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Effects of Smoking Successive Low- and High-Nicotine Cigarettes on Hypothalamic–Pituitary–Adrenal Axis Hormones and Mood in Men

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Smoking one cigarette produces rapid nicotine dose-related increases in hypothalamic–pituitary–adrenal (HPA) axis hormones, mood, and heart rate, but relatively little is known about the effects of smoking several cigarettes successively. Twenty-four healthy adult men who met Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria for nicotine dependence provided informed consent. After overnight abstinence from smoking, men smoked three low- or high-nicotine cigarettes for 4 min each at 60 min intervals. Samples for nicotine and hormone analysis, Visual Analog Scale (VAS) ratings of subjective effects and heart rate were collected at 4, 8, 12, 16, 20, 30, 40, and 50 min after each cigarette. After low-nicotine cigarettes, nicotine levels, adrenocorticotropin hormone, and heart rate did not increase significantly, cortisol and dehydroepiandrosterone decreased significantly, and positive VAS ratings were lower but parallel to ratings after high-nicotine cigarette smoking. After high-nicotine cigarettes, peak nicotine levels increased monotonically. HPA axis hormones increased after smoking, but peak levels did not differ significantly after successive high-nicotine cigarettes. Positive VAS ratings and heart rate increased after each high-nicotine cigarette, but peak levels were lower after smoking the second and third cigarette. 'Craving' decreased significantly after smoking both low- and high-nicotine cigarettes, then gradually increased during the 60 min interval between cigarettes. These data are consistent with clinical reports that the first cigarette after overnight nicotine abstinence is most salient. Tolerance to the subjective and cardiovascular effects of nicotine developed rapidly during repeated cigarette smoking, but nicotine-stimulated increases in HPA axis hormones did not change significantly.

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INTRODUCTION

Addiction to cigarette smoking is a leading cause of death and disease (CDC, 2000, 2004) and relapse rates remain high despite the availability of many pharmacological approaches to treatment (Henningfield *et al*, 2005). Nicotine's reinforcing effects are mediated in part by activation of the mesolimbic dopamine system to stimulate dopamine release (Corrigall *et al*, 1992; Di Chiara, 2000; Watkins *et al*, 2000). Activation of the hypothalamic–pituitary–adrenal (HPA) axis is also thought to contribute to drug abuse at several phases of the addictive process (Contoreggi *et al*, 2003; Heinrichs and Koob, 2004; Koob and Le Moal, 2001; Marinelli and Piazza, 2002; Sinha, 2001). Adrenocorticotropin hormone (ACTH) stimulation by corticotropin releasing factor (CRF) is one component of the integrated physiological response to 'stress' (Nemeroff, 1996; Tsigos and Chrousos, 2002). However, little is known about the possible influence of HPA axis hormones on the abuse-related effects of cigarette smoking. One intriguing finding is that increases in ACTH covary with rapid increases in positive subjective effects ratings and ascending levels of nicotine, as well as cocaine (Mendelson *et al*, 2002, 2005). Moreover, preclinical evidence suggests that stimulation of CRF, as inferred from increases in ACTH and corticosterone, may be important for the acquisition and maintenance of cocaine self-administration in rodents (Goeders, 1997, 2002a, b; Mantsch and Katz, 2007 (in press); Mantsch *et al*, 2000; Mello and Mendelson, 2002).

We recently reported that smoking one high-nicotine cigarette produced significant increases in HPA axis hormones, heart rate, and ratings of positive subjective effects (Mendelson *et al*, 2005). Moreover, comparison of the effects of smoking a low- and a high-nicotine cigarette demonstrated that these biological and subjective effects were nicotine dose-dependent (Mendelson *et al*, 2005). Significant increases in ACTH were followed by significant

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elevations in the adrenal hormones cortisol and dehydroepiandrosterone (DHEA) (Mendelson *et al*, 2005). Although the role of cortisol in nicotine dependence is unclear, a decrease in cortisol after smoking cessation appears to predict a higher risk for relapse (al'Absi *et al*, 2004; Rasmusson *et al*, 2006). DHEA has recently been suggested as a medication to facilitate smoking cessation, in part because levels of its sulfated derivative, DHEAs, were inversely correlated with several measures of nicotine dependence and negative affect in smokers (Marx *et al*, 2006).

The present study was designed to examine the effects of smoking three successive cigarettes on nicotine and HPA axis hormone levels, heart rate and reports of subjective effects. One goal was to determine if the pattern of changes in these measures observed after smoking one cigarette increased, decreased, or remained the same after smoking a second and third cigarette. The temporal covariance between these dependent variables during repeated cigarette smoking is unknown, and may be important for understanding the development of nicotine tolerance.

There have been relatively few clinical laboratory studies of the effects of smoking successive cigarettes, and most studies have not examined HPA axis hormones in relation to nicotine levels, heart rate, and reports of subjective effects. A number of behavioral studies have examined the effects of *ascending doses* of nicotine, and dose-related increases in positive subjective effects are usually reported (Perkins *et al*, 1994a, 2001; Sofuoglu *et al*, 2006; Stein *et al*, 1998). When the *same dose* of nicotine is repeatedly administered within a session, the extent to which tolerance develops varies as a function of the procedure, the dose of nicotine and the route of administration. No change and progressive increases and decreases in subjective effects and heart rate have been reported. Acute tolerance, defined as a diminished response to the subjective effects of nicotine, developed after repeated administration of nicotine nasal spray within a session (Perkins *et al*, 1993, 1995). When the same dose of nicotine nasal spray (0, 7.5, or 15 μg/kg) was given every 30 min for 2 h, there was a dose-related decrease in Visual Analog Scale (VAS) ratings of 'Light Headed', 'Dizzy', and 'Head Rush' in response to a challenge dose of 30 μg/kg nicotine administered 30 min after the session (Perkins *et al*, 1993). However, repeated administration of nicotine nasal spray (10 or 20 μg/kg) once every 30 min for 2 h also produced increases in heart rate and ratings of 'Rush' and 'Dizzy' that paralleled increases in plasma nicotine levels (Perkins *et al*, 1994b). When subjects smoked their usual cigarette or a low-yield cigarette for ~2.5 min once every 30 min for 2 h, increases in subjective ratings of 'Rush' and 'Dizzy' and heart rate also paralleled increases in plasma nicotine levels (Perkins *et al*, 1994b). Increasing the interval between nicotine exposure (20 μg/kg nicotine spray every 30 min for 2 h) and a challenge dose of the same dose of nicotine suggested that acute tolerance to cardiovascular and some subjective effects persisted for at least 2 h (Perkins *et al*, 1995).

The interval between smoking successive cigarettes is another determinant of subjective responses to nicotine. When subjects smoked their preferred brand of cigarettes for 15 min at intervals of 30, 60, 120, and 360 min during a 6 h session, 'Desire to Smoke' increased as a function of the length of the inter-cigarette interval (Schuh and Stitzer, 1995). Reports of 'Dizzy', 'Light Headed', and 'Tingling', and increases in heart rate were also greatest after 6 h of no smoking (Fant *et al*, 1995). The first clinical study to examine the effects of repeated IV nicotine administration (0.75, 1.5, and 3.0 mg per injection) reported orderly dose-related patterns of nicotine self-administration (Henningfield *et al*, 1983). At the end of a 3 h session, subjects reported drug liking, but also dysphoric effects and nausea (Henningfield *et al*, 1983). When the same dose of IV nicotine (2 μg/kg once a min for 10 min) was administered six times at 30 min intervals, there was a progressive decrease in ratings of positive subjective reactions, but cardiovascular measures remained elevated for 3 h (Rosenberg *et al*, 1980).

The primary goal of the present study was to assess the effects of smoking three successive low- or high-nicotine cigarettes on the relationship between nicotine levels, anterior pituitary, and adrenal hormone levels, VAS reports of positive and negative subjective effects and cardiovascular measures. The time course and direction of changes in these measures should clarify the extent and rate of acute tolerance development. A second goal was to determine if the subjective, hormonal, and cardiovascular effects of smoking successive cigarettes were nicotine dose related. The effects of smoking low- or high-nicotine cigarettes were compared under identical experimental conditions, and all dependent measures were sampled at 4 min intervals immediately following smoking each cigarette.

MATERIALS AND METHODS

Subjects

Twenty-four healthy adult men were recruited through newspaper advertisements and provided written informed consent for pre-study screening procedures and for participation in this study. The study was approved by the Institutional Review Board of the McLean Hospital. All men fulfilled American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria for current nicotine dependence (305.1). Volunteers with any lifetime DSM-IV Axis 1 disorder other than nicotine dependence were excluded. Men who were seeking treatment for nicotine dependence, or who were wearing a nicotine patch, were also excluded. All men selected for this study were in good physical health and had normal medical and laboratory screening examinations. Screening tests included complete blood count, hepatitis panel, chemistry panel including electrolytes and liver enzymes, and an electrocardiogram (EKG). Obese and underweight men were excluded (body mass index (BMI) below 21.4 or above 29).

Twelve men were randomly assigned to smoking a high-nicotine cigarette and twelve men were assigned to smoking a low-nicotine cigarette. Subjects in the high- and low-nicotine groups did not differ significantly with respect to age, years of smoking, number of cigarettes smoked per day, or BMI (Table 1). Their scores on the Fagerstrom test averaged 6.50 ± 0.47 and 6.25 ± 0.43 (Fagerstrom and

Table 1 Subject Characteristics

	Age (years)*	Years of smoking*	Cigarettes smoked per day*	BMI (kg/M^2) ($\bar{x}\pm$SEM)*	Baseline CO levels*	Baseline plasma nicotine levels*
High-nicotine cigarette group ($N=12$)	25.7 ± 1.2	7.5 ± 1.3	21.0 ± 2.0	23.3 ± 0.5	5.6 ± 0.8 p.p.m.	3.31 ± 1.05 ng/ml
Low-nicotine cigarette group ($N=12$)	27.9 ± 1.46	9.5 ± 1.6	20.8 ± 1.8	23.8 ± 0.6	6.0 ± 0.8 p.p.m.	3.06 ± 0.42 ng/ml
ANOVA results	*$P=0.25$ (df = 1, F = 1.41)	*$P=0.35$ (df = 1, F = 0.94)	*$P=0.95$ (df = 1, F = 0.004)	*$P=0.54$ (df = 1, F = 0.39)	*$P=0.72$ (df = 1, F = 0.13)	*$P=0.83$ (df = 1, F = 0.049)

Demographic data, carbon monoxide levels, and baseline plasma nicotine levels were analyzed using a one-factor ANOVA.

Schneider, 1989). A between-subjects design was used because an interval of at least 8 weeks (56 days) between successive studies was required due to the volume of blood withdrawn for plasma nicotine and hormone analyses. It is usually difficult to maintain contact with subjects over this period.

The study procedures were explained during initial screening and again on the study day, and any questions or concerns were discussed. Subjects were admitted to the clinical research ward on the morning of the study day. Studies were conducted at the same time of day, beginning at 10:00. Subjects were told they would be asked to smoke three successive cigarettes with a low- or high-nicotine content under controlled smoking conditions. After completion of the study, subjects remained on the clinical research ward for 2 or more hours. A light meal was provided and vital signs were measured at 30 min intervals. Once subjects were medically stable, they were discharged and taxi transportation was provided. Subjects were paid to participate in the study, consistent with NIH regulations. Subjects earned $50 for the initial screening visit and $250 for the study day.

Smoking-Abstinence Requirements

Subjects were asked to abstain from cigarette smoking and caffeinated beverage consumption after midnight on the night before the study. Carbon monoxide (CO) levels were measured with a Vitalograph Breath CO Monitor (Vitalograph Inc., Lenexa, KS) to assess compliance with smoking-abstinence requirements. Cigarette smokers with a CO level above 10 p.p.m. were not allowed to participate in the study. As shown in Table 1, CO levels averaged 5.6 ± 0.8 and 6.0 ± 0.8 p.p.m. in the high- and low-nicotine cigarette groups, respectively. It is important for subject safety, as well as to avoid confounding of the dependent variables, to ensure that subjects have not used any drugs before cigarette smoking. On the morning of each study day, urines were collected and analyzed with a Triage® screen. The Triage® Panel for Drugs of Abuse (Biosite Diagnostics, San Diego, CA) is a rapid multiple immunoassay system for the qualitative detection of the major metabolites of drugs of abuse in urine at concentrations as recommended by the Substance Abuse and Mental Health Services Administration. If a subject tested positive on the Triage® screen, he was not allowed to participate in the study.

Nicotine Dose Selection

A commercially available, high-nicotine cigarette and a low-nicotine cigarette were studied. The high-nicotine cigarette (Marlboro Red, Philip Morris brand, Philip Morris, Richmond, VA, USA) contained 15.48 mg of nicotine and 16 mg of tar based on analysis by the Massachusetts Department of Public Health. The low-nicotine cigarettes contained 1.1 mg of nicotine and 2.8 mg of tar based on analyses provided by the manufacturer, Murty Pharmaceuticals Inc., Lexington, KY. We refer to this cigarette as low-nicotine rather than as denicotinized, because most 'denicotinized' cigarettes contain trace amounts of nicotine (< 0.06–0.16 mg) (Pickworth *et al*, 1999; Robinson *et al*, 2000; Shahan *et al*, 1999). These cigarettes were identical to the high- and low-nicotine cigarettes used in our previous study (Mendelson *et al*, 2005).

Nicotine Administration Procedures

These studies were carried out on a clinical research ward. Subjects were studied in a semi-supine position. Men smoked three high- or low-nicotine cigarettes at 60 min intervals. We selected a 60 min inter-cigarette interval on the basis of our previous studies in which subjective, hormonal, and cardiovascular endpoints usually returned to baseline within 50 min after smoking a single high-nicotine cigarette (Mendelson *et al*, 2005). Cigarettes were administered using a controlled smoking procedure designed to standardize puff volume and duration of inhalation (Griffiths *et al*, 1982). A controlled smoking procedure was used because it is well established that nicotine deprivation as well as the nicotine content of cigarettes alters the topography of smoking behavior (Henningfield and Griffiths, 1979, 1980; Zacny and Stitzer, 1988). Subjects smoked each cigarette for 4 min and took one 5 s puff every 30 s. Over a 4 min smoking period, subjects took eight puffs with an inter-puff interval of 25 s.

Cardiovascular Measures and Safety Precautions

Heart rate, blood pressure, and EKGs were continuously monitored with a Criticare Scholar™II Patient Monitor (Model 507EP) for 10 min before smoking the first cigarette and for 3 h during cigarette administration. A physician certified in cardiopulmonary resuscitation was present during each study, and a cardiac defibrillator and

appropriate emergency treatment medications were located in the study room.

Sample Collection Procedures

A catheter for blood collection was placed in the antecubital vein of one arm. Baseline samples for analysis of ACTH, cortisol, DHEA, and nicotine levels were collected 10 min before smoking the first cigarette began, then at 4, 8, 12, 16, 20, 30, 40, and 50 min after smoking each successive cigarette. This sampling frequency was based upon our previous observations that nicotine levels in plasma increase rapidly after smoking begins and usually reach peak levels within 14 min (Mendelson *et al*, 2005, 2003). The catheter site and collection tubes were covered throughout the study so that smoke in the air would not contaminate the plasma samples. Blood samples for hormone analysis and for nicotine analysis were collected in vacutainer tubes without preservative. All blood samples were iced immediately, centrifuged, and plasma or serum was removed and frozen at -70°C.

Subjective Effects Measures

Subjects rated their reactions to smoking on a Visual Analog Scale (VAS) that ranged from 0 to 100. Subjects were asked to rate how 'High' they felt, if they felt a 'Rush', how much they 'Liked' nicotine, and how much they 'Craved' cigarettes. Subjects were also asked to rate feelings of 'Stimulated', 'Sick', 'Jittery', 'Good Feeling', 'Bad Feeling', 'Dizzy', and 'Alert'. Subjects were asked to rate these subjective effects before smoking and every 4 min for the first 20 min after cigarette smoking began, then at 30, 40, and 50 min following smoking onset.

Assay Procedures

ACTH. Plasma ACTH concentrations were measured in duplicate using an immunoradiometric assay kit purchased from Nichols Institute (San Juan Capistrano, CA). The assay sensitivity was 0.24 pmol/l and the intra- and interassay coefficients of variation (CVs) were 5.6 and 8.0%, respectively.

Cortisol. Plasma cortisol concentrations were measured in duplicate by the GammaCoat RIA kit purchased from DiaSorin (Stillwater, MN). The assay sensitivity was 5.5 nmol/l and intra- and interassay CVs were 6.5 and 9.6%, respectively.

DHEA. Plasma DHEA concentrations were determined in duplicate by Coated Tubes RIA method using kits purchased from Diagnostic Systems Laboratories Inc. (Webster, TX). The assay sensitivity was 0.02 ng/ml and intra- and interassay CVs were 3.2 and 11.2%, respectively.

Nicotine analysis. Plasma nicotine levels were measured in duplicate using a gas chromatography-mass spectrometry method described by Jacob and co-workers (Jacob *et al*, 2000). The nicotine assay sensitivity was 5.0 ng/ml and the intra- and interassay CVs were 4.6 and 11.5%, respectively.

Data Analysis

Comparisons between the subjective and physiological effects of the first, second, and third cigarettes in the high- and low-nicotine dose groups were analyzed with analysis of variance (ANOVA) for repeated measures. Within each nicotine dose group, changes in each dependent variable from baseline were assessed with a one factor repeated measures ANOVA. If significant main effects were detected, one-way ANOVAs were performed to determine which points differed significantly from baseline. Plasma nicotine levels, hormone levels, subjective measures, and cardiovascular parameters in the low- and high-nicotine groups were analyzed by a two factor (dose group $\times$ time) repeated measures ANOVA with time post-smoking as the repeated factor. If significant main effects were detected, one-way ANOVAs were performed to identify the time points that differed significantly between the two nicotine dose groups. The statistical significance of the temporal covariance between heart rate, plasma nicotine and VAS measures and between cortisol and DHEA, after low- and high-nicotine cigarette smoking was evaluated with a Pearson Product Moment Correlation analyses.

RESULTS

Nicotine Levels before and after Smoking Three Low- or High-Nicotine Cigarettes

There were no significant differences between low- and high-nicotine groups in baseline nicotine levels (Figure 1) or baseline CO levels (Table 1). Nicotine levels increased significantly within 4 min, or 8 puffs on the first high-

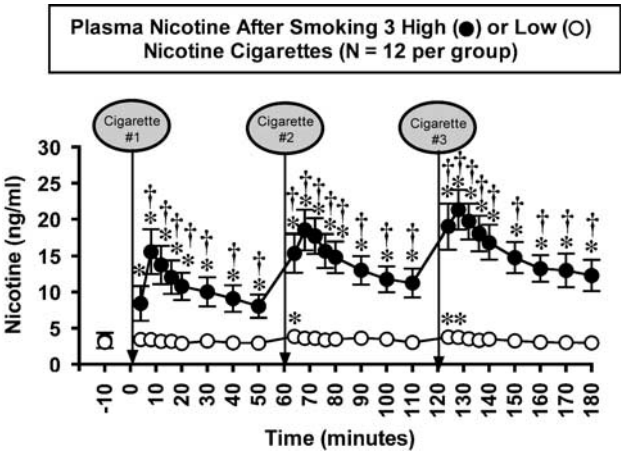

Figure 1 Plasma nicotine levels after smoking three low- or high-nicotine cigarettes. Plasma nicotine levels after smoking a high-nicotine cigarette (filled circles) and a low-nicotine cigarette (open circles) are shown on the left ordinates. Time (min) is shown on the abscissa. Points above baseline were collected 10 min before smoking the first cigarette began at time 0. A vertical line and an arrow indicate each 4 min cigarette-smoking period. Each data point is the average ($\pm$SEM) of 12 men. Statistical analyses indicated significant changes in plasma nicotine levels from baseline in both low- (df = 25, F = 2.4, $P = 0.04$) and high-nicotine cigarette groups (df = 25, F = 21.1, $P < 0.0001$). *Significant changes from the pre-smoking baseline ($P = 0.05 - < 0.0001$). †Points at which plasma nicotine levels after smoking a low-nicotine cigarette differed from plasma nicotine levels after smoking a high-nicotine cigarette (df = 1, F = 4.2–43.5, $P = 0.05 - < 0.0001$).

nicotine cigarette, and remained significantly above baseline throughout the 180 min sampling period ($P = 0.05$–< 0.0001) (Figure 1). Peak nicotine levels were detected within 8 min after smoking each high-nicotine cigarette and increased monotonically across successive cigarettes. Peak nicotine levels averaged 15.6 ± 3.1, 18.56 ± 2.7, and 21.3 ± 2.7 ng/ml after the first, second, and third cigarette and were significantly higher after the third cigarette than after the first cigarette ($P = 0.005$). Nicotine levels gradually decreased to 8.1 ± 1.6, 11.3 ± 2.0, and 12.3 ± 2.2 ng/ml at the end of each successive smoking period (Figure 1). During low-nicotine cigarette smoking, nicotine levels averaged between 2.94 ± 0.45 and 3.86 ± 0.43 ng/ml and were significantly lower than after high-nicotine cigarette smoking throughout the 120 min sampling period ($P = 0.05$–< 0.0001). Repeated measures ANOVA indicated significant interactive effects (nicotine dose × time) ($df = 25$, $F = 18.5$, $P < 0.0001$).

HPA Hormones after Smoking Three Low- or High-Nicotine Cigarettes

ACTH levels. Before smoking began, average baseline ACTH levels were equivalent in the low- and high-nicotine cigarette groups (Figure 2). After smoking low-nicotine cigarettes, ACTH levels did not change significantly from baseline at any time point. Smoking the first high-nicotine cigarette produced a significant increase in ACTH above baseline within 12 min and ACTH remained significantly above baseline for 8 min ($P = 0.03$–0.02). ACTH did not increase after smoking the second high-nicotine cigarette, but after smoking the third high-nicotine cigarette, ACTH increased significantly within 12 min and remained elevated for 18 min ($P = 0.05$–0.03). Repeated measures ANOVA indicated significant interactive effects (nicotine dose × time) ($df = 25$, $F = 2.4$, $P < 0.05$).

Cortisol levels. Baseline cortisol levels were equivalent before low- and high-nicotine cigarette smoking. Cortisol levels were significantly higher after smoking high-nicotine cigarettes than after smoking low-nicotine cigarettes ($P = 0.02$–0.009). Cortisol levels decreased significantly from baseline within 12 min after smoking the first low-nicotine cigarette ($P = 0.04$), then continued to decrease, and remained significantly below baseline throughout the 180 min sampling period ($P = 0.04$–< 0.0001). Cortisol initially decreased after smoking each high-nicotine cigarette ($P = 0.05$–0.003), then increased to peak levels within 20–30 min. Peak cortisol levels after smoking the third cigarette were lower than after the first cigarette ($P = 0.06$). Repeated measures ANOVA indicated significant interactive effects (nicotine dose × time) ($df = 25$, $F = 4.1$, $P < 0.002$).

DHEA levels. Baseline DHEA levels were higher in the low-nicotine group before low- and high-nicotine cigarette smoking, but these differences were not significant. The pattern of changes in DHEA was similar to those of cortisol in both the low-nicotine group ($r = 0.663$, $P < 0.001$) and the high-nicotine group ($r = 0.624$, $P < 0.001$). After low-nicotine cigarette smoking, DHEA levels decreased significantly from baseline within 16 min and remained significantly below baseline until 40 min after the third cigarette ($P = 0.04$–0.0008). After smoking each high-nicotine cigarette, DHEA initially decreased, then increased to peak levels within 20–30 min ($P = 0.02$–0.009). Repeated measures ANOVA indicated significant interactive effects (nicotine dose × time) ($df = 25$, $F = 4.4$, $P < 0.003$).

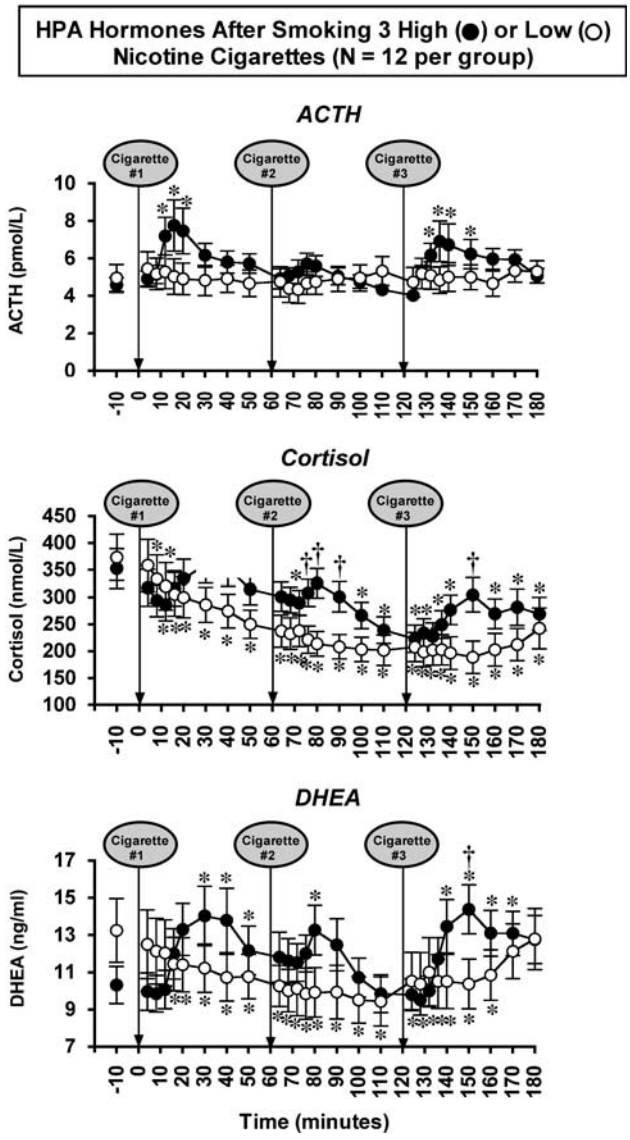

Figure 2 Adrenocorticotropin hormone (ACTH), cortisol and dehydroepiandrosterone (DHEA) levels after smoking three low- or high-nicotine cigarettes. Hormone levels after smoking a high-nicotine cigarette (filled circles) or a low-nicotine cigarette (open circles) are shown on the left ordinates. Time (min) is shown on the abscissa. Points above baseline were collected 10 min before cigarette smoking began at time 0. A vertical line and an arrow indicate each 4 min cigarette-smoking period. Each data point is the average (±SEM) of 12 men. Data are shown for ACTH (pmol/l) cortisol (nmol/l) and DHEA (ng/ml). Statistical analyses indicated significant changes from baseline in ACTH levels ($df = 25$, $F = 2.8$, $P = 0.04$), cortisol levels ($df = 25$, $F = 4.8$, $P = 0.006$), and DHEA levels ($df = 25$, $F = 4.0$, $P = 0.007$) after high-nicotine cigarette smoking. *Significantly different from baseline ($P = 0.05$–< 0.0001). †Significantly different after high-nicotine cigarette smoking than after low-nicotine cigarette smoking, DHEA ($df = 1$, $F = 5.7$, $P = 0.026$), and cortisol ($df = 1$, $F = 5.9$–8.3, $P = 0.02$–0.009).

Cardiovascular Effects of Smoking Three Low- or High-Nicotine Cigarettes

Heart rate. There were no significant differences in heart rate between the groups before smoking began (Figure 3). Heart rate increased significantly within 4 min after smoking the first cigarette in both the low- ($P=0.02$) and high- ($P<0.0001$) nicotine groups. Heart rate remained significantly higher after smoking each of the high-nicotine cigarettes than after smoking the low-nicotine cigarettes ($P=0.05$–0.0009), however, the pattern of changes was very similar ($r=0.753$, $P<0.0001$). After smoking each high-nicotine cigarette, peak heart rates averaging 78.7 ± 2.2–86.2 ± 2.9 b.p.m. were detected within 4 min ($P=0.001$–<0.0001), and heart rate remained significantly above baseline for 12–20 min ($P=0.038$–<0.0001). After low-nicotine cigarette smoking, peak heart rates of 68.4 ± 1.3–75.5 ± 2.4 b.p.m. were detected within 4 min, but heart rate remained equivalent to baseline levels throughout the low-nicotine cigarette smoking period. Increases in heart rate were significantly correlated with increases in reports of 'High' ($r=0.88$; $P<0.0001$), 'Rush' ($r=0.87$; $P<0.0001$), 'Stimulated' ($r=0.67$; $P=0.0002$), and increases in nicotine levels ($r=0.41$; $P=0.04$). Repeated measures ANOVA indicated significant interactive effects (nicotine dose × time) (df = 25, F = 2.3, $P<0.05$).

Systolic and diastolic blood pressure. Baseline systolic and diastolic blood pressure did not differ before low- and high-nicotine cigarette smoking (data not shown). At 10 min before smoking began, systolic blood pressure averaged between 116.33 ± 2.33 and 115.82 ± 2.86 mmHg and diastolic blood pressure averaged between 75.75 ± 2.44 and 75.18 ± 1.89 mmHg. There were no significant changes in systolic or in diastolic blood pressure after smoking either low- or high-nicotine cigarettes (data not shown).

Subjective Effects after Smoking Three Low- or High-Nicotine Cigarettes

VAS ratings of 'High', 'Rush', 'Liking', and 'Craving' before and after smoking three successive low- or high-nicotine cigarettes are shown in Figure 4. Repeated measures ANOVA indicated significant interactive effects for 'High' and 'Rush' (nicotine dose × time) (df = 25, F = 2.4, $P<0.05$), (df = 25, F = 2.4, $P<0.05$), respectively. Significant main effects were detected for 'Like' and 'Craving' (df = 1, F = 4.5, $P<0.05$, df = 1, F = 12.5, $P<0.002$), respectively. Baseline levels of these subjective measures did not differ significantly between the high- and low-nicotine cigarette groups. The pattern of changes in each of these VAS ratings was very similar after low- and high-nicotine cigarette smoking ('High', $r=0.823$, $P<0.001$; 'Rush', $r=0.816$, $P<0.0001$; 'Liking', $r=0.652$, $P<0.001$). After smoking each of the high-nicotine cigarettes, VAS ratings of 'High', 'Rush', and 'Liking' increased significantly to peak levels within the first 4 min or after eight puffs ($P<0.0001$), and were significantly higher than after low-nicotine cigarette smoking ($P=0.04$–0.005), ($P=0.46$–0.01), and ($P=0.05$–0.03), respectively. After low-nicotine cigarette smoking, the VAS ratings of 'High', 'Rush' and 'Liking' also increased significantly above baseline ($P=0.02$–0.0003).

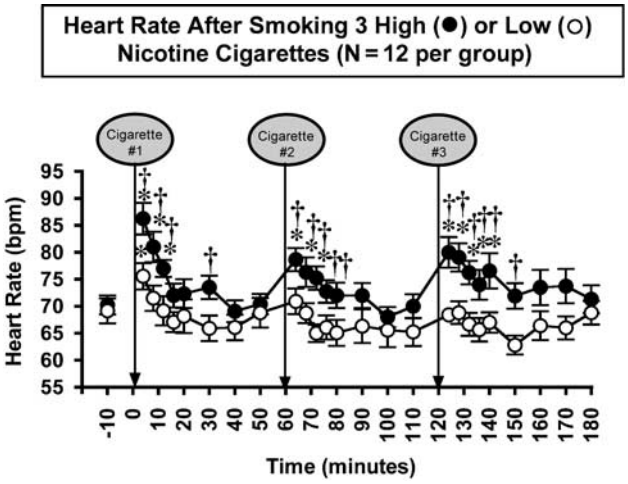

Figure 3 Heart rate after smoking three low- or high-nicotine cigarettes. Heart rate (b.p.m.) after smoking a high-nicotine cigarette (filled circles) or a low-nicotine cigarette (open circles) is shown on the left ordinate. Time (min) is shown on the abscissa. Points above baseline were collected 10 min before smoking began at time 0. A vertical line and an arrow indicate each 4 min cigarette-smoking period. Each data point is the average (±SEM) of 12 men. Statistical analyses indicated significant changes in heart rate levels from baseline in both low- (df = 25, F = 2.8, $P=0.02$) and high-nicotine cigarette groups (df = 25, F = 7.5, $P<0.0001$). *Significantly different from baseline ($P=0.05$–<0.0001). †Significantly different after high-nicotine cigarette smoking than after low-nicotine cigarette smoking (df = 1, F = 4.3–14.6, $P=0.05$–0.0009).

Baseline VAS ratings of 'craving' for nicotine were high in both the high- and low-nicotine dose groups and averaged 65.8 ± 8.3 and 61.2 ± 9.6. Craving ratings decreased significantly within 4–8 min after smoking each low- or high-nicotine cigarette ($P=0.03$–0.0001). High-nicotine cigarette smoking decreased 'Craving' scores significantly more than low-nicotine cigarette smoking at most time points ($P=0.05$–0.006), but 'Craving' scores in the two groups were significantly correlated ($r=0.840$, $P<0.0001$). During the last 30 min of each of the three post-smoking periods, craving scores after smoking low-nicotine cigarettes were significantly higher than after smoking high-nicotine cigarettes ($P=0.05$–0.008).

Baseline VAS ratings of 'Sick', 'Bad Feeling', 'Jittery', and 'Dizzy' before and after smoking three successive high- and low-nicotine cigarettes did not differ significantly between the low- and high-nicotine groups. There were no significant differences between the low- and high-nicotine dose groups in ratings of 'Sick', 'Jittery', and 'Dizzy'. However, ratings of 'Bad Feeling' were higher in the low-nicotine group than in the high-nicotine group at 30 and 40 min after smoking the first cigarette ($P<0.05$) (data not shown).

DISCUSSION

This is the first clinical laboratory study to examine the effects of smoking three successive cigarettes on nicotine levels, HPA axis hormones, heart rate, and subjective effects measures using rapid (4 min) sampling procedures. One major finding of this study was that nicotine levels

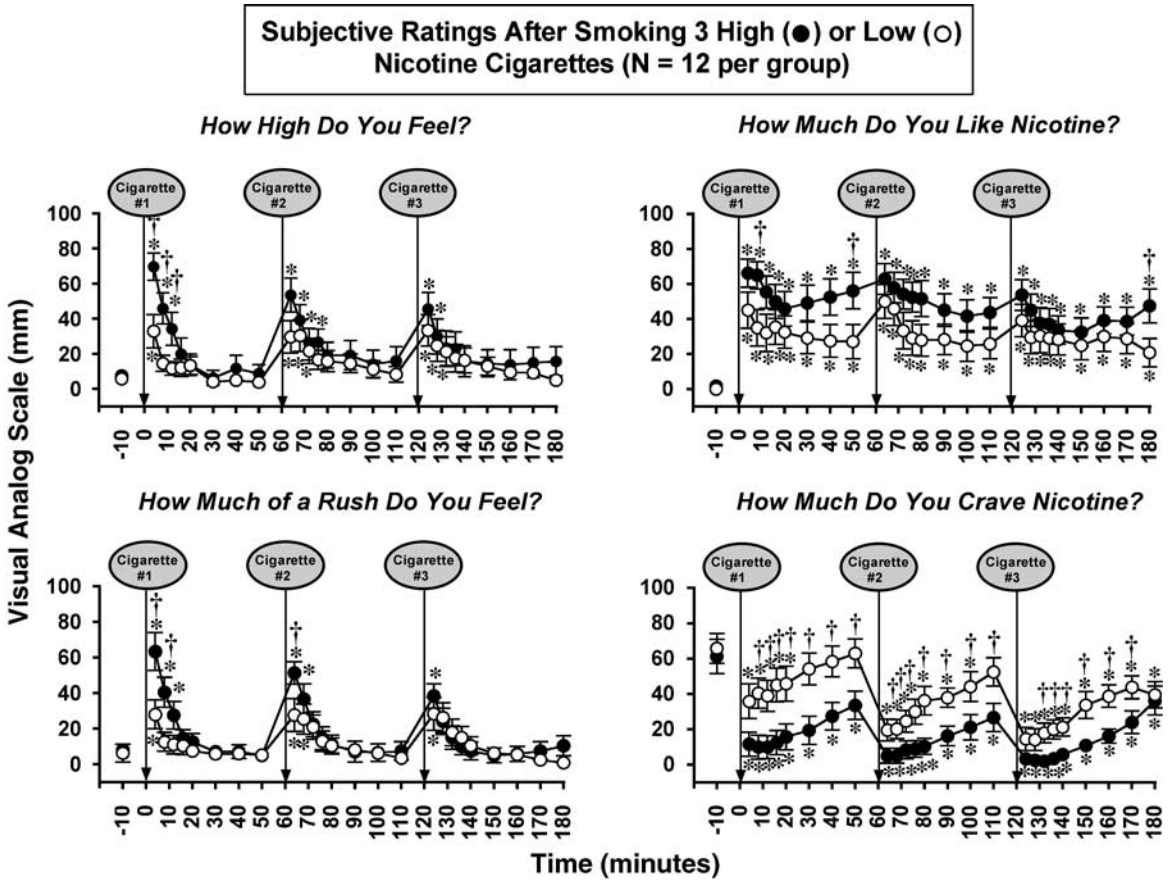

Figure 4 Reports of subjective effects after smoking three low- or high-nicotine cigarettes. Subjective ratings on a Visual Analog Scale (VAS) (0–100) after smoking a high-nicotine cigarette (filled circles) or a low-nicotine cigarette (open circles) are shown on the left ordinates. Time (min) is shown on the abscissa. Points above baseline were collected 10 min before smoking began at time 0. Each data point is the average (±SEM) of 12 men. A vertical line and an arrow indicate each 4 min cigarette-smoking period. *Significantly different from baseline ($P = 0.05 - < 0.0001$). Statistical analyses indicated significant changes from baseline in reports of 'High' after high-nicotine cigarettes ($df = 25$, $F = 9.1$, $P < 0.0001$) and low-nicotine cigarettes ($df = 25$, $F = 4.0$, $P = 0.01$); reports of 'Liking' after high-nicotine cigarettes ($df = 25$, $F = 5.8$, $P < 0.0001$) and low-nicotine cigarettes ($df = 25$, $F = 3.6$, $P = 0.01$); reports of 'Rush' after high-nicotine cigarettes ($df = 25$, $F = 10.3$, $P < 0.0001$) and low-nicotine cigarettes ($df = 25$, $F = 4.0$, $P = 0.02$); and reports of 'Craving' after high-nicotine cigarettes ($df = 25$, $F = 7.5$, $P < 0.0001$) and after low-nicotine cigarettes ($df = 25$, $F = 6.3$, $P = 0.0007$). †Significantly different after high-nicotine cigarette smoking than after low-nicotine cigarette smoking 'High' ($df = 1$, $F = 4.9–9.6$, $P = 0.04–0.005$), 'Liking' ($df = 1$, $F = 4.1–5.4$, $P = 0.05–0.03$), 'Rush' ($df = 1$, $F = 4.4–7.9$, $P = 0.46–0.01$), and 'Craving' ($df = 1$, $F = 4.2–9.2$, $P = 0.05–0.006$).

increased significantly after smoking each of the three high-nicotine cigarettes at 1 h intervals, and were significantly higher after the third cigarette than after the first cigarette. However, the cumulative increases in peak nicotine levels were not accompanied by progressive increases in peak positive subjective effects ratings, or peak heart rate, ACTH, cortisol, or DHEA. Rather, these measures gradually decreased or remained the same after smoking each successive cigarette. Although the magnitude of changes in VAS ratings and heart rate were nicotine-dose related, the pattern of changes in these measures was similar after low- and high-nicotine cigarette smoking. HPA axis hormone levels increased after smoking high-nicotine cigarettes, but no changes or a significant decrease occurred after smoking low-nicotine cigarettes. The relations of these findings to earlier reports, and to our previous study of the subjective, cardiovascular and endocrine effects of smoking a single high- or low-nicotine cigarette (Mendelson *et al*, 2005) are described below.

Nicotine Levels after Smoking Three Successive Cigarettes

The progressive increase in peak nicotine levels after smoking three successive high-nicotine cigarettes presumably reflects some accumulation of nicotine. A similar pattern of increases in nicotine levels was reported when subjects smoked five cigarettes at 30 min intervals (Isaac and Rand, 1972) and seven cigarettes at 1 h intervals (Russell, 1985). However, subjective effects and cardiovascular measures were not reported in those studies. Progressive increases in nicotine levels, comparable to those observed in the present study, were also reported after successive IV nicotine injections (Rosenberg *et al*, 1980). In contrast, smoking three low-nicotine cigarettes did not result in cumulative increases in nicotine levels.

One limitation of the present study is that nicotine and hormone levels were measured in venous rather than arterial blood. Arterial nicotine levels are 2–3- or 6–10-fold

higher than venous nicotine levels (Benowitz, 1996; Rose *et al*, 1999). After the first 5 s puff, nicotine from cigarette smoke reached peak levels in the arterial circulation within 15–20 s and continued to increase after the second and third puff (Rose *et al*, 1999). Although significant increases in subjective ratings and plasma nicotine were detected after eight puffs in the present study, arterial nicotine, CRF, and ACTH levels may have increased significantly after a single puff.

Tolerance to Nicotine's Biological and Subjective Effects

There is an extensive literature indicating that acute tolerance develops to some effects of nicotine under a number of conditions, and chronic tolerance may persist for weeks or even years after abstinence from smoking (Benowitz *et al*, 1990; Fant *et al*, 1995; Perkins *et al*, 1991, 1993, 1994b, 1995, 2001; Rosenberg *et al*, 1980; West and Russell, 1987). As discussed below, decreases in VAS subjective reports and cardiovascular measures after successive cigarettes are consistent with this interpretation. It is generally agreed that nicotine is the primary addictive agent in cigarette smoking (APA, 1994; Benowitz, 1996; CDC, 2001; Henningfield *et al*, 1995; Jaffe, 1990). Yet there was a clear dissociation between the monotonic increases in peak nicotine levels, and the progressive decreases in peak VAS ratings of positive subjective effects and peak heart rate. The high subjective, cardiovascular, and hormonal response to the first cigarette is consistent with the usual observation that after a period of nicotine abstinence, the first cigarette of the day is the most reinforcing (Fant *et al*, 1995; Pomerleau and Pomerleau, 1992a; West and Russell, 1987). However, it is not clear if the subsequent decreases in peak subjective and cardiovascular effects primarily reflect acute tolerance to these effects of nicotine or relief of nicotine withdrawal symptoms after overnight abstinence from smoking. Because these men had been abstinent from nicotine for at least 12 h (as evidenced by CO levels below 6 p.p.m. and nicotine levels below 4 ng/ml) and were well matched in years of smoking and nicotine dependence, it is not possible to distinguish between these two alternatives with certainty (cf. Pillitteri *et al*, 1997; West and Russell, 1987). It is interesting to compare these effects of repeated cigarette smoking with previous clinical laboratory studies of cocaine, where drug abstinence symptoms were not an issue. As in the present study, repeated administration of cocaine did not result in cumulative increases in ratings of positive subjective effects or heart rate (Dudish *et al*, 1996; Evans *et al*, 1999; Fischman and Schuster, 1982; Fischman *et al*, 1985; Foltin and Fischman, 1991; Sofuoglu *et al*, 1999; Ward *et al*, 1997). Only ratings of negative subjective effects increased after repeated doses of IV cocaine (Foltin and Fischman, 1998) whereas, in the present study, peak ratings of negative effects after smoking tended to decrease or stay about the same.

Tolerance to the Effects of Successive Cigarette Smoking on Heart Rate and HPA Axis Hormones

Heart rate. Heart rate increased significantly after smoking each high-nicotine cigarette, but the greatest increase occurred after the first high-nicotine cigarette. These data suggest that tolerance to nicotine's stimulation of heart rate developed, even though peak nicotine levels increased with each successive cigarette. Tolerance to the acute cardiovascular stimulating effects of nicotine has also been observed after repeated injections of the same dose of intravenous nicotine (Benowitz *et al*, 1990; Rosenberg *et al*, 1980) and nicotine nasal spray (Perkins *et al*, 1989, 1991, 1995).

ACTH, cortisol, and DHEA. ACTH increased significantly after smoking the first high-nicotine cigarette and the time course was similar to our previous report of the effects of smoking a high-nicotine cigarette for 12 min (Mendelson *et al*, 2005). In that study, cortisol and DHEA levels also increased significantly after high-nicotine cigarettes, and reached peak levels within 30–40 min after smoking when ACTH levels were decreasing (Mendelson *et al*, 2005). This time course is consistent with established feedback relationships between ACTH and cortisol (Yen *et al*, 1999). In the present study, ACTH levels did not increase significantly after the second cigarette, and this may have reflected negative feedback from the sustained elevation in cortisol at the time of smoking. The plausibility of this explanation is strengthened by the fact that cortisol levels were much lower when the third cigarette was smoked, and this was followed by a significant increase in ACTH. Interpretation of these data, in relationship to behavioral and cardiovascular measures, is complicated by the feedback relationships between ACTH and cortisol (see for review, Yen *et al*, 1999).

After each low-nicotine cigarette, ACTH did not increase, and cortisol and DHEA decreased significantly, and these data also are consistent with our previous study (Mendelson *et al*, 2005). The decrease in cortisol and DHEA may have reflected the fact that smoking low-nicotine cigarettes did not stimulate ACTH at any time point, and/or the normal circadian rhythm of cortisol release in the absence of nicotine/ACTH stimulation. Peak levels of cortisol occur between 0800 and 0900 hours, then gradually decline to a nadir between 0100 and 0200 hours the next morning (Gianoulakis *et al*, 2005; Selmaoui and Touitou, 2003). Low levels of cortisol and DHEA have also been observed after smoking cessation (Oncken *et al*, 2002) and during nicotine withdrawal. Decreases in cortisol on the first day of smoking abstinence and a decrease in the plasma DHEA/cortisol ratio were associated with a higher rate of relapse (al'Absi *et al*, 2004; Rasmusson *et al*, 2006). The high craving scores reported by the low-nicotine group suggests that subjects remained in a state of relative nicotine deprivation throughout the study.

DHEA is believed to enhance feelings of well-being and sexuality, although the evidence is conflicting (see for review, Nair *et al*, 2006; Spark, 2002). Two placebo-controlled clinical trials support the notion that DHEA treatment improves mood and alleviates depression (Morales *et al*, 1994; Schmidt *et al*, 2005). In the present study, peak levels of DHEA after smoking successive high-nicotine cigarettes averaged between 13 and 14.4 ng/ml or 46–50 nmol/l or 1328–1440 ng/dl. These values were over three times higher than average DHEA levels achieved after 3 months of DHEA replacement in men (14.72 ± 1.4 nmol/l) (Morales *et al*, 1994), and higher than average DHEA

levels after 6 weeks of high dose DHEA treatment (1047.2 ± 709.1 ng/dl) (Schmidt *et al*, 2005). Interestingly, IV cocaine also stimulated significant increases in DHEA and cortisol that were similar in time course and magnitude to smoking a high-nicotine cigarette (Mendelson *et al*, 2002, 2003). We have suggested previously that the significant nicotine-induced increases in cortisol and DHEA may contribute to the abuse-related effects of cigarette smoking (Mendelson *et al*, 2005) and DHEA has also been suggested as a potential medication for smoking cessation (Marx *et al*, 2006).

The present study confirms the generality of earlier findings that ACTH increases after cigarette smoking under a number of conditions where samples for analysis were collected once a day or at relatively infrequent intervals, often without concurrent plasma nicotine measurement (Baron *et al*, 1995; Coiro and Vescovi, 1999; del Arbol *et al*, 2000; Pickworth and Fant, 1998; Seyler *et al*, 1984, 1986). In addition, these data confirm and extend observations that cigarette smoking usually induces an increase in plasma cortisol levels (Cryer *et al*, 1976; Gossain *et al*, 1986; Seyler *et al*, 1984; Spohr *et al*, 1979; Wilkins *et al*, 1982; Winternitz and Quillen, 1977; see for review Pickworth and Fant, 1998), and smokers tend to have higher basal cortisol and DHEA levels than nonsmokers (al'Absi *et al*, 2003; del Arbol *et al*, 2000; Field *et al*, 1994; Pomerleau *et al*, 1992b).

Tolerance to the Subjective Effects of Smoking Successive Cigarettes

Although VAS ratings of positive subjective effects were significantly higher after high- than low-nicotine cigarette smoking, nicotine levels did not appear to be the sole determinant of ratings of 'High', 'Rush', and 'Liking'. The successive decrease in positive VAS ratings after smoking is consistent with decreases in subjective responses to a challenge dose of nicotine nasal spray, after repeated nicotine administration (Perkins *et al*, 1993, 1995). The decrease in peak positive VAS ratings after successive cigarettes appeared to be unrelated to changes in the aversive properties of smoking. Ratings of 'Sick' and 'Bad Feeling' did not change significantly from baseline after high-nicotine cigarette smoking, and peak ratings of 'Dizzy' and 'Jittery' decreased after successive cigarettes. The initial nicotine dose-dependent differences in 'High', 'Rush', and 'Liking' suggests that subjects could detect relative nicotine levels within eight puffs, yet VAS ratings continued to increase significantly after each successive low-nicotine cigarette. Nicotine-dependent smokers appear to be quite sensitive to relative nicotine levels and were able to discriminate plasma nicotine levels of 2.6 ng/ml from placebo after nicotine or placebo nasal spray (Perkins *et al*, 2001). In the present study, nicotine levels after low-nicotine cigarette smoking averaged between 2.9 and 3.8 ng/ml.

Ratings of cigarette 'Craving' decreased rapidly after smoking, and the time course of changes in 'Craving' was virtually identical in the low- and high-nicotine groups. 'Craving' ratings decreased most within the first 20 min after smoking, then increased to peak levels within 50 min. The extent to which increases in 'Craving' may prompt the next smoking episode during unconstrained smoking is unclear. However, during several weeks' residence on a clinical research ward, men usually initiated smoking within 20–30 min after the last cigarette (Mello *et al*, 1985, 1980; Mutschler *et al*, 2002). In other naturalistic smoking conditions, inter-cigarette intervals of 30–35 min are often reported (Hatsukami *et al*, 1988). Findings in the present study are consistent with previous reports that 'Craving' often decreases after smoking low-nicotine cigarettes that contain <0.06–0.1 mg of nicotine (Gross *et al*, 1997; Mendelson *et al*, 2005; Pickworth *et al*, 1999; Robinson *et al*, 2000; Rose *et al*, 2000). The salient effects of low-nicotine cigarettes on reports of 'Craving', 'Liking', and reduction of acute withdrawal symptoms are usually interpreted to illustrate the importance of complex sensory cues in maintaining cigarette-smoking behavior (Butschky *et al*, 1995; Gross *et al*, 1997; Pickworth *et al*, 1999; Rose *et al*, 2000; Shahan *et al*, 1999). The effectiveness of low-nicotine cigarettes in reducing 'Craving' may also offer a way to facilitate smoking cessation (see for review Rose, 2006).

Conclusions

Smoking a high-nicotine cigarette consistently stimulated increases in HPA axis hormones, heart rate, and VAS reports of positive subjective effects. However, peak levels of heart rate and VAS reports gradually diminished after smoking three successive cigarettes, suggesting that tolerance developed rapidly to these measures. It is often postulated that behavioral and biologic indicators of tolerance reflect desensitization of nicotinic acetylcholine receptors, and this concept is supported by a number of preclinical studies of endocrine (Sharp and Beyer, 1986; Sharp and Matta, 1993) and behavioral endpoints (James *et al*, 1994; Robinson *et al*, 2006). Speculation about receptor mechanisms of acute tolerance is beyond the scope of this clinical report. In contrast to heart rate and VAS reports, nicotine's effects on HPA axis hormones were relatively constant, suggesting that acute tolerance did not develop to these measures. Peak levels of ACTH and DHEA did not differ significantly across successive cigarettes and peak levels of cortisol were lower after the third cigarette. These changes reflected both the direct effects of nicotine and the feedback relationships between the pituitary hormone ACTH, and the adrenal hormones cortisol and DHEA. The sustained activation of HPA axis hormones after smoking successive cigarettes, as well as the temporal concordance between these hormones and nicotine levels, is consistent with the hypothesis that the HPA axis hormones may contribute to the abuse-related effects of cigarette smoking.

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DISCLOSURE STATEMENT

None.

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