Attenuated adrenocortical and blood pressure responses to psychological stress in ad libitum and abstinent smokers

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Abstract

Chronic smoking may alter physiological systems involved in the stress response. This study was designed to examine the effects of ad libitum smoking and abstinence on adrenocortical and cardiovascular responses to acute psychological stress in dependent cigarette smokers. We evaluated differences among abstinent smokers, smokers who continued to smoke at their normal rate, and nonsmokers in salivary cortisol concentrations, systolic and diastolic blood pressure (BP), heart rate (HR), and mood reports. Measurements were obtained during rest and in response to acute psychological stress (public speaking) in one session (stress session) and during continuous rest in a control session. Thirty-eight smokers (21 women) and 32 nonsmokers (18 women) participated. Smokers were assigned to either abstain from smoking the night prior to and the day of each session, or to continue smoking at their normal rate before each session. All groups showed significant stress-induced changes in BP and HR. Smokers, regardless of their assigned condition, showed attenuated systolic BP responses to the public-speaking stressor when compared to nonsmokers. While resting cortisol levels were greater among smokers than nonsmokers, no cortisol response to the acute stressor was demonstrated in either ad libitum or abstinent smokers. These results indicate that chronic smoking diminishes adrenocortical and cardiovascular responses to stress, and that short-term abstinence does not correct these alterations.
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1. Introduction

Acute stress activates adrenocortical and sympathetic systems (McEwen and Stellar, 1993; Chrousos and Gold, 1992; Mills and Dimsdale, 1992). Under stress, the hypothalamic–pituitary–adrenocortical (HPA) system produces corticotropin-releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus, which in turn stimulates the release of the adrenocorticotropic hormone (ACTH) from the anterior pituitary (Owens and Nemeroff, 1991; Dallman, 1993; al'Absi and Arnett, 2000; Koob et al., 1993). ACTH travels through the circulatory system to the

adrenal cortex, stimulating the release of cortisol (Petrudz and Merchenthaler, 1992). Cortisol plays a significant role as a modulator of the central nervous system during stress (McEwen and Sapolsky, 1995; Kreek and Koob, 1998; al'Absi et al., 2002b). It interacts with several neurotransmitters that are modulated by nicotine or mediate nicotine's effects, including acetylcholine, norepinephrine, dopamine, vasopressin, and beta-endorphin (Koob and Le Moal, 1997). Stress also produces various sympathetic changes, including increased blood pressure (BP), heart rate (HR), and catecholamine production (Mills and Dimsdale, 1992; Lovallo et al., 1990; Cacioppo, 1994; Christensen, 1994), which possibly mediate effects of stress on smoking and relapse (Epping-Jordan et al., 1998; Kreek and Koob, 1998; Pomerleau and Pomerleau, 1991; Roth et al., 1988).

The acute effects of nicotine on adrenocortical and cardiovascular functions have been investigated in several

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laboratory studies (Fuxe et al., 1989; Davis, 1999; Davis and Matthews, 1990; Pomerleau and Pomerleau, 1991; Pomerleau et al., 1983; Seyler et al., 1984; Dembroski et al., 1985; MacDougall et al., 1986; Wilkins et al., 1982; Houlihan et al., 1999). An additive effect of nicotine and acute stress has been documented on cortisol production (Pomerleau and Pomerleau, 1991) and BP (Dembroski et al., 1985; MacDougall et al., 1986). Effects of nicotine on the HPA axis are mediated by nicotine's effects on multiple central nervous system pathways, although specific mechanisms have not been elucidated. Nicotine stimulates vasopressin secretion, which, in combination with CRH, leads to ACTH release. It also stimulates cholinergic receptors in the hypothalamus, particularly the PVN, causing the release of CRH, which starts the HPA cascade, leading to the production of cortisol from the adrenal cortex (Fuxe et al., 1989; Pomerleau and Pomerleau, 1991; Seyler et al., 1984). Chronic administration may lead to prolonged HPA activation, although the degree to which tolerance develops to the HPA effects of nicotine is not clear.

Little is known about cortisol responses to stress after extended periods of smoking or how these responses may be modified by short-term abstinence. It is possible that adrenocortical responses to stress in smokers are altered after abstinence, and that these abstinence-related changes enhance the intensity of withdrawal symptoms under acute stressful events, contributing to relapse (Kreek and Koob, 1998; Piazza and Le Moal, 1998). Determining the stress response profile in abstinent smokers should help elucidate the role of stress-related physiological changes in withdrawal symptoms. This should also provide information related to the extent to which acute stress may contribute to alteration in the rewarding properties of nicotine (Piazza and Le Moal, 1998).

While some studies have reported that smokers show smaller salivary cortisol responses to laboratory stressors when compared to nonsmokers (Kirschbaum et al., 1993b; Roy et al., 1994), others report no differences (Baron et al., 1995; Tersman et al., 1991). These discrepancies may stem from differences in experimental design. For example, smokers may vary in terms of the level of nicotine dependence and comorbid psychopathology. It is also possible that the inconsistent cortisol findings reflect variability in the length of abstinence and, therefore, the severity of withdrawal symptoms. Focusing on BP responses, one study (Tsuda et al., 1996) attempted to address this issue and found that smokers who were abstinent from tobacco overnight had a lower diastolic BP baseline, but greater responses to behavioral stressors than ad libitum smokers and nonsmokers. We recently reported that smokers had greater systolic BP responses to cognitive challenges after overnight abstinence than after the ad libitum condition in a within-subject, abstinent-smoking, counterbalanced design (al'Absi et al., 2002a). There was no difference in cortisol concentrations between the abstinence and ad libitum smoking conditions (al'Absi et al., 2002a).

Studies that have addressed these questions so far have suffered from several limitations, including small sample size, exclusion of women, and minimal or no control of effects of time of day on the dependent measures, especially cortisol. Effects of acute stress and smoking abstinence have also not been directly compared with those of nonsmokers, and no systematic work has focused on separating the pharmacological effects of smoking from effects of abstinence. Furthermore, only brief cognitive challenges were used in the earlier studies. A better assessment of the effects of acute stress requires the use of stressors that are socially relevant with significant effects on the HPA axis (al'Absi et al., 1997). Socially relevant stressors are more ecologically valid challenges compared with structured and brief psychomotor or mental challenges. Socially salient stressors may better simulate situations where smokers may encounter interpersonal conflicts or challenges that might increase their risk of smoking or relapse. Assessment of effects of stress on cortisol production also requires the use of rest day control design to provide appropriate within-subject control that accounts for effects of time of the day on HPA activity. This is an important element of control in light of the clear diurnal variation of cortisol productions (Weitzman et al., 1971).

The purpose of this study was to determine alterations in psychophysiological and adrenocortical responses to behavioral stress in dependent smokers compared to nonsmokers, and to assess effects of short-term abstinence on responses to acute stress. We predicted that, compared to ad libitum smokers and nonsmokers, abstinent smokers would exhibit enhanced responses to stress. Smokers and nonsmokers participated in two counterbalanced sessions (rest and stress) separated by a minimum of 2 days. Smokers were assigned to one of two conditions: abstinence from smoking and all nicotine-containing products the night before and the day of each laboratory session, or smoking ad libitum.

2. Method

2.1. Participants

Thirty-eight smokers (17 men and 21 women) and 32 nonsmokers (14 men and 18 women) were recruited by newspaper advertisements and posters placed around the university community. Subjects underwent a screening session, which included a brief medical history, assessment of behavioral habits (including history of smoking, alcohol, and drug use), and measurement of height and weight. Participants had to meet the following criteria: (1) no regular use of prescribed or over-the-counter medications; (2) no current or prior treatment for hypertension; (3) weight within ±30% of Metropolitan Life Insurance norms; (4) consumption of ≤ 2 alcoholic drinks a day; (5) no history of

a chronic illness or psychiatric disorder. Smokers were included if they smoked ≥ 15 cigarettes a day for at least 1 year. Smokers were assigned to one of two conditions: abstinence from smoking and all nicotine-containing products ($n = 21$), or smoking ad libitum ($n = 17$) for 18 h prior to each laboratory session.

Smokers who were assigned to the abstinence condition must demonstrate a measurement of carbon monoxide (CO) level ≤ 10 ppm at the beginning of each session. All smokers and nonsmokers were instructed to have a light meal approximately 2 h before each laboratory session. Participants signed a consent form approved by the Institutional Review Board of the University of Minnesota, and they received a monetary incentive for participation.

2.2. Self-report measures

We used a modified version of the Minnesota Nicotine Withdrawal Scale (MNWS) (Hughes and Hatsukami, 1986, 1998), which included the following items: irritability, anger, anxiety, difficulty concentrating, restlessness, depressed or sad mood, and hunger. MNWS scores were calculated without the item of craving. We changed the wording of the item "craving" to "desire to smoke," and its mean scores were analyzed separately in light of recent evidence suggesting distinct patterns of craving from other withdrawal symptoms (Hughes and Hatsukami, 1998). To assess physical effects of abstinence, we included symptoms previously discussed as related to smoking abstinence (Hughes et al., 1991) and which might be influenced by acute stress. These included headache, sweating, tremor, stomachache, drowsiness, fatigue, and coughing. We also assessed activation and distress—two factors previously shown to be sensitive to acute stress and to have sound psychometric properties (al'Absi et al., 1994, 1998). In the current study, the Cronbach's α values for positive affect and distress were .85 and .82, respectively. Positive affect was assessed using items of cheerfulness, content, calmness, controllability, and interest. Distress was assessed using items of anxiety, irritability, impatience, and restlessness. All items were included in one form titled "Subjective State." Each item referenced a seven-point scale anchored by the end points, *Not at All* and *Very Strong*. Participants were asked to mark each rating scale at the point that best describes how they felt during the previous 30 min. During the screening session, participants completed the Fagerström Test of Nicotine Dependence (FTND) (Heatherton et al., 1991). We also assessed depression using the Center for Epidemiologic Studies—Depression (CES-D) scale and anxiety trait using the State–Trait Anxiety Inventory (Trait Form, STAI; Speilberger et al., 1983). The 10-item version of the Perceived Stress Scale (PSS) (Cohen et al., 1983) was administered to measure appraisals of the stressfulness of life events. Participants also provided information about their daily caffeine and alcohol intake, physical activity, and level of education.

2.3. Adrenocortical and cardiovascular measures

Cortisol concentrations were measured in saliva. The subject produced 1–2 ml of saliva by chewing on a cotton swab and depositing it into a plastic tube (Salivette tubes; Sarstedt, Rommelsdorf, Germany). All samples were stored at -70 °C until transferred for assay. Salivary cortisol assays were conducted in duplicate using a time-resolved immunoassay with fluorometric end point detection. The assay has a minimum sensitivity of 0.4 nmol/l (Dressendorfer et al., 1992). Cortisol assays were conducted at the University of Düsseldorf (Germany). Cotinine assay was conducted at Hennepin County Medical Center (Minneapolis, MN). Cotinine levels were measured from the last saliva sample obtained during each session by gas chromatography with a nitrogen–phosphorus detector (Jacob et al., 1981).

Systolic BP, diastolic BP, and HR were measured using a Dinamap oscillometric monitor system (Critikon, Tampa, FL).

2.4. Procedures

Each participant attended two counterbalanced sessions (rest and stress) separated by two or more days. All sessions started at approximately 1:00 p.m. To confirm abstinence from smoking, collection of an expired air sample for CO measurement was conducted upon arrival at the laboratory using MicroCO monitors (Micro Direct, Auburn, ME). Afterwards, the participant was seated in a semirecumbent position and a BP cuff was placed on the nondominant arm. The participant then completed forms about diet and sleep to verify compliance with dietary restrictions that included 24-h abstinence from alcohol and 48-h abstinence from any over-the-counter medications. To eliminate the possible effects of caffeine withdrawal in habitual coffee drinkers, we limited the caffeine restriction to 4 h prior to each laboratory session.

The protocol included a 30-min baseline rest period, followed by the public-speaking challenges (30 min) or rest (the rest session), and a 30-min recovery period. Participants provided saliva samples and completed Subjective State ratings when they first arrived at the laboratory (initial assessment), after baseline, after the public-speaking task or rest, and after 30 min of recovery. During the baseline, rest, and recovery periods, participants had a choice of reading general interest magazines or watching nature videos selected for their emotionally neutral content. BP and HR were obtained every 2 min during the public-speaking stressor and every 3 min during baseline and recovery, and during the rest control session.

2.5. Public-speaking stressor

The public-speaking stressor involved three scenarios in which participants were asked to construct and deliver a 4-

Table 1
Subjects' characteristics

	Nonsmokers ($n = 32$)	Smokers (smoking) ($n = 17$)	Smokers (abstinent) ($n = 21$)	P
Age (years)	23.2 (1.4)	22.3 (2.0)	23.5 (1.7)	NS
Percent of women	56	59	48	NS
Height (m)	1.72 (0.02)	1.72 (0.02)	1.72 (0.02)	NS
Weight (kg)	69.3 (2.2)	70.2 (3.1)	69.3 (2.7)	NS
BMI (kg/m^2)	23.4 (0.6)	23.8 (0.8)	23.6 (0.7)	NS
Education	14.7 (0.3)	14.2 (0.4)	14.1 (0.3)	NS
Caffeine (drinks/day)	1.2 (0.43)	3.4 (0.60)	4.1 (0.52)	.0001
Physical activities (h/week)	5.9 (0.8)	3.8 (1.2)	2.9 (1.0)	.06
Depression (CES-D)	11.6 (1.6)	13.0 (2.2)	11.7 (2.1)	NS
Anxiety trait (STAI)	33.1 (1.5)	35.5 (2.1)	34.6 (2.0)	NS
Perceived stress (PSS)	13.3 (1.1)	13.9 (1.5)	15.1 (1.4)	NS
FTND	NA	6.8 (0.48)	5.9 (0.42)	NS
Cigarettes/day	NA	21 (1.8)	17 (1.6)	NS
Duration at present rate	NA	5.3 (1.4)	3.3 (1.2)	NS

Entries show mean (S.D.).
BMI, body mass index; CES-D, Center for Epidemiologic Studies—Depression scale; STAI, State–Trait Anxiety Inventory (Trait); PSS, Perceived Stress Scale; FTND, Fagerström Test of Nicotine Dependence.

min speech after 4 min of silent preparation. They were instructed that their speeches would be videotaped and then evaluated by three staff members from the experimental team. The three scenarios were presented in a counterbalanced order. In one scenario, participants were presented with a controversial social issue and were asked to introduce their positions and defend them. In another scenario, participants were given an article about an issue of general interest and were asked to construct a presentation based on this article. In a third scenario, participants were asked to imagine a hypothetical situation where they were being accused of shoplifting. Participants were asked to construct arguments for a speech to defend themselves. Before each scenario, participants were asked to make their statements specific and precise, since the evaluation of performance was going to be based on how convincing, organized, articulate, and enthusiastic they were during each presentation. This task has been shown to be a potent laboratory stressor, inducing significant cardiovascular, endocrine, and mood changes (al'Absi et al., 1997). Similar evaluative tasks have been found to be effective in elevating cortisol (Kirschbaum et al., 1993a).

2.6. Data analysis

Dependent variables were salivary cortisol, systolic and diastolic BP, HR, and scores on the MNWS, physical symptoms, positive affect, and distress factors. Salivary cotinine, from the last salivary sample, and CO were also assessed in smokers in both sessions. A one-way analysis of variance (ANOVA) was conducted to determine differences among groups (abstinent smokers, ad libitum smokers, and

nonsmokers) in demographic and psychological variables. The two groups of smokers (abstinent and ad libitum smoking) were compared on smoking history, average daily cigarettes, and level of nicotine dependence using FTND scores. Salivary cotinine and CO levels obtained during both sessions were compared between the two smoking groups using 2 sessions (stress, rest) × 2 groups (abstinent, ad libitum smokers) ANOVA. Salivary cortisol and mood ratings assessed at the beginning of each session (initial assessment) were analyzed using 2 sessions (stress, rest) × 3 groups (smokers who abstained from smoking, smokers who continued to smoke ad libitum, and nonsmokers) ANOVA.

BP and HR measures collected during baseline, preparation for each public-speaking scenario, delivery of speeches, and during the rest recovery period were averaged to obtain respective means. Repeated multivariate analyses of variance (MANOVAs) were conducted to analyze these variables. These analyses included three groups (smokers who
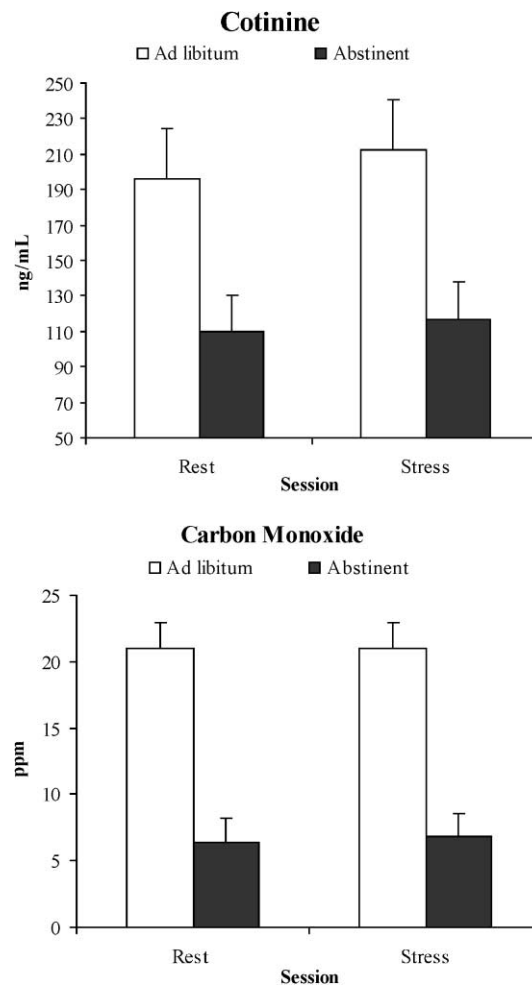

Fig. 1. Cotinine (ng/ml) and CO (ppm) measures obtained from smokers who were abstinent overnight and the day of each session (rest and stress) and from smokers who continued to smoke at their regular rate (ad libitum). Both measures were higher in the ad libitum smoking groups than the abstinent smokers in both sessions ($Ps < .001$).

abstained from smoking, smokers who continued to smoke ad libitum, and nonsmokers) as a between-subject factor and two within-subject factors: two sessions (rest and stress) and four sampling periods of cardiovascular measures (baseline, preparation, delivery of speeches, and recovery). Similar MANOVAs were conducted on cortisol and mood ratings using three sampling periods (baseline, public-speaking stressor or rest, and recovery). Order of session (Stress–Rest vs. Rest–Stress) was included as a factor in these analyses, but patterns of results were similar across orders. We, therefore, report results collapsed across orders. All the repeated analyses used Wilk's λ correction to test time effect and to correct for repeated measures.

Due to loss of samples, low quantity of saliva for cortisol and cotinine assay, and incomplete questionnaire data, variations exist between sample size and degrees of freedom for the reported variables. Two of the subjects in the ad libitum smoking group and three in the abstinent smoking group did not have complete cortisol samples in both sessions. Five nonsmokers attended only one session (three completed the rest session only and two completed stress sessions only). As a result, data from these participants were excluded from the analysis.

3. Results

3.1. Participant characteristics

Table 1 shows participants' characteristics. All three groups did not differ in age, height, weight, or education ($Fs < 1$). The two smoking groups reported drinking more coffee than nonsmokers [$Fs(2,60) > 10.30$, $Ps < .001$]. There was also a trend toward greater physical activity in nonsmokers [$F(2,60) = 3.00$, $P = .06$]. Groups did not differ in
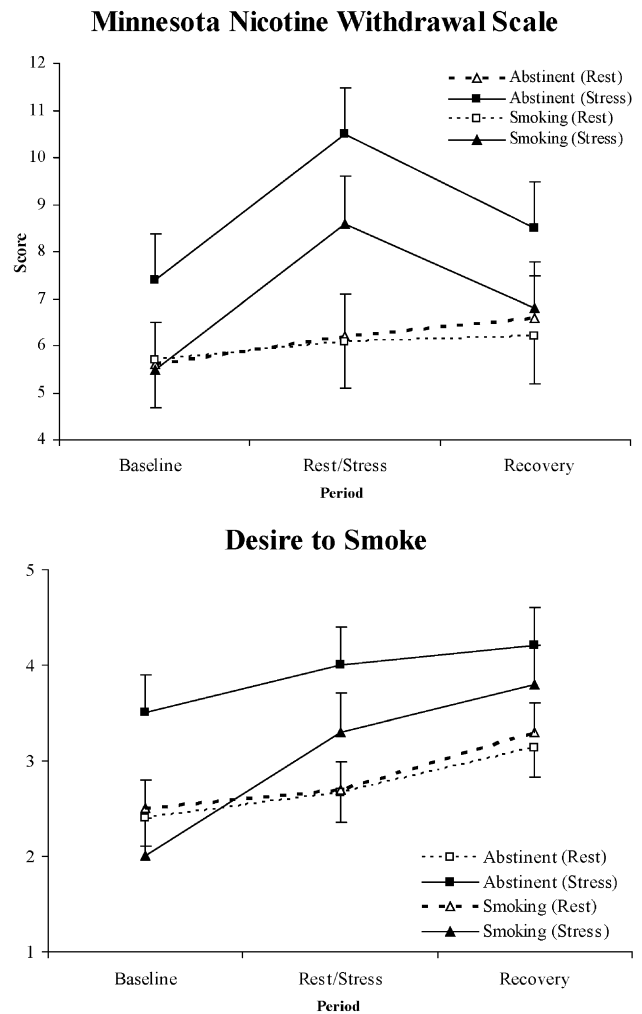

Fig. 2. Mean scores on the modified MNWS (upper) and reported desire to smoke (lower) and S.E.M. before, following the stressor/rest, and following a recovery period.

Table 2
Mood self-reports

Group	Nonsmokers		Smokers (smoking)		Smokers (abstinent)		P	
Session	Rest	Stress	Rest	Stress	Rest	Stress	Group	Periods × Sessions[a]
Distress								
Baseline	3.0 (0.64)	4.7 (0.77)	4.8 (0.82)	4.2 (0.98)	4.3 (0.75)	5.2 (0.90)	NS	.01
Rest/stress	3.7 (0.74)	5.3 (0.86)	4.9 (0.94)	6.6 (1.09)	5.5 (0.86)	7.5 (1.00)		
Recovery	4.8 (0.76)	4.6 (0.71)	5.2 (0.97)	5.3 (0.90)	5.7 (0.89)	6.3 (0.83)		
Positive affect								
Baseline	20.1 (1.2)	18.8 (1.4)	18.1 (1.5)	18.2 (1.7)	14.9 (1.4)	16.5 (1.6)	.04	NS
Rest/stress	19.1 (1.3)	17.9 (1.3)	16.8 (1.6)	16.7 (1.6)	14.9 (1.5)	12.9 (1.5)		
Recovery	18.4 (1.2)	18.6 (1.3)	16.9 (1.6)	16.3 (1.7)	13.4 (1.4)	14.5 (1.5)		
Physical symptoms								
Baseline	4.8 (0.77)	5.2 (0.77)	5.4 (0.99)	5.6 (0.98)	5.8 (0.91)	5.4 (0.90)	NS	.0001
Rest/stress	5.5 (0.76)	1.9 (0.52)	4.7 (0.97)	2.4 (0.66)	5.8 (0.89)	2.6 (0.61)		
Recovery	5.0 (0.67)	4.2 (0.63)	4.0 (0.86)	3.7 (0.80)	5.3 (0.79)	4.1 (0.73)		

Entries show mean (S.E.) of positive affect, distress, and physical symptoms factors reported during baseline, after performing the public-speaking task (on the stress session), and after recovery.

[a] P values reflect the Period-by-Session interaction, indicating a change following the public-speaking stressor observed during the stress session.
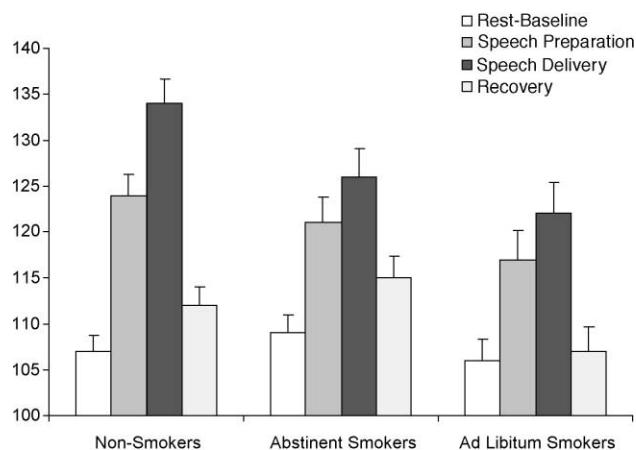
Systolic Blood Pressure

Fig. 3. Mean systolic BP and S.E.M. (mm Hg) during rest, preparation, delivery of the speeches, and recovery. Smokers in both groups (abstinent and ad libitum) showed attenuated responses to the public-speaking stressor ($P < .01$).

reported depression, anxiety, or perceived stress ($Fs < 1$), as shown in Table 1.

The two smoking groups did not differ in level of nicotine dependence as assessed by FTND, average of cigarettes smoked, or duration of smoking ($Fs < 1.7$).

3.2. Cotinine and CO

Abstinent smokers had less salivary cotinine on both days than ad libitum smokers [$F(1,24) = 7.29$, $P < .01$]. No differences between the two sessions were found ($F < 1$). It should be noted that cotinine concentrations in the abstin-

ence group were about 40% less than concentrations in the ad libitum group. This is similar to the decline observed in another study in which a within-subjects, abstinence-smoking design was used (al'Absi et al., 2002b). The reduction is consistent with data on cotinine half-life (Curvall et al., 1990) and confirms the compliance with the smoking abstinence restriction. Similarly, expired CO levels were smaller in the abstinence group than in the ad libitum condition [$F(1,30) = 31.25$, $P < .0001$]. This difference was similar on both days (see Fig. 1). Abstinent smokers reported greater desire to smoke than ad libitum smokers [$Fs(1,32) > 5.90$, $Ps < .02$].

3.3. Mood and withdrawal symptoms

Comparing self-report measures obtained at the beginning of each session (initial assessment) showed that abstinent smokers reported less positive affect than the other two groups [$F(2,57) = 4.62$, $P < .01$]. There were no differences between smoking groups in MNWS scores or physical symptoms ($Fs < 2.30$, $P > .12$).

As shown in Table 2, performing the public-speaking stressor significantly increased reported distress in all groups, as shown by a significant Period main effect [$F(2,57) = 8.27$, $P < .001$, and Periods-by-Session interaction [$F(2,57) = 4.70$, $P < .01$], indicating that the increase was specific to the period following the public-speaking challenge on the stress day. Smokers reported less positive affect than nonsmokers [$F(2,58) = 3.37$, $P < .05$]. Reported positive affect was reduced across time in each session, as evidenced by a main effect of Periods [$F(2,57) = 8.77$, $P < .0001$].

All smokers reported greater withdrawal symptoms following the laboratory stressor, as shown by the Periods-by-

Table 3
Cardiovascular activity

Group	Nonsmokers		Smokers (smoking)		Smokers (abstinent)		P	
Session	Rest	Stress	Rest	Stress	Rest	Stress	Group	Periods × Sessions[a]
SBP (mm Hg)								
Baseline	108 (1.8)	107 (1.8)	107 (2.3)	106 (2.3)	111 (2.2)	110 (2.1)	NS	.0001
Rest/prep	108 (1.8)	124 (2.4)	105 (2.3)	117 (3.1)	111 (2.1)	122 (2.8)		
Rest/delivery	108 (1.9)	134 (2.7)	106 (2.5)	122 (3.5)	110 (2.3)	127 (3.2)		
Recovery	108 (1.9)	112 (2.0)	106 (2.4)	107 (2.6)	110 (2.3)	115 (2.4)		
DBP (mm Hg)								
Baseline	62 (1.5)	62 (1.5)	63 (2.0)	62 (2.0)	63 (1.8)	63 (1.9)	NS	.0001
Rest/prep	63 (1.4)	72 (1.5)	62 (1.8)	72 (1.9)	65 (1.7)	74 (1.8)		
Rest/delivery	63 (1.3)	77 (1.4)	62 (1.6)	75 (1.9)	64 (1.5)	75 (1.7)		
Recovery	64 (1.5)	64 (1.5)	62 (2.0)	63 (2.0)	65 (1.8)	66 (1.9)		
HR (beats/min)								
Baseline	63 (2.0)	64 (2.1)	74 (2.5)	74 (2.7)	70 (2.3)	67 (2.5)	.04	.0001
Rest/prep	62 (2.0)	77 (2.3)	70 (2.6)	83 (3.0)	67 (2.4)	77 (2.8)		
Rest/delivery	63 (2.0)	81 (2.3)	71 (2.5)	84 (3.0)	69 (2.3)	78 (2.8)		
Recovery	62 (2.0)	64 (2.1)	70 (2.7)	71 (2.7)	68 (2.4)	68 (2.5)		

Entries show mean (S.E.) of SBP, systolic blood pressure (mm Hg); DBP, diastolic blood pressure (mm Hg); and HR, heart rate (beats/min) during baseline, during preparation (prep) and delivery of the public-speaking stressor or rest, and during recovery.

[a] P values reflect the Period-by-Session interaction, indicating a change following the public-speaking stressor observed during the stress session.

Session interaction on the MNWS scores [$F(2,32) = 5.71$, $P < .01$; see Fig. 2]. Reported physical symptoms were reduced on the stress day, specifically following performance of the public-speaking stressor, as evidenced by Periods-by-Sessions interaction [$F(2,32) = 11.67$, $P < .0001$].

3.4. Desire to smoke

During the initial assessment in each session, reported desire to smoke was stronger in the abstinent smokers than in smokers who smoked ad libitum [$F(1,32) = 5.86$, $P < .02$]. Desire to smoke beyond this point continued to increase across periods in both groups [$F(2,32) = 26.10$, $P < .001$]. Furthermore, as shown in Fig. 2, the public-speaking stressor led to a greater increase in desire to smoke, as shown by Periods-by-Sessions interaction [$F(2,32) = 3.40$, $P < .05$].

3.5. Cardiovascular measures

Baseline diastolic and systolic BP did not differ between smokers and nonsmokers ($Fs < 1.0$). HR during baseline was greater among smokers who continued to smoke ad libitum compared with nonsmokers [$F(2,62) = 5.33$, $P < .01$]. All participants showed significant diastolic and systolic BP responses to the public-speaking stressor [$Fs(3,57) > 45.30$, $Ps < .0001$]. However, as depicted in Fig. 3, smokers in both conditions showed significantly smaller systolic BP responses than nonsmokers, demonstrating a significant Periods-by-Groups interaction [$F(6,114) = 3.57$, $P < .005$]. The public-speaking stressor produced significant HR increases in all participants [$F(3,57) = 52.30$, $P < .0001$] (Table 3).

3.6. Salivary cortisol concentrations

Comparing cortisol concentrations at the beginning of each session showed that only the ad libitum smoking group exhibited greater levels in the stress day compared with rest day levels [$F(2,57) = 8.00$, $P < .01$]. Comparing cortisol levels across all periods and in both sessions showed that smokers in both conditions had greater cortisol concentrations than nonsmokers, as documented by a significant main
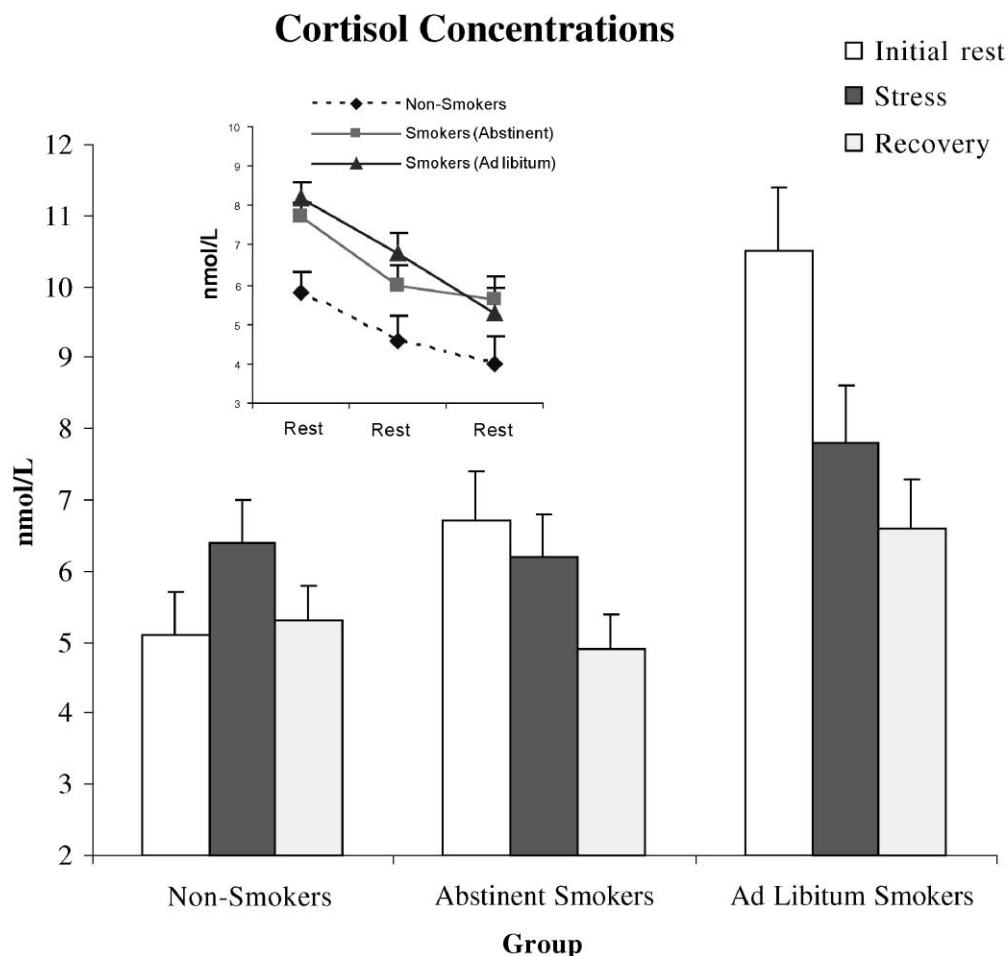

Fig. 4. Mean salivary cortisol concentrations and S.E.M. (nmol/l) before, immediately following the public-speaking stressor, and after 30 min of recovery rest period. While nonsmokers exhibited an upward trend in cortisol production following the stressor, smokers in both groups (abstinent and ad libitum) showed a steady decline in cortisol concentrations. The small figure shows salivary cortisol concentration in the rest session in all groups. Smokers had greater cortisol concentrations than nonsmokers ($P < .01$).

effect of Group $[F(2,53) = 5.99, P < .005]$. However, the Periods-by-Groups interaction $[F(4,104) = 5.47, P < .0001]$ and Periods-by-Sessions-by-Groups interaction $[F(4,104) = 4.02, P < .005]$ were also significant (see Fig. 4). Therefore, we conducted simple interaction effect tests to determine differences between groups on each day (rest and stress) separately. On the rest day, both smoking groups had overall greater cortisol concentrations than nonsmokers $[F(2,57) = 6.97, P < .001]$. Significant decline across time was also obtained on this day $[F(2,56) = 45.68, P < .0001]$, reflecting expected diurnal cortisol changes.

On the stress day, smokers who continued to smoke ad libitum showed significantly greater cortisol than the other two groups $[F(2,59) = 6.24, P = .01]$. However, this difference was qualified by a significant Periods-by-Groups interaction $[F(4,114) = 5.45, P < .001]$. Further tests comparing cortisol values before and after the public-speaking stressor in each group separately showed a decline in cortisol concentrations in both smoking groups, with a significant linear $(Fs > 11.70, Ps < .001)$, but not quadratic $(Fs < 2.30, Ps > .14)$, trend found in these groups. Tests in the nonsmoking group showed a significant quadratic trend $[F(1,27) = 10.60, P < .005]$, but nonsignificant linear trend $(F < 1)$, reflecting changes in cortisol concentrations following the public-speaking stressor. These data indicate that while both smoking groups had greater basal cortisol than nonsmokers, only nonsmokers showed appreciable cortisol changes during the public-speaking stressor.

Because of the difference in caffeine consumption between smokers and nonsmokers, and based on the previously documented effects of caffeine on cortisol during stress (al'Absi et al., 1995, 1998), we conducted analyses of covariance using the reported amount of daily caffeine consumption as a covariate. The pattern of the findings was similar to the above reported findings. Similarly, the results were not altered when cortisol levels at the beginning of the stress session were used as a covariate.

4. Discussion

This study compared adrenocortical and cardiovascular responses to stress among smokers who continued to smoke ad libitum, abstinent smokers, and nonsmokers, and included a rest day control session to account for diurnal changes in salivary cortisol. While both groups of smokers showed higher prevailing cortisol levels than nonsmokers, neither showed an appreciable cortisol response to stress. Smokers also showed attenuated systolic BP responses to the public-speaking stressor. Although abstinent smokers reported less positive affect than minimally deprived smokers, they showed comparable cortisol concentrations in both sessions.

Findings from this study extend previously reported results indicating that smokers have higher cortisol levels than nonsmokers (Field et al., 1994; Gossain et al., 1986). By including a separate rest session, the design of this study

allowed for the control of the diurnal effects on the physiological measures, especially cortisol, and as such provided a more accurate method to assess effects of acute stress. Although the sample size was not large enough to specifically evaluate gender differences in the variables measured in this study, more than half of the participants were women, strengthening the generalizability of the results. The manipulation of the smoking status prior to each session and the inclusion of the nonsmoking group further strengthened the design and helped in demonstrating the heightened tonic cortisol production among smokers.

The sources of the heightened adrenocortical activation in smokers have not been determined. One possible mechanism is that smokers may have been under heightened levels of stress produced by nicotine withdrawal (Parrott, 1999), which may result in enhanced activation of the CRH (Koob and Le Moal, 1997; Kreek and Koob, 1998) and therefore increased cortisol levels. However, this explanation is inconsistent with the equally elevated cortisol seen in smokers who were minimally deprived in this study.

The most striking finding in this study is the diminished systolic BP and cortisol responses to the public-speaking stressor in smokers. Both groups of smokers exhibited attenuated BP responses and an absence of cortisol response, suggesting that these alterations were independent of acute effects of withdrawal. The extent to which absent cortisol response is due to an enhanced negative feedback caused by the higher basal cortisol concentrations or due to attenuated sensitivity to stress-related physiological activation is not yet clear. A post-hoc examination was conducted to test whether smokers with lower initial cortisol values had greater cortisol response to stress. This examination yielded a positive relationship between initial cortisol levels and concentrations obtained after performing the public-speaking task. This suggests that high baseline cortisol concentrations were associated with high cortisol levels after the public-speaking stressor. Another possible explanation is that the public-speaking task may not have produced the presumed effect in smokers; instead, it may have ameliorated abstinence effects by distracting individuals from these symptoms. Mood reports obtained on both days, however, do not support this possibility. It should also be noted that ancillary correlation analyses conducted on subjective reports and cortisol concentrations from all periods did not reveal consistent patterns of associations in either session.

The attenuated systolic BP responses to the public-speaking stressor seen in smokers are in contrast to previous work showing greater BP responses to the mental arithmetic challenge in smokers than nonsmokers (Tersman et al., 1991; Tsuda et al., 1996). It is possible that the nature of the challenge used here (i.e., public-speaking social stressor vs. mental arithmetic used in previous studies) may have influenced the pattern of responses seen in the present study. Considering the paucity of work comparing BP responses to socially salient stressors in smokers and nonsmokers, the BP findings should be considered preliminary.

The present study showed alteration of the stress response among smokers, regardless of the acute effects of nicotine. These findings were obtained in ad libitum smokers and in smokers who were abstinent for at least 18 h, when acute pharmacological effects of nicotine should be at minimum. The results are in agreement with findings reported in a smaller sample of male smokers, minimally deprived of smoking (Kirschbaum et al., 1993b). These findings seem incongruent with the stimulating effects of acute doses of nicotine on the HPA axis and cardiovascular system rate that have been documented in several laboratory studies (Kirschbaum et al., 1992; Pomerleau et al., 1983; Wilkins et al., 1982; Houlihan et al., 1999; Davis and Matthews, 1990). Furthermore, the acute effect of nicotine and acute stress has been shown to be additive on cortisol production and BP (Dembroski et al., 1985; MacDougall et al., 1986). These acute nicotine effects on the HPA axis seem to be centrally mediated, since nicotine stimulates vasopressin secretion, which, in combination with CRH, leads to ACTH release. Effects of acute nicotine administration on other neurochemical pathways within the PVN of the hypothalamus, such as cholinergic receptors, may cause the release of CRH, leading to the production of cortisol from the adrenal cortex (Pomerleau and Pomerleau, 1991; Seyler et al., 1984). It is possible that a frequent and prolonged stimulation of the HPA in response to nicotine leads to enhanced HPA activation, but reduced sensitivity to effects of other stimuli not related to nicotine (Kirschbaum et al., 1994). The obtained results suggest that the effect of nicotine on the HPA does not habituate. The absence of any changes in response to the stressor, on the other hand, suggests that the chronic effect of nicotine may disrupt the ability of the HPA system to respond to other challenges.

Potential central mechanisms involved in the altered stress response include the possible reduction of number or affinity of receptors mediating effects of nicotine in PVN and other CNS structures that integrate the neuroendocrine stress response. These possibilities are speculative at this time, and await further research. Also, the extent to which long-term abstinence normalizes basal adrenocortical activation and responses to stress is not clear. The present study shows that abstinence overnight and the day of each session failed to correct these alterations.

The current study has some limitations that should be noted. Only cortisol, BP, and HR data were obtained during the laboratory stressors. Information on the hemodynamic changes and ACTH would have allowed a more complete characterization of the underlying mechanisms responsible for the cortisol and BP response alterations in smokers. Also, abstinent smokers in this study were not interested in quitting, and it is not clear if this pattern of results would be influenced by intention to quit. The length of time of abstinence may have been short, and may have obscured effects of smoking deprivation in this design. Due to the small sample size, it was not possible to conduct detailed analyses examining gender differences or the effects of menstrual cycle and contraceptive use in women. We should also note that the sample included relatively young, well-educated, healthy participants, and smokers had a relatively short history of smoking. Nevertheless, this study has several strengths, including the use of multimethod assessment to assess effects of the acute stressful challenge, use of a socially relevant laboratory stressor, use of multiple methods to verify abstinence, conducting a rest day control session, and including a nonsmoking control group.

In summary, results from this experiment indicate that chronic smoking was associated with adrenocortical hypo-responsiveness and attenuated pressor effects of stress. Smokers showed greater prevailing levels of cortisol, but diminished BP and cortisol responses to acute stress compared with nonsmokers. The extent to which these alterations contribute to or mediate the exacerbation of stress-related withdrawal symptoms among smokers is currently under investigation. Identifying alterations in the stress-related adrenocortical and psychophysiological activity could lead to a better understanding of mechanisms of stress that might contribute to increased risk for smoking or relapse.

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