothesized that sensitization processes play a pivotal role in the early stages of the development of drug addictions. Robinson and Berridge proposed that the sensitization of neural systems that attribute incentive salience to drug-associated stimuli causes a pathological motivation to use drugs (Robinson and Berridge, 1993; Robinson and Berridge, 2003). Repeated nicotine administration leads to a potentiation of nicotine-induced dopamine release in the nucleus accumbens (Benwell and Balfour, 1992; Shoaib et al., 1994). Pretreatment with nicotine also potentiates the locomotor response to nicotine (Clarke and Kumar, 1983; Domino, 2001). The acute administration of nicotine to rats leads to a brief period of hypoactivity, ~20 min, which is followed by a more prolonged period of hyperactivity. Pretreatment with nicotine attenuates the nicotine-induced hypoactivity and potentiates the nicotine-induced hyperactivity (Clarke and Kumar, 1983; Domino, 2001). Conflicting findings have been reported with regard to the effects of pretreatment with nicotine on the locomotor response to other drugs of abuse. Experimental evidence suggests that pretreatment with nicotine does not affect the locomotor response to cocaine or morphine (Schenk et al., 1991; Vezina et al., 1992). One study

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doi:10.1016/j.pbb.2011.08.005

investigated the effects of pretreatment with nicotine on the locmotor response to amphetamine (AMP) in adolescent male and female rats and adult male and female rats (Collins et al., 2004). It was shown that pretreatment with nicotine does not affect the locomotor response to AMP in adolescent female rats and adult male and female rats. In contrast, nicotine pretreatment potentiated the locomotor response to AMP in adolescent male rats (Collins et al., 2004). Another study reported that pretreatment with nicotine potentiates the locomotor response to AMP in late adolescent male rats (Schoffelmeer et al., 2002).

Several studies have investigated the effect of pretreatment with nicotine on the locomotor response to nicotine and AMP (Clarke and Kumar, 1983; Collins et al., 2004; Domino, 2001; Schoffelmeer et al., 2002). However, at this point, it is not known if repeated exposure to tobacco smoke potentiates the locomotor response to tobacco smoke, nicotine or AMP. There are several reasons that it is important to investigate the effects of inhaled tobacco smoke on drug induced locmotor responses. First, it is not known if repeated exposure to tobacco smoke has the same effect on locomotor activity as repeated systemic injections with nicotine. Nicotine injections lead to a rise and then drop in nicotine levels whereas in the tobacco smoke exposed animals nicotine levels are elevated for hours each day (Ghosheh et al., 1999; Harris et al., 2010). Second, there are compounds in tobacco smoke that may potentiate the effects of nicotine (Fowler et al., 2003; Talhout et al., 2007). Therefore, pre-exposure to tobacco smoke could possibly have a greater effect on the locomotor response to drugs of abuse than injections with nicotine. Acetaldehyde is one of the compounds in tobacco smoke that has been suggested to contribute to the development of tobacco addiction. Acetaldehyde is self-administered by rodents and induces conditioned place preference (Brown et al., 1979; Myers et al., 1982; Smith et al., 1984). In addition, acetaldehyde potentiates the positive reinforcing effects of nicotine (Belluzzi et al., 2005). Tobacco smoke also contains high levels of the beta-carboline alkaloids norharman and harman, which inhibit monoamine oxidase (MAO)-A and B in the brains of smokers (Fowler et al., 1998; Fowler et al., 1996; Herraiz and Chaparro, 2005). MAO inhibitors are used for the treatment of depression in humans and norharman and harman have antidepressant-like effects in rodents (Aricioglu and Altunbas, 2003; Farzin and Mansouri, 2006; Yamada and Yasuhara, 2004). Finally, it is also important to investigate the effects of tobacco smoke on the brain because nicotine by itself is not abused in humans and the majority of smokers do not prefer a nicotine-spray above placebo (Perkins et al., 1997).

Taken together, the aforementioned studies indicate that pretreatment with nicotine potentiates the locomotor response to nicotine. Conflicting findings have been reported with regard to the effects of pretreatment with nicotine on the locomotor response to AMP (Collins et al., 2004; Schoffelmeer et al., 2002). The aim of the present studies was to investigate whether pretreatment with tobacco smoke affects the locomotor response to tobacco smoke, nicotine, and AMP. These studies may provide insight into whether passive exposure to high doses of secondhand tobacco smoke or experimenting with cigarettes leads to neuronal adaptations that potentiate the psychomotor effects of tobacco smoke, nicotine, and AMP. These neuroadaptations may contribute to the transition from experimenting with cigarettes to developing a drug addiction.

2. Methods

2.1. Subjects

Adult male Wistar rats (Charles River, Raleigh, NC, USA) weighing 250–300 g at the beginning of the experiments were used. Animals were group-housed (two per cage) in a temperature- and humidity-controlled vivarium and maintained on a 12-hour light–dark cycle (lights off at 8 AM). All testing occurred at the beginning of the dark cycle. Food and water were available ad libitum in the home cages. All subjects were treated in accordance with the National Institutes of Health guidelines regarding the principles of animal care. Animal facilities and experimental protocols were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and approved by the University of Florida Institutional Animal Care and Use Committee.

2.2. Drugs

Nicotine hydrogen tartrate salt and D-amphetamine hemisulfate were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA) and dissolved in saline (0.9% sodium chloride). Nicotine was administered subcutaneously (s.c.) at a dose of 0.04 or 0.4 mg/kg nicotine base (0.11 or 1.14 mg/kg nicotine salt, pH ~ 3.4). AMP was administered intraperitoneally (i.p.) at a dose of 0.1 or 0.5 mg/kg. Research cigarettes (3R4F) were purchased from the University of Kentucky (College of Agriculture, Reference Cigarette Program, Lexington, KY). Drug doses are expressed as salt with the exception of the nicotine dose which is expressed as free base.

2.3. Tobacco smoke exposure

The rats were exposed to tobacco smoke in standard polycarbonate rodent cages (38 × 28 × 20 cm; L × W × H) with corncob bedding and a wire top as previously described by our research group (Small et al., 2010; Yamada et al., 2010). The rats were not restrained (whole body exposure) during the tobacco smoke exposure sessions and water was freely available. The rats were moved to the exposure cages immediately before the tobacco smoke exposure sessions and returned to their home cages after the exposure sessions. Tobacco smoke was generated using a microprocessor-controlled cigarette smoking-machine (model TE-10, Teague Enterprises, Davis, CA) (Teague et al., 1994). Tobacco smoke was generated by burning filtered 3R4F reference cigarettes using a standardized smoking procedure (35 cm^3 puff volume, 1 puff/min, 2 s/puff). Mainstream and sidestream smoke was transported to a mixing and diluting chamber. The smoking machine produced a mixture of approximately 10% mainstream smoke and 90% sidestream smoke; based on total suspended particle matter. The smoke was aged for 2–4 min and diluted with air to a concentration of 30–100 mg of total suspended particles/m^3 before being introduced into the exposure chambers. The concentration of the smoke was dependent on the stage of the experiment. Exposure conditions were monitored for carbon monoxide (CO) and total suspended particulate matter. CO levels were assessed using a continuous CO analyzer that accurately measures CO levels between 0 and 2000 parts/million (Monoxor II, Bacharach, New Kensington, PA USA). In order to measure the total suspended particle matter in the exposure chamber, smoke was pumped out of the chamber into a chemical hood through a pre-weighed filter (Pallflex Emfab Filter, Pall Corporation, Port Washington, NY, USA) for 5 min. The total suspended particulate matter per cubic meter was calculated by dividing the weight increase of the filter by the volume of the smoke. The rats in the tobacco group were exposed to tobacco smoke for 2 h/day for 13 or 14 days. The average total suspended particulate matter was about 100 mg/m^3 and the CO level about 350 ppm. Previous studies by our research group and others demonstrated that passive exposure to tobacco smoke with a particulate matter of about 100 mg/m^3 leads to increased levels of nicotine and the nicotine metabolite cotinine (Anderson et al., 2004; Small et al., 2010). Passive exposure to tobacco smoke leads to nicotine dependence as indicated by mecamylamine precipitated affective and somatic withdrawal signs and an upregulation of nAChRs (Small et al., 2010; Yamada et al., 2010).

2.4. Plasma nicotine and cotinine levels

The rats were anesthetized with isoflurane and then decapitated. The blood samples were collected in plastic BD Vacutainer Blood Collection Tubes (BD, Franklin Lakes, NJ, USA). The samples were centrifuged at 1300 *g* for 10 min and then the plasma was stored in a −80 °C freezer until further use. Nicotine and cotinine levels were determined as described previously (Small et al., 2010). A validated high-performance liquid chromatography-tandem mass spectrometry (HPLC/MS/MS) method was used to determine plasma nicotine and cotinine levels. Plasma proteins, which could interfere with the HPLC/MS/MS analysis, were precipitated by adding 150 μL methanol to 100 μL plasma. This mixture was vortexed for 30 s and then centrifuged at 1500 *g* for 15 min. The clear supernatant (100 μL) was carefully transferred into series 200 Perkin Elmer auto sampler vials for HPLC/MS/MS analysis. Nicotine and cotinine were separated by reversed phase chromatography using a Prodigy 5u, 100×4.6 mm, C18 column (Phenomenex, Torrance, CA, USA) that was fitted with a C18 pre-column and an isocratic mobile phase composed of 10 mM ammonium acetate buffer in 75% methanol delivered at 1 mL/min by a series 200 Perkin Elmer HPLC pump (Waltham, MA, USA). The injection volume was 10 μL and the chromatographic run time was 4 min. The column eluent was directed to the mass spectrometer by atmospheric pressure ionization (API) source. The mass spectrometer (API 4000 LC-MS-MS system, Applied Biosystems/MDS SCIEX, Foster City, CA, USA) was operated in electro spray positive ion mode (ESI^+) and quantitation was performed using multiple-reaction monitoring (MRM). The MRM transitions that were used for the quantification of nicotine and cotinine were *m/z* 163.1>132.0 and *m/z* 177.1>146.1, respectively. High purity nitrogen was used as curtain and collision gas and zero grade air was used as the source gas. The API source was operated at 300 °C and the ion spray voltage was set at 5 kV. Data acquisition and quantitation were performed using Analyst software version 1.4.2 (Applied Biosystems/MDS SCIEX, Foster City, CA, USA). During the sample analyses quality control samples were interspaced with test samples to ensure the accuracy and reliability of the assay procedure.

2.5. Experiment 1: Tobacco smoke exposure and locomotor activity

Prior to the onset of the open field test and tobacco smoke exposure sessions, the rats were handled for 5 min/day on 3 consecutive days. Then the rats (air n = 9, tobacco n = 10) were habituated to the tobacco smoke exposure chambers. The rats were placed in the exposure chambers for 30 min/day for 3 days. Once the rats had been habituated to the exposure chambers, they were habituated to the circular open field (diameter, 55 cm; height, 42 cm). The open field was dark blue with a black bottom. Immediately before the open field habituation sessions the rats were placed in the exposure chambers for 30 min and then placed into the open field for 15 min. The rats were exposed to the open field for 4 days and locomotor activity was assessed with Noldus Ethovision Software (Wageningen, The Netherlands). The tobacco smoke exposure sessions began when the rats had been habituated to the tobacco smoke exposure chambers and the open field. Animals in the air group were placed in the exposure chambers for 30 min/day with the smoking machine turned off. The rats in the tobacco group were exposed to tobacco smoke for 2 h/day. Locomotor activity was measured for 90 min immediately following tobacco smoke exposure after 1, 7, and 14 days of smoke exposure. After day 14, the subjects were not exposed to tobacco smoke or the open field for 3 weeks. After the 3-week off period, the rats were re-exposed to tobacco smoke for 2 h and placed in the open field for 90 min. For all the experiments, body weights were recorded daily. On days that the rats were exposed to tobacco smoke, body weights were recorded before the tobacco smoke exposure session. In order to determine plasma nicotine and cotinine levels, a separate group of animals (n = 6) was exposed to tobacco smoke for 2 h/day 7 days and decapitated immediately after the last smoke exposure session. The tobacco smoke exposure conditions for this experiment were the same as for all the other experiments.

2.6. Experiments 2–5: Effect of tobacco smoke exposure on the locomotor response to nicotine and amphetamine

At the beginning of the experiment 2, the animals (air–saline n = 10, air–nicotine n = 10, tobacco–saline n = 10, tobacco–nicotine n = 10) were handled and habituated to the smoke exposure chamber and open field as described under experiment 1. All the experimental manipulations were conducted as described under experiment 1 with the exception that the rats were habituated to the open field for three days instead of four days before the smoke exposure sessions. The rats were exposed to tobacco smoke for 2 h/day for 13 days. The tobacco smoke levels were similar as in the first experiment. One day after the last smoke exposure session, day 14, the rats received an injection with saline or nicotine (0.4 mg/kg nicotine base). Immediately after the injection the rats were placed in the open field for 90 min. Experiments 3, 4 and 5 were conducted in a similar manner as experiment 2, with the exception that the rats received an injection with a low dose of nicotine (Exp. 3; 0.04 mg/kg nicotine base; air–saline n = 10, air–nicotine n = 10, tobacco–saline n = 10, tobacco–nicotine n = 9), AMP (Expt. 4; 0.5 mg/kg AMP; air–saline n = 10, air–AMP n = 10, tobacco–saline n = 10, tobacco–AMP n = 10; Expt. 5; 0.1 mg/kg AMP; air–AMP n = 10, tobacco–AMP n = 10) immediately before being placed in the open field. A new group of animals was used for each experiment. The 0.5 mg/kg dose of AMP was based on previous studies that showed that this dose of AMP increases locomotor activity and induces a greater increase in locomotor activity in drug sensitized animals than in control animals (Cadoni et al., 2000; Schoffelmeer et al., 2002; Wolf et al., 1993). Experiment 5 was conducted to rule out the possibility that tobacco smoke exposed animals are more sensitive to an extremely low dose of AMP (0.1 mg/kg) than control rats. This dose AMP does not affect locomotor activity in control rats but increases locomotor activity in rats that are pretreated with nicotine (Birrell and Balfour, 1998). Experiment 1 did not investigate if pre-exposure to tobacco smoke (2 weeks) leads to a long-term increase in baseline locomotor activity in drug-free animals. Therefore, all the rats of experiment 3 were tested in the open field for 90 min in a drug-free state three weeks after the last tobacco smoke exposure session.

2.7. Data analyses

The absolute body weights of the animals in the tobacco groups and the control groups were compared with one-way analyses of variance (ANOVA) before the onset of tobacco smoke exposure. In order to analyze the effects of tobacco smoke exposure on body weight gain, the body weights of the rats were expressed as a percentage of the body weights on the day prior to tobacco smoke exposure. The effect of tobacco smoke exposure on body weight gain was analyzed with a two-way repeated-measures ANOVA with exposure condition (air or tobacco smoke) as the between subjects factor and time as the within subjects factor. In experiment 1, the effect of tobacco smoke exposure on locomotor activity was analyzed with a two-way repeated-measures ANOVA with exposure condition (air or tobacco smoke) as the between subjects factor and time (15-minute blocks) as the within subjects factor. Total locomotor activity (90 min) was analyzed with a one-way ANOVA with exposure condition (air or tobacco smoke) as the between subjects factor. In experiments 2–4, the effect of tobacco smoke and acute nicotine (Expt. 2, 3)/AMP (Expt. 4) administration on locomotor activity was analyzed with a three-way repeated-measures ANOVA with exposure

Table 1
Effect of tobacco smoke exposure on body weights.

Experiment	Baseline		Day 14		P-value
	Air	Tobacco	Air	Tobacco	
Expt. 1 (Tobacco)	297.2 ± 4.7	290.9 ± 4.1	376.2 ± 7.9	356.2 ± 8.1	P<0.0001
Expt. 2 (Nicotine, 0.4)	252.3 ± 2.7	244.4 ± 3.1	346.2 ± 4.0	296.3 ± 14.7	P<0.0001
Expt. 3 (Nicotine, 0.04)	281.9 ± 3.3	280.3 ± 3.4	368.0 ± 4.1	332.6 ± 6.3	P<0.0001
Expt. 4 (AMP, 0.5)	268.0 ± 3.9	264.0 ± 2.2	352.2 ± 4.9	326.3 ± 4.3	P<0.0001
Expt. 5 (AMP, 0.1)	268.0 ± 4.6	266.0 ± 2.6	360.6 ± 5.1	308.9 ± 4.4	P<0.0001

Data are expressed as means (± S.E.M.). Baseline refers to body weights obtained one day prior to the onset of smoke exposure. Nicotine doses; 0.04 and 0.4 mg/kg; Amphetamine doses; 0.1 and 0.5 mg/kg. Post refers to body weights obtained on day 14 of smoke exposure. Experiments 1 and 5, n = 9–10/group and Experiments 2–4, N = 19–20/group. P-values (P<0.0001) indicate lower body weight gain in the tobacco smoke exposed rats than in the control rats.

condition (air or tobacco smoke) and drug treatment (nicotine base 0.04, 0.4 mg/kg/AMP 0.5 mg/kg or saline) as the between subjects factors and time (15-minute blocks) as the within subjects factor. Total locomotor activity was analyzed with a two-way ANOVA with exposure condition (air or tobacco smoke) and drug treatment (nicotine/AMP or saline) as the between subjects factors. In experiment 5, the effect of tobacco smoke exposure on the locomotor response to AMP (0.1 mg/kg) was analyzed with a two-way ANOVA with exposure condition (air or tobacco smoke) as the between subjects factor and time (15-minute blocks) as the within subjects factor. The effect of AMP (0.1 mg/kg) on total locomotor activity was analyzed with a one-way ANOVA with exposure condition (air or tobacco smoke) as the between subjects factor. Newman–Keuls post hoc tests were conducted when the ANOVA revealed statistically significant effects. For all the experiments, the criterion for significance was set at 0.05. The statistical analyses were performed using PASW Statistics version 18.

3. Results

3.1. Experiment 1: Tobacco smoke exposure and locomotor activity

There were no differences in body weights between the air group and the tobacco group prior to the onset of the tobacco smoke exposure sessions (Table 1; F1,18 = 1.05, n.s.). Exposure to tobacco smoke decreased body weight gain during the 14-day exposure period (Table 1; Time: F13,221 = 486.25, P<0.0001; Tobacco: F1,17 = 116.92, P<0.003; Time × Tobacco: F13,182 = 15.19, P<0.0001). The rats were habituated to the open field for 4 consecutive days. There was no difference in locomotor activity between the air group and the tobacco group during the last habituation session (Table 2; F1,18 = 0.05, n.s.). The effect of tobacco smoke on locomotor activity was investigated after the 1st, 7th, and 14th exposure sessions and after one exposure session following a three-week off period. The first tobacco smoke exposure session had a very small effect on locomotor activity (Fig. 1A; Time: F5,85 = 105.15, P<0.0001; Tobacco: F1,17 = 0.09, n.s.; Time × Tobacco: F5,85 = 2.55, P<0.03). A close look at the data suggests that the time × treatment interaction was due to the fact that locomotor activity in the tobacco group was slightly higher during the 30–45 minute period and slightly lower during the 75–90 minute period. Post hoc tests did not reveal any significant differences between the tobacco group and the control group. Seven days of tobacco smoke exposure session also had a small effect on locomotor activity (Fig. 1B; Time: F5,85 = 57.06, P<0.0001; Tobacco: F1,17 = 0.84, n.s.; Time × Tobacco: F5,85 = 2.56, P<0.03). Posthoc analysis indicated that the tobacco smoke exposed rats had a slightly higher activity during the 15–30 min period compared to the control rats. The greatest difference in locomotor activity between the tobacco smoke exposed rats and the control rats was detected after 14 days of tobacco smoke exposure (Fig. 1C; Time: F5,85 = 13.01, P<0.0001; Tobacco: F1,17 = 0.84, P<0.04; Time × Tobacco: F5,85 = 0.82, n.s.). The tobacco smoke exposed rats had a higher activity compared to the control rats during the 30–45 minute period and the 45–60 minute period. After the tobacco smoke exposure session on day 14, the rats were not exposed to tobacco smoke for three weeks and then the rats were re-exposed to tobacco smoke for a single 2-hour session. Immediately after this exposure session, locomotor activity was higher in the tobacco group than in the control group (Fig. 1D; Time: F5,85 = 158.91, P<0.0001; Tobacco: F1,17 = 5.43, P<0.03; Time × Tobacco: F5,85 = 1.91, n.s.). Posthoc analysis indicated that locomotor activity in the tobacco group was higher than in the control group during the 0–15, 15–30, and 30–45 minute periods. In separate analysis, the effect of tobacco smoke on the total distance traveled was analyzed. Exposure to tobacco smoke increased total locomotor activity after the exposure session on day 14 (Fig. 1E, F1,18 = 5.06, P<0.04). Exposure to tobacco smoke did not affect total locomotor activity after the exposure session on day 1 (F1,18 = 0.09, n.s.), day 7 (F1,18 = 0.84, n.s.), and after the exposure session that followed the three week off period (F1,18 = 3.32, n.s.). Plasma nicotine and cotinine levels after exposure to tobacco smoke were 38.5 ± 6.7 ng/mL and 130.0 ± 7.8 ng/mL, respectively. Taken together, this study indicates that daily exposure to tobacco smoke leads to a gradual increase in the locomotor response to tobacco smoke.

3.2. Experiments 2 and 3: Effect of tobacco smoke exposure on the locomotor response to nicotine

In experiment 2, the effect of a challenge dose of nicotine (0.4 mg/kg) on locomotor activity in rats in the air group and the tobacco group was investigated. There were no differences in body

Table 2
Locomotor activity during last day of open field habituation.

Expt. 1 (Tobacco)	Air	Tobacco		
	3113 ± 204	3173 ± 189		
Expt. 2 (Nic, 0.4)	Air–Saline	Air–Nicotine	Tobacco–Saline	Tobacco–Nicotine
	2180 ± 88	2100 ± 137	2227 ± 101	2397 ± 79
Expt. 3 (Nic, 0.04)	Air–Saline	Air–Nicotine	Tobacco–Saline	Tobacco–Nicotine
	3638 ± 187	3482 ± 244	3548 ± 175	3800 ± 226
Expt. 4 (AMP, 0.5)	Air–Saline	Air–AMP	Tobacco–Saline	Tobacco–AMP
	2276 ± 158	2353 ± 125	2350 ± 152	2574 ± 197
Expt. 5 (AMP, 0.1)	Air–AMP	Tobacco–AMP		
	3551 ± 100	3478 ± 114		

Data are expressed as means (± S.E.M.). Nicotine doses; 0.04 and 0.4 mg/kg; Amphetamine doses; 0.1 and 0.5 mg/kg. N = 9–10/group. Abbreviations: AMP, amphetamine.

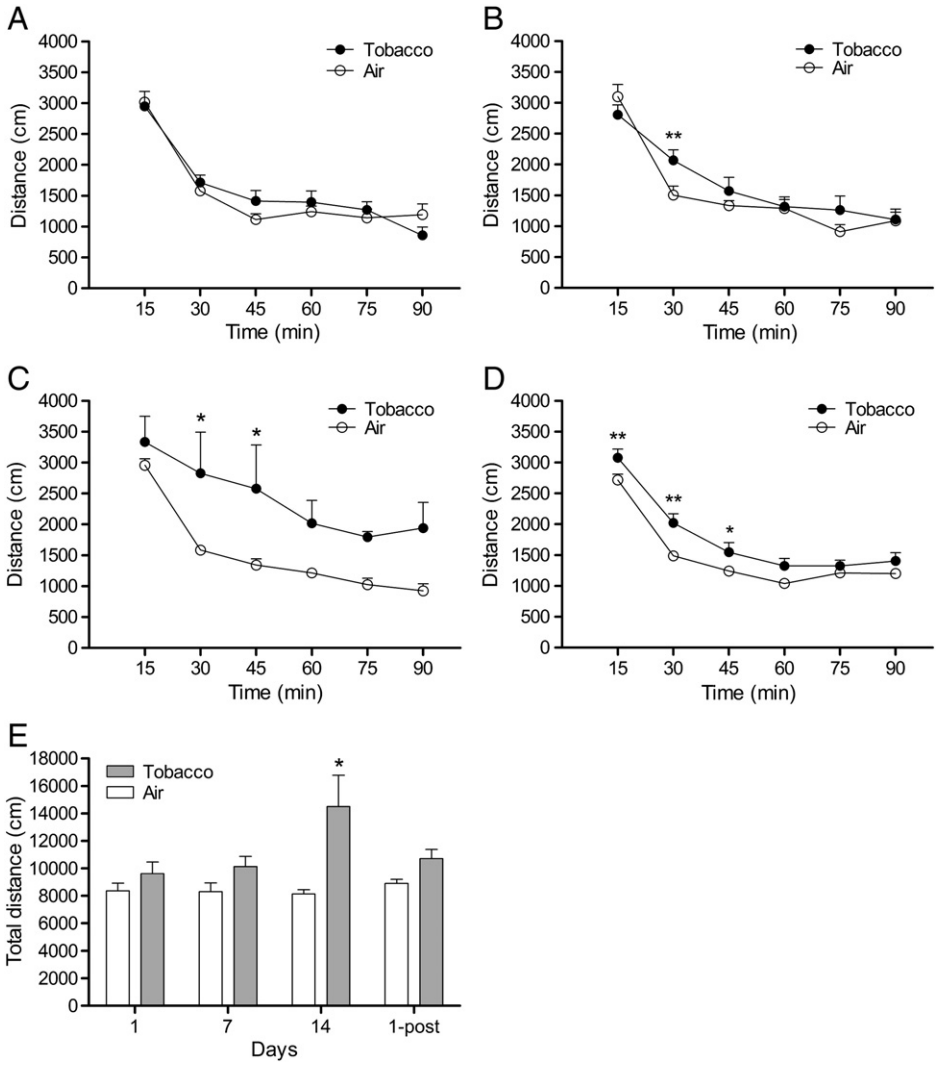

Fig. 1. Effect of repeated exposure to tobacco smoke on the locomotor response to tobacco smoke (A–E; air n = 9, tobacco n = 10). The figures depict locomotor activity in 15-minute intervals in rats that have been exposed to tobacco smoke for 1-day (Fig. 1A), 7-days (Fig. 1B), 14-days (Fig. 1C), or re-exposed to tobacco smoke for 2 h after a 3-week off period (Fig. 1D). Fig. 1E depicts the total distance traveled during the 90-minute open field test in the tobacco smoke exposed rats and the control rats. Asterisks (* $P<0.05$; ** $P<0.01$) indicate increased locomotor activity in the tobacco smoke exposed rats compared to the air-control rats.

weights between the air group and the tobacco group prior to the onset of the tobacco smoke exposure sessions (Table 1; $F3,39 = 1.20$, n.s.). Exposure to tobacco smoke decreased body weight gain (Table 1; Time: $F13,494 = 116.92$, $P<0.0001$; Tobacco: $F1,38 = 35.07$, $P<0.0001$; Time × Tobacco: $F13,494 = 4.14$, $P<0.0001$). There were no differences in locomotor activity between the 4 groups during the last habituation session (Table 2; $F3,39 = 1.46$, n.s.). On day 14, rats in the air group and rats in the tobacco group were injected with nicotine or saline. Exposure to tobacco smoke and the administration of nicotine affected locomotor activity (Fig. 2A. Time: $F5,180 = 69.88$, $P<0.0001$; Tobacco: $F1,36 = 4.88$, $P<0.03$; Time × Tobacco: $F5,180 = 2.82$, $P<0.02$; Time × Nicotine: $F5,180 = 28.38$, $P<0.0001$; Time × Tobacco × Nicotine: $F5,180 = 2.28$, $P<0.048$). Because of the multitude of effects, the results of the posthoc tests were depicted in several figures. Fig. 2B indicates that the administration of nicotine to the animals in the air group induced a period of hypolocomotion (0–15 min) that was followed by a period of hyperlocomotion (60–75 min). Fig. 2C indicates that the administration of nicotine to the tobacco smoke exposed rats also induced a hypolocomotion (0–15 min) that was followed by a period of hyperlocomotion (0–45, 45–60, 60–75, 75–90 min). Fig. 2D demonstrates that nicotine induced a greater increase in locomotor activity in the tobacco smoke exposed rats than in the air-control rats. These findings indicate that nicotine induces a brief period of hypoactivity which is followed by a period of hyperactivity. Repeated exposure to tobacco smoke attenuated the nicotine-induced hypoactivity and potentiated the hyperactivity. The effect of tobacco smoke exposure and nicotine on the total distance traveled is depicted in Fig. 2E (Tobacco: $F1,36 = 4.88$, $P<0.03$). The posthoc analysis indicated that the animals in the tobacco–nicotine group had a higher activity than the rats in all the other groups.

In experiment 3, the effect of exposure to tobacco smoke on the locomotor response to a low dose of nicotine (0.04 mg/kg) was investigated. There were no differences in body weights between the air group and the tobacco before the onset of the tobacco smoke exposure sessions (Table 1; $F1,38 = 0.11$, n.s.). Exposure to tobacco smoke decreased body weight gain (Table 1; Time: $F13,481 = 549.97$, $P<0.0001$; Treatment: $F1,37 = 16.85$, $P<0.0001$; Time × Treatment: $F13,481 = 26.53$, $P<0.0001$). There were no differences in locomotor activity between the 4 groups during the last habituation session (Table 2; $F3,38 = 0.42$, n.s.). Exposure to tobacco smoke and nicotine affected locomotor activity (Fig. 3A. Time: $F5,175 = 165.51$, $P<0.0001$; Tobacco: $F1,35 = 6.90$, $P<0.01$; Time × Nicotine: $F5,175 = 3.34$, $P<0.007$). Posthoc analysis indicated that during the first 15 min in the open field, the locomotor activity of the tobacco–saline group and the tobacco–nicotine group were increased compared to the activity

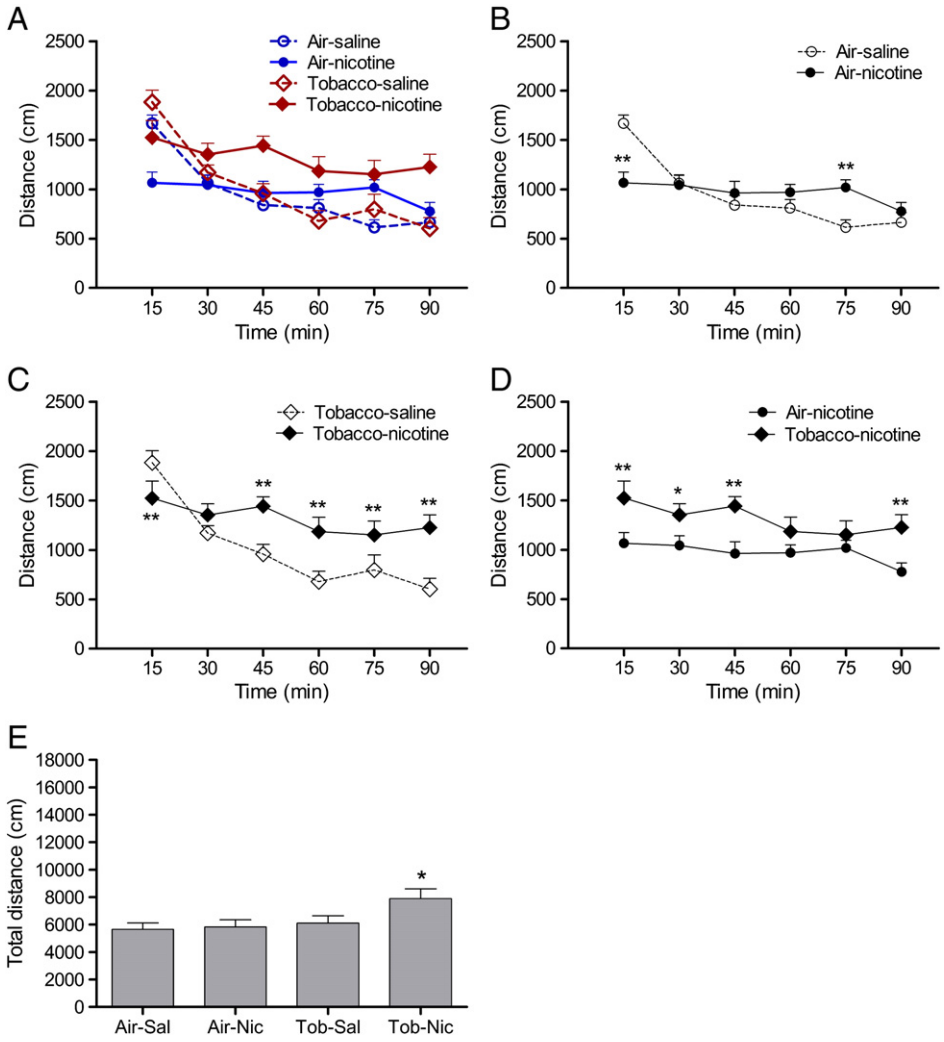

Fig. 2. Effect of repeated exposure to tobacco smoke on the locomotor response to a high dose of nicotine (A; air–saline n = 10, air–nicotine n = 10, tobacco–saline n = 10, tobacco–nicotine n = 10; Nicotine base, 0.4 mg/kg). The results of the post hoc analyses are depicted in Fig. 2B (air–saline and air–nicotine), 2C (tobacco–saline and tobacco–nicotine), and 2D (air–nicotine and tobacco–nicotine). Fig. 2A–D depict the distance traveled in 15-minute intervals. Fig. 2E depicts the total distance traveled during the 90-minute open field test. In Fig. 2A–D, asterisks (* $P<0.05$; ** $P<0.01$) indicate a difference in the locomotor activity between the two groups depicted in the figure. In Fig. 2E, asterisks (* $P<0.05$) indicate a difference in locomotor activity between the tobacco–nicotine group and all other groups. Abbreviations: Nic, nicotine; Sal, saline; Tob, tobacco.

of the air–saline and air–nicotine group (Fig. 3A). Posthoc tests also revealed that the animals in the tobacco–nicotine group traveled a greater distance during the 90-minute test period than the animals in the tobacco–saline group (Fig. 3B). The tobacco–saline animals did not travel a greater distance than the air–saline animals during the 90-minute test period. This finding suggests that exposure to tobacco smoke potentiates the locomotor response to a low dose of nicotine. In order to investigate the long-term effect of tobacco smoke exposure on locomotor activity, all the rats were tested again in the open field 3 weeks after the last smoke exposure session. The rats were tested in a drug-free state and did not receive nicotine or tobacco smoke for 3 weeks. Prior exposure to tobacco smoke or nicotine did not affect locomotor activity of the rats in the open field (Table 3. Time: $F5,175 = 202.57$, $P<0.0001$; Tobacco: $F1,35 = 2.42$, n.s.; Nicotine: $F5,175 = 0.04$, n.s.).

3.3. Experiments 4 and 5: Effect of tobacco smoke exposure on the locomotor response to amphetamine

In experiment 4, the effect of a challenge dose of AMP (0.5 mg/kg) on locomotor activity in rats in the air group and the tobacco group was investigated. There were no differences in body weights between the 4 groups prior to the onset of the tobacco smoke exposure sessions (Table 1; $F3,39 = 2.19$, n.s.). Exposure to tobacco smoke decreased body weight gain (Table 1; Time: $F13,494 = 722.72$, $P<0.0001$; Tobacco: $F1,38 = 14.32$, $P<0.0005$; Time × Tobacco: $F13,494 = 30.19$, $P<0.0001$). There were no differences in locomotor activity between the 4 groups during the last habituation session (Table 2; $F3,51 = 0.86$, n.s.). On day 14, the air-control rats and the rats in the tobacco group were injected with AMP or saline. AMP increased locomotor activity in the control group and the tobacco group to a similar degree (Fig. 4A; Time: $F5,180 = 138.79$, $P<0.0001$; Tobacco: $F1,36 = 0.18$, n.s.; AMP: $F1,36 = 67.55$, $P<0.0001$; Time × AMP: $F5,180 = 5.56$, $P<0.0001$). Posthoc analysis indicated that at all time points the locomotor activity of the AMP treated air-control rats and tobacco rats was increased compared to the corresponding saline-treated control groups. An additional analysis was conducted to investigate the effects of tobacco smoke and AMP on total locomotor activity during the 90-minute observation period. Previous exposure to tobacco smoke did not affect total locomotor activity (Fig. 4B; Tobacco: $F1,36 = 0.18$, n.s.). AMP increased locomotor activity to a similar degree in the air-control rats and the tobacco smoke exposed rats (AMP: $F1,36 = 67.55$, $P<0.0001$).

In experiment 5, the effect of a very low dose of AMP (0.1 mg/kg) on locomotor activity in tobacco smoke exposed rats and air-control rats was investigated. There were no differences in body weights

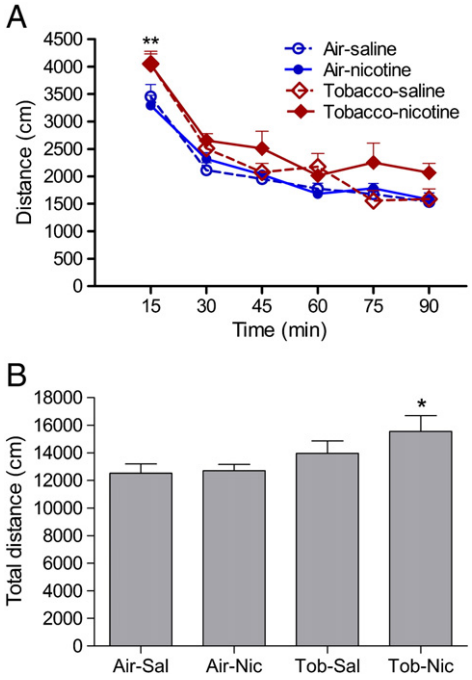

Fig. 3. Effect of repeated exposure to tobacco smoke on the locomotor response to a low dose of nicotine (A; air–saline n = 10, air–nicotine n = 10, tobacco–saline n = 10, tobacco–nicotine n = 9; Nicotine base, 0.04 mg/kg). Fig. 3A depicts the distance traveled in 15-minute intervals and Fig. 3B depicts the total distance traveled during the 90-minute open field test. In Fig. 3A, asterisks (** P<0.01) indicate that the rats in the tobacco–saline and tobacco–nicotine group traveled a greater distance than rats in the corresponding air groups. In Fig. 3B, asterisks (* P<0.05) indicate a difference in locomotor activity between the tobacco–nicotine group and the air–nicotine group. Abbreviations: Nic, nicotine; Sal, saline; Tob, tobacco.

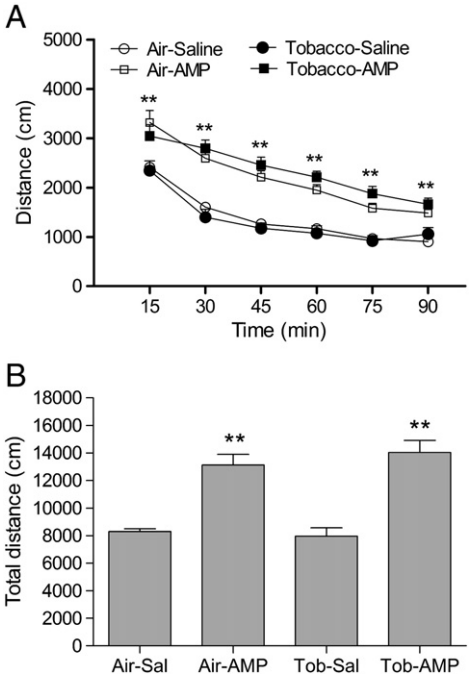

Fig. 4. Effect of repeated exposure to tobacco smoke on the locomotor response to amphetamine (A; air–saline n = 10; air–AMP n = 10, tobacco–saline n = 10, tobacco–AMP n = 10; AMP, 0.5 mg/kg,). Fig. 4A depicts the distance traveled in 15-minute intervals. Fig. 4B depicts the total distance traveled during the 90-minute open field test. In Fig. 4A and B, asterisks (** P<0.01) indicate a difference in the locomotor activity between the AMP-treated rats and their corresponding control group. Abbreviations: AMP, amphetamine; Sal, saline.

between the air–AMP group and the tobacco–AMP group prior to the onset of the tobacco smoke exposure sessions (Table 1; F1,19 = 0.14, n.s.). Exposure to tobacco smoke decreased body weight gain (Table 1; Time: F13,234 = 144.26, P<0.0001; Treatment: F1,18 = 65.03, P<0.0001; Time × Treatment: F13,234 = 12.87, P<0.0001). There were no differences in locomotor activity between the air–AMP and the tobacco–AMP group during the last habituation session (Table 2; F1,19 = 0.23, n.s.). Statistical analysis indicated that there was no difference in locomotor activity between the air group and the tobacco group after the administration of AMP (Table 4; Time: F5,90 = 94.6, P<0.0001; Tobacco: F1,18 = 0.11, n.s.). This indicates that repeated exposure to tobacco smoke does not enhance the locomotor response to a very low dose of AMP.

4. Discussion

The aim of the present experiments was to investigate the effects of pretreatment with tobacco smoke on the locomotor response to tobacco smoke, nicotine, and AMP. It was shown that repeated exposure to tobacco smoke led to a potentiation of the locomotor response to smoke. The smoke-induced potentiation of locomotor activity developed gradually over time. The second experiment showed that repeated exposure to tobacco smoke potentiates the locomotor response to a high dose of nicotine (0.4 mg/kg). The tobacco smoke exposed animals were less sensitive to the depressant effects of nicotine and repeated exposure to tobacco smoke potentiated the stimulant effects of nicotine. A low dose of nicotine (0.04 mg/kg) did not affect locomotor activity in the air-control rats but increased locomotor activity in the tobacco smoke exposed rats. Finally, it was shown that repeated exposure to tobacco smoke did not affect the locomotor response to AMP. These studies demonstrate that repeated exposure to tobacco smoke potentiates the locomotor response to tobacco smoke and nicotine but not AMP. In the present study, exposure to tobacco smoke led to a plasma nicotine level of 38.5 ng/mL and a cotinine level of 130.0 ng/mL. Similar nicotine and cotinine levels have been found in moderate and heavy smokers (Benowitz, 1988; Lawson et al., 1998; Wall et al., 1988).

The results of the first experiment indicated that repeated exposure to tobacco smoke potentiates the locomotor response to tobacco smoke. The posthoc analysis did not reveal any significant

Table 3
Effect of exposure to tobacco smoke and nicotine on locomotor activity 3 weeks later.

Time (min)	Air–Saline	Air–Nicotine	Tob–Saline	Tob–Nicotine
0–15	3524 ± 179	3224 ± 159	3544 ± 188	3588 ± 129
15–30	2120 ± 161	2020 ± 134	2105 ± 112	2356 ± 118
30–45	1967 ± 153	1717 ± 114	1832 ± 131	1999 ± 114
45–60	1509 ± 153	1714 ± 105	1847 ± 119	1731 ± 156
60–75	1532 ± 116	1574 ± 99	1670 ± 161	1806 ± 142
75–90	1473 ± 92	1481 ± 114	1535 ± 97	1691 ± 133
Total (0–90)	12,127 ± 653	11,730 ± 582	12,532 ± 624	13,171 ± 473

Animals (N = 9–10/group) were re-tested in the open field 3 weeks after exposure to tobacco smoke (14 days) and/or nicotine (0.04 mg/kg). Abbreviations: Tob, tobacco. Data are expressed as means (± S.E.M.).

Table 4
Effect of amphetamine on locomotor activity in tobacco smoke exposed rats.

Time (min)	Air–AMP	Tobacco–AMP
0–15	3277 ± 192	3275 ± 197
15–30	2142 ± 138	2191 ± 178
30–45	1899 ± 89	2105 ± 172
45–60	1737 ± 113	1795 ± 139
60–75	1732 ± 112	1623 ± 171
75–90	1476 ± 184	1638 ± 171
Total (0–90)	12,263 ± 681	12,627 ± 860

Data are expressed as means (± S.E.M.). Amphetamine dose; 0.1 mg/kg. N = 10/group.

differences between the tobacco group and the air group after the first tobacco smoke exposure session. After 7 days of tobacco smoke exposure, the smoke exposed rats had a slightly higher activity during the 15–30 minute period than the control rats. After 14 days, the greatest effect of tobacco smoke on locomotor activity was observed. The tobacco smoke exposed rats had a higher locomotor activity at all the time points. However, due to the large variability between animals the difference in locomotor activity between the two groups was only significant during the 15–30 and 30–45 minute intervals. After 2 weeks of smoke exposure the rats were left undisturbed for 3 weeks and then re-exposed to tobacco smoke and placed in the open field. In this session, the smoke exposed rats had a higher locomotor activity than the air-control rats during the 0–15, 15–30, and 30–45 minute periods. This experiment did not investigate if exposure to tobacco smoke has a long-term effect on baseline locomotor activity. However, the results of experiment 3 showed that rats do not have an increased activity in the open field test when tested in a drug-free state three weeks after tobacco smoke exposure. It is interesting to note that after the first tobacco smoke exposure session there was no difference in locomotor activity between the tobacco group and the air group. However, when the rats were re-exposed to tobacco smoke after a three week off period, the animals in the tobacco group had a higher activity than the air-control rats. This suggests that repeated exposure to tobacco smoke induces long term-changes in the brain that potentiate stimulant effects of tobacco smoke. Previous studies have shown that the administration of nicotine to nicotine-naive animals leads to an initial decrease in locomotor activity (Clarke and Kumar, 1983; Domino, 2001). In the present study, exposure to tobacco smoke did not suppress locomotor activity in the open field test. The discrepancy between this tobacco smoke experiment and the nicotine experiments may be due to the time course of smoke and nicotine administration. In the present study, the animals inhaled tobacco smoke for 2 h before they were placed in the open field whereas nicotine is administered immediately before the animals are placed in the open field (Clarke and Kumar, 1983; Domino, 2001). Because the suppressant effects of nicotine last only about 20 min, it is most likely that the suppressant effects of nicotine had dissipated by the time the tobacco smoke exposed animals were placed in the open field.

In experiment 2, the effect of repeated exposure to tobacco smoke on the locomotor response to nicotine (0.4 mg/kg) was investigated. In the animals in the air group, nicotine suppressed locomotor activity during the first 15 min of the open field test and increased locomotor activity during the second half of the open field test. This observation is in line with previous studies that reported that nicotine has both locomotor suppressant and stimulating effects in nicotine-naive animals (Clarke and Kumar, 1983). Repeated exposure to tobacco smoke led to the development of tolerance to the locomotor suppressant effects of nicotine. This is indicated by the fact that there was no difference in locomotor activity between the air–saline group and the tobacco–nicotine during the first 15 min of the open field test. Furthermore, repeated exposure to tobacco smoke led to a potentiation of the locomotor stimulant effect of nicotine. This is illustrated by the fact that the animals in the tobacco–nicotine group had a higher activity than the animals in the air–nicotine group during the first 45 min (0–15, 15–30, and 30–45 min) of the open field test and during the last 15 min (75–90 min) of the open field test. The present findings are in line with previous studies that reported that pretreatment with nicotine prevents the acute hypolocomotor effects of nicotine and leads to a potentiation of the stimulant effects of nicotine (Clarke and Kumar, 1983; Domino, 2001).

In a follow-up experiment, the effect of chronic exposure to tobacco smoke on the locomotor response to a very low dose of nicotine (0.04 mg/kg) was investigated. This low dose of nicotine did not affect the total distance traveled (90-minute period) in the control rats, but significantly increased the total distance traveled in the tobacco smoked exposed rats. A previous study by another research group also showed that 0.04 mg/kg of nicotine does not increase locomotor activity in drug-naive control rats (Whiteaker et al., 1995). Locomotor activity of rats pretreated with nicotine and acutely treated with 0.04 mg/kg of nicotine was about twice as high as the activity of saline-treated control rats (Whiteaker et al., 1995). However, this effect did not reach statistical significance. A significant effect might have been detected with a larger number of animals per group and/or a more prolonged open field test. In experiment 3, the activity of the tobacco–saline animals was slightly higher than the activity of the air–saline animals during the first 15 min of the open field test (Fig. 3A). The open field test was conducted 24 h after the last smoke exposure session and at this time point all the nicotine was metabolized (half-life of nicotine is about 1 h) (Ghosheh et al., 1999; Miller et al., 1977). At this point, it is not known what caused this delayed effect of tobacco smoke exposure on locomotor activity and it is not known why this effect was observed in only one experiment. It is speculated that the increased activity (first 15 min) was due to some remaining cotinine in the brains of the smoke exposed animals. The half-life of cotinine is about 6 h and cotinine binds to brain $\alpha4\beta2$ and $\alpha3/\alpha6\beta2$ nicotinic acetylcholine receptors (nAChRs) (Ghosheh et al., 1999; Miller et al., 1977; O'Leary et al., 2008). Cotinine also stimulates the release of dopamine in the striatum and dopamine release in this brain area plays a role in locomotor activity (Dwoskin et al., 1999 3195 /id).

A previous study reported that repeated exposure to tobacco smoke does not affect locomotor activity or the locomotor response to nicotine in rats (Harris et al., 2010). However, in the aforementioned study, the animals were exposed to tobacco smoke for a shorter duration (45 min vs. 2 h) and a lower level of tobacco smoke than in the present study. Repeated exposure to a high dose of nicotine leads to a greater and more prolonged increase in locomotor activity than repeated exposure to a low dose of nicotine (Domino, 2001). Therefore, it is most likely that Harris et al. (2010) would have observed an effect of tobacco smoke exposure on locomotor activity and on the locomotor response to nicotine if the animals had been exposed to a higher level of tobacco smoke and for a longer period of time.

The tobacco smoke and nicotine-induced sensitization of locomotor responses may have been due to nicotine-induced changes in the ventral tegmental area and in two of its projection areas, namely the nucleus accumbens and the prefrontal cortex. Nicotinic acetylcholine receptors are located in the ventral tegmental area and in brain areas that project to the ventral tegmental area such as the pedunculopontine tegmental nucleus (Deutch et al., 1987). Extensive evidence indicates that nicotine stimulates dopaminergic neurons in the VTA and increases the release of dopamine in the nucleus accumbens (Calabresi et al., 1989; Nisell et al., 1994a). Repeated administration of nicotine leads to a potentiation of the locomotor response to nicotine and a potentiation of the nicotine-induced release of dopamine in the nucleus accumbens (Benwell and Balfour, 1992). The administration of nAChR antagonists into the ventral tegmental area blocks the nicotine-induced release of dopamine in the nucleus accumbens (Nisell et al., 1994b) and the nicotine-induced increase in locomotor activity in rats chronically treated with nicotine (Corrigall et al., 1994). Drug induced changes in the prefrontal cortex have also been implicated in sensitization processes (Steketee, 2003). The systemic administration of nicotine leads to a greater release of dopamine in the prefrontal cortex of nicotine sensitized animals than in control animals (Nisell et al., 1996). Additional evidence for a role of the prefrontal cortex in drug sensitization is provided by studies that investigated the role of this brain site in AMP and cocaine sensitization. For example, it has been shown that lesioning of the prefrontal cortex with ibotenic acid prevents the development of cocaine and AMP- induced locomotor sensitization (Cador et al., 1999; Li et al., 1999). A recent fMRI study supports the hypothesis that the ventral tegmental area, the nucleus accumbens, and the prefrontal

cortex play a role in nicotine sensitization (Li et al., 2008). It was shown that nicotine sensitized animals display a more prolonged fMRI BOLD (blood-oxygenation level dependent) response in the ventral tegmental area, the nucleus accumbens, and the prefrontal cortex in response to nicotine compared to animals that received nicotine for the first time.

In the fourth and fifth experiments, the effect of repeated exposure to tobacco smoke on AMP-induced locomotor activity was investigated. In experiment 4, it was shown that AMP increased locomotor activity to a similar degree in the tobacco group and the air group and there was no difference in locomotor activity between the air–AMP group and the tobacco–AMP group. This indicates that repeated exposure to tobacco smoke does not affect the locomotor response to a challenge dose of AMP. An additional experiment was conducted to investigate whether tobacco smoke exposed animals are more sensitive to an extremely low dose of AMP (0.1 mg/kg) than control animals. In the present study, there was no difference in locomotor activity between the animals in the tobacco–AMP and the animals in the air–AMP group. This suggests that repeated exposure to tobacco smoke does not affect the locomotor response to an extremely low dose of AMP. The present results are in line with a study that investigated the effect of pretreatment with nicotine (7 days, twice daily 0.4 mg/kg of nicotine base) on the locomotor response to AMP (0.056–0.56 mg/kg) in adult male rats (Collins et al., 2004). There was an effect of the dose of AMP on locomotor activity but pretreatment with nicotine did not affect the locomotor response to AMP. In contrast to the aforementioned findings, another study reported that pretreatment with nicotine sensitizes the locomotor response to AMP (Birrell and Balfour, 1998). In this study the rats were pretreated with nicotine for 5 days (once daily 0.4 mg/kg of nicotine base) and on day 6 the rats were treated with AMP. It was shown that pretreatment with nicotine potentiated the locomotor response to 0.1, 0.2, ad 0.5 mg/kg of AMP. It is not completely clear what caused the discrepancies between these studies. It might be possible that the discrepancy is caused by differences in the test environments. In the present smoke exposure experiment and in the study by Collins et al., (2004), locomotor activity was assessed in an open field. In contrast, in the study in which pretreatment with nicotine sensitized the locomotor response to AMP, locomotor activity was assessed in a 4-arm maze. The open field test and the arm maze test were developed to measure different behavioral parameters. The arm maze test was originally developed to measure place learning and memory (Hodges, 1996). The open field test was developed to measure exploratory behavior and emotional reactivity (Walsh and Cummins, 1976). It has been suggested that drug such as AMP do not directly increase locomotion but increase the exploration of stimuli (Stewart and Vezina, 1988). Therefore, AMP may have a greater effect on locomotor activity in a complex environment such as the 4-arm maze than in an open field test.

It cannot be ruled out that repeated exposure to tobacco smoke would have sensitized the locomotor response to AMP if the rats had been exposed to tobacco smoke during adolescence or at an earlier time in development. Collins et al., (2004) showed that the administration of nicotine to adolescent male rats (postnatal day 30) but not to adult male rats leads to a potentiation of the locomotor response to AMP. Another study also reported that the administration of nicotine to young rats (140–160 g) leads to a potentiation of the locomotor response to AMP (Schoffelmeer et al., 2002). Although the age of the animals was not reported in the aforementioned study, the body weights of the rats correspond to those of late adolescent rats (postnatal days 38–42) (Spear, 2000).

Taken together, the present studies indicate that repeated passive exposure to tobacco smoke leads to a potentiation of the locomotor response to tobacco smoke and nicotine but not AMP in adult rats. Experimental evidence suggest that sensitization processes, which are reflected in enhanced locomotor responses to drugs of abuse, play a role in the development of drug addictions (Robinson and Berridge, 1993). Therefore, these studies suggest that passive exposure to high doses of secondhand tobacco smoke or experimenting with cigarettes induces adaptations in the brain that potentiate the stimulant effects of nicotine and increase the risk for developing a tobacco addiction.

Acknowledgments

This research was funded by a Flight Attendant Medical Research Institute Young Clinical Scientist Award (Grant no. 52312) to Adrie Bruijnzeel.

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