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Stress response dysregulation and stress-induced analgesia in nicotine dependent men and women

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Abstract

Alterations in the stress response and endogenous pain regulation mechanisms may contribute directly and indirectly to maintenance of nicotine dependence and relapse. We examined the extent to which nicotine dependence alters endogenous pain regulatory systems, including the hypothalamic-pituitary-adrenocortical axis, cardiovascular activity, and stress-induced analgesia. Smokers and nonsmokers attended a laboratory session that included assessment of hormonal and cardiovascular responses to stress. Smokers smoked at their regular rate prior to the session. The hand cold pressor and heat thermal pain tests were completed twice, once after acute stress (public speaking and math tasks) and the other after rest. While smokers and nonsmokers exhibited significant hormonal and cardiovascular responses to stress, smokers exhibited blunted stress responses relative to nonsmokers. They also exhibited diminished stress-induced analgesia. Results demonstrate altered stress response and diminished stress-induced analgesia among chronic smokers, and suggest that these dysregulated physiological responding may contribute to altered endogenous pain regulation.

Keywords

Pain; Stress-induced analgesia; Tobacco dependence; Cardiovascular; Cortisol

1. Introduction

Animal studies have demonstrated analgesic effects of nicotine (Bannon et al., 1998; Caggiula et al., 1995; Khan et al., 1998; Mandillo and Kanarek, 2001), and studies of acute nicotine administration in humans have shown an increase in pain threshold, regardless of participants' smoking status (Perkins et al., 1994). Nicotine's effects on multiple neurobiological systems, including the endogenous opioid system, the hypothalamic-pituitary-adrenocortical (HPA) axis, and the sympathetic nervous system, likely contribute to this analgesic effect. Nicotine directly stimulates dopaminergic, cholinergic, noradrenergic, and serotonergic pathways (Benwell et al., 1990; Fu et al., 2001; Rose and Corrigan, 1997; Salokangas et al., 2000) and the HPA axis (Kirschbaum et al., 1992; Seyler et al., 1984). Stimulation of cholinergic receptors in the hypothalamus, particularly in the

paraventricular nucleus, trigger the HPA cascade by the release of corticotropin-releasing factor (CRF). CRF acts on the corticotrope cells of the anterior pituitary, stimulating synthesis of proopiomelanocortin, and subsequent release of adrenocorticotrophic hormone (ACTH) and beta-endorphin. Nicotine also activates the sympatho-adreno-medullary axis, leading to the increased production of catecholamines and increasing cardiac and vascular activity. These effects, in turn, are associated with reduced pain sensitivity in animals (Maixner et al., 1982; Randich and Maixner, 1986) and in humans (al'Absi et al., 1996, 2000; Fillingim and Maixner, 1996; France and Stewart, 1995). On the other hand, chronic smoking leads to various neurobiological changes that are manifested by alterations in the stress response (Fung et al., 1996) and related neurochemical and neurophysiological correlates (al'Absi et al., 2003; Geraciotti et al., 1999; Gilbert et al., 1999; Wise and Munn, 1995).

Research has demonstrated that acute stress exposure may attenuate pain perception (al'Absi and Rokke, 1991; Janssen et al., 2001), although clinical research has demonstrated that chronic environmental stress may exacerbate pain perception (Bansevicius et al., 1997; Caceres and Burns, 1997; Lundberg, 1999). Research addressing potential endogenous mechanisms mediating stress-related analgesia has focused on changes in blood pressures (BP), baroreceptor activity, HPA, and endogenous opioid functions during stress (al'Absi and Petersen, 2003; al'Absi et al., 2002; Ghione, 1996). Considering this and the demonstrated dysregulation of the HPA and cardiovascular responses to stress in dependent smokers, endogenous regulation of pain perception may also be altered in this population (Girdler et al., 2005).

Extending previous findings, we specifically examined the extent to which chronic smoking (i.e., nicotine dependence) alters pain perception and endogenous pain regulatory mechanisms, including cardiovascular and neuroendocrine responses to stress and stress-induced analgesia. We predicted that smoking dependence would be associated with altered pain-related endogenous mechanisms, including blunted response to stress relative to nonsmokers. This prediction was based on previous studies (cited above) indicating effects of nicotine on pain and previous research demonstrating dysregulation of the stress response systems in smokers dependent to nicotine (al'Absi et al., 2003, 2008).

2. Methods

2.1. Participants

Male and female smokers and nonsmokers were recruited through community newspapers and flyers in Duluth and Minneapolis, MN. Interested participants completed telephone screening queries regarding medical and psychiatric history, including medication and tobacco use. Those meeting inclusion criteria were given an appointment for the informed consent process and on-site screening. These participants were recruited as part of a larger study to examine predictors of smoking relapse. Participants were enrolled in the study if they met the following criteria: (1) no current major or chronic medical illness or current psychiatric disorder; (2) no routine use of prescription medications except for contraceptives; (3) weight within $\pm 30\%$ of Metropolitan Life Insurance norms; and (4) two or less alcohol drinks consumed/day. All methods, advertisements and consent documents were approved by the Institutional Review Board of the University of Minnesota. One hundred sixteen smokers (10 cigarettes per day for at least 2 years; 52 women) and 38 nonsmokers (19 women) were included in the study.

2.2. Apparatus and measures

2.2.1. Physiological measures—A Dinamap oscillometric monitor system (Critikon, Tampa, FL) was used to assess systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR). An intravenous catheter was used to collect blood samples. Blood samples were separated and plasma samples were stored in -70°C until assayed. Plasma cortisol was assayed using enzyme immunoassay (EIA; DSL, Sinsheim, Germany) with a lower sensitivity of $0.1\text{ }\mu\text{g/dl}$. ACTH was assayed using radioimmunoassay kits (IBL, Hamburg, Germany) with a lower sensitivity of 0.46 pg/ml . Inter- and intra-assay coefficients of variance for these assays were below 10%. Salivary samples were collected for assay of cortisol and cotinine concentrations. Participants placed cotton dental rolls in their mouth until saturated and collected the rolls into a plastic tube (Salivette® tubes, Sarstedt, Rommelsdorf, Germany). Cotinine levels in saliva were measured by EIA (DRG Diagnostics, Marburg, Germany). Inter- and intra-assay variations were below 12%. MicroCO™ monitors (Micro Direct Inc., Auburn, Maine) were used to measure expired carbon monoxide (CO). Both salivary cotinine and CO were used to verify smoking abstinence.

2.2.2. Pain measures—The cold pressor and thermal heat pain induction procedures were implemented. The cold pressor test (CPT) consisted of a 1-gallon container filled with a ice-water slurry ($0-1^{\circ}\text{C}$). The participant was instructed to remove his or her hand when he or she could no longer tolerate the pain or if the maximum time of 4 min was reached. Eight nonsmokers and 12 smokers reached maximum tolerance time (4 min) after rest, and 10 nonsmokers and eight smokers reached maximum tolerance time after stress.

Pain tolerance was defined as the amount of time (in seconds) until the termination of the task. After CPT, the short version of the McGill pain questionnaire (MPQ) (Melzack, 1975) was administered to assess the participant's global pain experience.

Heat pain stimuli were delivered to the skin on the left volar forearm using a computer-controlled 2 cm^2 Peltier contact thermode affixed in place with a Velcro strap. Temperature was monitored by a contactor-contained thermistor (Medoc TSA 2001, Minneapolis, MN). The thermode was returned to the adapting temperature (35°C) between trials by active cooling at a rate of 10°C/s . Heat pain threshold and tolerance were assessed using an ascending method of limits with a staircase ramp of 1°C/s , following procedures similar to those described previously (Fillingim et al., 1998). Participants were instructed to press a button when the thermal stimulus first felt painful (threshold) and again press the button when the pain became intolerable (tolerance). The assessment was repeated four times and the average of the last three trials was calculated to determine both pain threshold and tolerance level. To avoid sensitization or habituation, the position of the thermode was moved approximately one cm after each trial (but still remained on the ventral forearm). The maximum temperature allowed was 52°C , and if participants failed to report pain threshold or pain tolerance before reaching the maximum, a value of 52°C was entered for that variable. Only one smoker and reached the maximum temperature (52°C) after rest and one smoker reached the max temperature after stress.

2.2.3. Mood measures—Smokers completed nicotine withdrawal self report instruments. Withdrawal symptoms were assessed by the Minnesota Nicotine Withdrawal Scale (MNWS; (Hughes and Hatsukami, 1986)). The scale includes items of irritability, anger, anxiety, difficulty concentrating, restlessness, depressed or sad mood, and hunger. Withdrawal severity was calculated without the item “craving” since it has been reported to be different from other withdrawal-related symptoms (Hughes and Hatsukami, 1986). In addition, the abbreviated version of the Questionnaire of Smoking Urges (QSU-B) (Cox et al., 2001;

Tiffany and Drobes, 1991) was used to measure two components of craving (factor 1: appetitive urges; factor 2: aversive urges).

We also assessed positive affect and distress, two factors previously shown to be reliable in tracking acute changes in mood states and have satisfactory psychometric properties (al'Absi et al., 1998, 1994; Lundberg and Frankenhaeuser, 1980). Positive affect included items such as cheerfulness, content, calmness, controllability, and interest. Negative affect consisted of anxiety, irritability, impatience, and restlessness. Each item had a 7-point scale anchored by the end points, "Not at All" and "Very Strong."

2.2.4. Other measures—Demographic information, stress levels (the Perceived Stress Scale; PSS) (Cohen et al., 1983), and total mood disturbance (i.e., sum of scores on subscales of tension, depression, anger, vigor, fatigue, confusion with vigor weighted negatively as measured by the Profile of Mood State questionnaire (POMS) (McNair et al., 1992) were completed during the screening session or before the first laboratory session. Levels of nicotine dependence (Fagerström Test of Nicotine Dependence; FTND) (Heatherton et al., 1991) and smoking history were also assessed among smokers.

2.3. Procedures

Participants completed a four-hour laboratory session initiated between 12 and 2:00 pm to control for diurnal patterns of hormonal plasma levels. For smokers, the session took place after ad libitum smoking. They smoked a cigarette of their preferred brand immediately before the lab session. Participants were instructed to refrain from caffeine for 4 h and from alcohol and exercise for 24 h prior to each session. Upon arrival at the lab, the participant was greeted and seated in a comfortable chair in the testing room. Expired CO was assessed, and the first saliva sample was collected. The participant was then fitted with a BP cuff and intravenous catheter insertion was completed.

The lab protocol time-line is summarized in Fig. 1. Systolic and diastolic BP and heart rate (HR) measures were obtained every 5 min for the last 20 min of the baseline phase (45 min). During this period watch common interest, nature video. This was followed by pain assessment under two different conditions counterbalanced across subjects: one was preceded by a 20 min rest period (i.e., rest–pain condition) and the other was preceded by an acute psychological stressor (i.e., stress–pain condition). The acute psychological stressor consisted of public speaking (4 min preparation and 4 min delivery) and a mental arithmetic task (10 min). Cardiovascular measures were collected every 2 min during each stress task. A recovery period (20 min) followed pain assessment completion where cardiovascular measures were recorded every 5 min. Saliva and blood samples, and mood, and withdrawal symptom measures (smokers only) were collected after each period. The participant was debriefed and paid for the participation following completion of the session.

2.4. Data analysis

Primary dependent variables included pain (maximum pain tolerance, MPQ total score for CPT and thermal pain), hormonal (plasma cortisol, ACTH, and salivary cortisol levels), cardiovascular (SBP, DBP, and HR), mood state (distress and positive affect), and withdrawal symptoms and craving. Hormonal samples collected after rest, after acute stress (public speaking and math tasks), after the pain assessment, and after recovery were used to examine effects of acute stress. Cardiovascular data were averaged for baseline rest, psychological stress, and recovery periods. Salivary cortisol data were log transformed to meet the normality assumption. A total of 154 participants (71 women) completed the stress and CPT assessment, and ninety-two participants (50 women) completed the thermal pain assessment.

Hormonal measures were analyzed using a series of 2 smoking group (smoker, nonsmoker) $\times$ 2 sex (men, women) $\times$ 4 time (rest, stress, stress–pain, recovery) multivariate analysis of covariance (MANCOVA) adjusting for task order and site. For cardiovascular and mood measures a series of 2 smoking group $\times$ 2 sex $\times$ 3 time (baseline, stress, recovery) MANCOVA was conducted. Pain measures were analyzed using 2 smoking group $\times$ 2 sex $\times$ 2 condition (rest–pain vs. stress–pain) MANCOVAs. Withdrawal symptoms and craving measures collected by MNWS and QSU-B were analyzed by 2 sex $\times$ 3 time (baseline, stress, recovery) MANCOVAs. Wilk's lambda criterion was used to test these models. Measures from the first hormonal samples, demographic variables, and trait mood measures (POMS, PSS) were analyzed using ANOVAs with smoking group and sex as between-subject factors as well as chi-square tests. Smoking variables (e.g., cigarettes per day) as well as carbon monoxide and salivary cotinine levels were compared between men and women using ANOVAs. Bonferroni adjustments were used for post hoc comparisons where appropriate.

3. Results

3.1. Participant characteristics

There were no differences between smokers and nonsmokers in body mass index, average hours of sleep, or marital status (p s $>$ 0.19; see Table 1). Smokers were older than nonsmokers and women were older than men (F s $>$ 3.98, p s $<$ 0.05). Smokers reported fewer years of education, higher daily caffeine consumption (F s $>$ 9.11, p s $<$ 0.01), and were more likely to be white and have past psychiatric history than nonsmokers ($\chi^2 >$ 7.75, p s $<$ 0.01). Smokers reported greater levels of mood disturbance and perceived stress than nonsmokers (F s $>$ 8.48, p s $<$ 0.05). Men exhibited higher levels of ACTH first sample than women (F (1, 135) = 5.09, $p <$ 0.05) though no group differences emerged ($p >$ 0.31). Male and female smokers did not differ in any of smoking-related variables (p s $>$ 0.31). Therefore, in addition to the MANCOVA models described above, we added the previous psychiatric history, and age, ethnicity, education, and caffeine as covariates where appropriate in subsequent analysis.

3.2. Effects of stress

3.2.1. Mood and withdrawal symptoms—Reported distress increased and positive affect decreased in response to stress (see Table 2; F s(2, 147) $>$ 20.3, p s $<$ 0.001). Positive affect during stress period was lower in women than in men as found by a sex $\times$ time interaction (F s(2, 147) = 5.89, $p <$ 0.01) with multiple comparisons ($p <$ 0.01). Smokers reported greater distress and lower positive affect than nonsmokers (F s(1, 148) $>$ 4.22, p s $<$ 0.05). These results remained significant after controlling for additional covariates (p s $<$ 0.01). In smokers, craving (F (2, 111) = 6.66, $p <$ 0.01) and withdrawal symptoms in MNWS (F (2, 111) = 40.9, $p <$ 0.001) as well as F2 of QSU-B (F (2, 110) = 9.53, $p <$ 0.001) increased in response to stress. Levels of QSU-B F1 decreased significantly during recovery (F (2, 110) = 5.94, $p <$ 0.01). These variables were comparable between men and women (p s $>$ 0.13).

3.2.2. Cardiovascular measures—Cardiovascular responses to stress were moderated by significant group $\times$ time interactions in SBP, DBP, and HR (F s(2, 147) $>$ 5.54, p s $<$ 0.01; see Table 3). This was supported further in that stress reactivity (baseline to stress) in DBP and HR was attenuated (F s(1, 148) $>$ 5.32, p s $<$ 0.05) and stress recovery (stress to recovery) in all cardiovascular measures were diminished (F s(1, 148) $>$ 8.56, p s $<$ 0.01) in smokers relative to nonsmokers (see Fig. 2). A significant group $\times$ sex effect was found in HR (F s(1, 148) = 4.37, p s $<$ 0.05) reflecting smaller response and slower recovery among female smokers relative to female nonsmokers (p s $<$ 0.01), however, this pattern was not observed among male participants (p s $>$ 0.42). A group $\times$ time interaction in DBP remained

significant when accounting for additional covariates ($p < 0.05$) while the effect was somewhat diminished in SBP and HR ($ps < 0.09$).

3.2.3. Hormonal measures—Plasma cortisol increased following stress induction ($F(3, 115) = 16.9, p < 0.001$; see Table 3). ACTH increased in response to stress ($F(3, 124) = 17.9, p < 0.001$) with men exhibiting greater increases than women ($F(1, 126) = 16.8, p < 0.001$). Salivary cortisol evidenced a group $\times$ time interaction ($F(3, 142) = 3.67, p < 0.05$). Change scores (from stress to pain period) yielded a group effect ($F(1, 144) = 5.77, p < 0.05$) reflecting attenuated cortisol response during stress in smokers relative to nonsmokers (see Fig. 3). These results remained significant with additional covariates ($ps < 0.05$).

3.2.4. Pain measures and stress-induced analgesia—Maximum pain tolerance to the CPT was lower in smokers than in nonsmokers ($F(1, 145) = 5.05, p < .05$) and women exhibited lower pain tolerance than men ($F(1, 145) = 6.57, p < 0.05$; see Table 4). There was no difference in pain tolerance between ratings after rest and after stress however ($p = 0.20$). With respect to thermal heat pain, acute stress was associated with increased pain tolerance ($F(1, 87) = 9.78, p < 0.01$), and women showed lower tolerance than men ($F(1, 87) = 6.08, p < 0.05$). No difference was observed in MPQ ($ps > 0.21$). MANCOVAs with additional covariates found a significant group $\times$ condition interaction in heat pain threshold ($F(1, 75) = 4.43, p < 0.05$) and tolerance ($F(1, 85) = 4.96, p < 0.05$). Follow-up tests revealed greater pain threshold and tolerance after stress than after rest in nonsmokers ($ps < 0.05$) but not in smokers ($ps > 0.15$; see Fig. 4). In addition, a group $\times$ sex effect in heat pain tolerance ($F(1, 85) = 4.39, p < 0.05$) suggested lower tolerance in nonsmoking women relative to nonsmoking men.

3.3. Association between stress response and pain perception

Correlational analyses were conducted to explore subjective and physiological correlates of pain. Greater SBP during stress was associated with higher pain tolerance to CPT ($r = 0.26, p < 0.01$). On the other hand, higher distress levels during baseline, stress, and recovery were correlated with higher MPQ scores ($rs > 0.34, ps < 0.001$). Nonsmokers exhibited positive associations between pain tolerance and baseline ACTH ($r = 0.36, p < 0.05$) and a trend of increased pain tolerance and salivary cortisol levels ($r = 0.29, p = 0.08$); however, these were not found in smokers ($ps > 0.19$). Increases in withdrawal symptoms and smoking urges (QSU-B factors 1 and 2) during baseline, stress, and recovery were associated with higher MPQ scores in smokers ($rs > 0.18, ps < 0.05$).

Evidence of association between stress-related physiological measures and thermal heat pain emerged. Greater SBP levels during baseline, stress, and recovery and greater salivary cortisol levels after stress were associated with higher pain tolerance ($rs > 0.21, ps < 0.05$). Lower plasma cortisol and ACTH during recovery were associated with higher MPQ scores ($rs > -0.23, ps < 0.05$). Reported distress during baseline, stress, and recovery were positively associated with MPQ scores ($rs > 0.36, ps < 0.001$). In smokers, higher withdrawal symptoms during recovery was associated with lower thermal heat pain threshold ($r > -0.35, p < 0.01$), and withdrawal symptoms and QSU-B factors were associated with increased MPQ scores ($rs > 0.25, ps < 0.05$).

4. Discussion

The primary findings for this study include the following. First, stress was associated with increased reported distress and increased plasma ACTH and cortisol, and with increased craving among smokers. Second, smokers exhibited deficiency in stress-related modulation of pain as indicated by the absence of stress-induced analgesia on the thermal heat pain test

compared with nonsmokers. Third, smokers exhibited blunted cardiovascular responses to stress. Fourth, smokers exhibited blunted cortisol response to stress compared to nonsmokers. Finally, the correlation analyses indicated enhanced pain tolerance with increased BP and cortisol production, increased pain ratings with increased distress, and increased pain rating with reduced cortisol, ACTH levels, and in smokers, with increased craving and withdrawal symptoms.

The convergent findings of altered stress response and altered pain regulatory mechanisms in smokers are novel and of specific importance in terms of identifying factors contributing to maintenance of nicotine dependence. While nonsmokers exhibited stress-induced analgesia on both thermal pain threshold and tolerance, smokers failed to show this effect. The absence of stress induced analgesia in smokers is in contrast to findings in healthy human demonstrating that acute stress exposure was associated with attenuated pain perception (al'Absi and Petersen, 2003; al'Absi and Rokke, 1991; Janssen et al., 2001). Mechanisms involved in mediating stress-induced analgesia include baroreceptor stimulation (France, 1999; Sheps et al., 1989; Simons et al., 2005), peripheral opioid peptides (al'Absi et al., 2004; Randich and Maixner, 1984), and stress cardiovascular reactivity (France and Stewart, 1995; Janssen, 2002). These mechanisms may be dysregulated as a function of chronic tobacco (presumably nicotine) use (Mandillo and Kanarek, 2001; Perkins et al., 1994). This is supported in the present study by the blunted cardiovascular response to stress among smokers.

Cumulative evidence suggests a link between high BP and pain insensitivity in animals and humans (Maixner et al., 1982). High BP is associated with higher pain thresholds in humans (Ghione, 1996), and pharmacologically induced increase in BP has been associated with increased pain tolerance in animal studies (Randich and Maixner, 1986). Mechanisms proposed to explain BP related hypoalgesia include enhanced hypothalamic-autonomic activation (al'Absi and Wittmers, 2003; France, 1999; Guasti et al., 2002). Chronic nicotine dosing/exposure resulting from smoking may lead to down regulation in baroreceptor and endogenous opioid activity and therefore reduced descending regulation of pain (al'Absi et al., 2008; Gerhard et al., 1999). This in turn could enhance sensitivity to pain and diminish stress-induced analgesia in smokers.

Blunted or dysregulated adrenocortical response to stress may also have contributed to increased pain sensitivity and absence of stress-induced analgesia among smokers. Previous work has documented an association between increased adrenocortical production and attenuated pain perception (al'Absi et al., 2002). A possible adrenocortical effect may be mediated by increased corticotropin-releasing factor (CRF) and cortisol (Lariviere and Melzack, 2000). While direct effects of CRF on pain sensitivity has not been established (Lautenbacher et al., 1999), its potential role in altering pain perception has been suggested by pharmacological observations demonstrating analgesic effects of CRF in dental patients (Hargreaves et al., 1987). Cortisol has also been found to correlate inversely with pain intensity and number of pain sites in chronic pain patients (Morrison et al., 2000). Reduced adrenocortical activity has been documented in patients with chronic pain (Valdes et al., 1989), abdominal pain (Alfven et al., 1994), chronic pelvic pain (Heim et al., 1998), chronic headache (Elwan et al., 1991), low back pain (Theorell et al., 2000), fibromyalgia (Crofford et al., 1994; Griep et al., 1993), rheumatoid arthritis (Chikanza et al., 1992; Hedman et al., 1992), and chronic fatigue syndrome (Demitrack and Crofford, 1998; Demitrack et al., 1991). In light of these interactions between HPA hormones and pain sensitivity and the observed blunted cortisol response to stress in smokers, we propose that the blunted stress response contributed to the enhanced pain sensitivity and absence of stress-induced analgesia in smokers.

Ultimately, the data reflect the interplay of multiple factors. Since smoking acutely increases cardiovascular and HPA activity, blunted cardiovascular and adrenocortical responses to stress indicate persistent consequences of chronic smoking on central stress response mechanisms. Arguably, this reflects adjustment processes across multiple neurobiological systems (Edwards and Koob, 2010; Koob and Kreek, 2007), which are supported by observations in humans reflecting down regulation of the adrenergic and HPA activity (Girdler et al., 2005; Shaw and al'Absi, 2010). Hyporesponsiveness during stress may also reflect alterations preexisting to smoking initiation in stress and emotion-related processing centers in the brain, such as forebrain and limbic functions (Lovallo, 2007; Robledo and Koob, 1993). Similarly, long-term, ongoing tobacco use may alter pain related neuronal processing (Ditre et al., 2011; Pomerleau et al., 1984; Silverstein, 1982). Central overlapping processes are likely involved in pain regulation and the stress response. Neurons within the paraventricular nucleus synthesize a variety of neuropeptides (e.g., enkephalins, dynorphin) that have analgesic effects (Akil et al., 1984; Lewis et al., 1986; Rossier et al., 1979), possibly mediating the nicotine-induced analgesia. This is supported by studies demonstrating that nicotine-induced analgesia is reduced by endogenous opioid antagonists (Aceto et al., 1993; Tripathi et al., 1982). It is now critical to elucidate the extent to which these processes directly contribute to observed changes in analgesia and stress response in smokers.

In conclusion, the results demonstrate altered stress response and diminished stress-induced analgesia among smokers following chronic nicotine exposure. This may represent another manifestation of altered central stress response systems. The possibility that these alterations contribute to increased sensitivity to withdrawal symptoms and increase risk for early smoking relapse needs to be elucidated.

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References

- Aceto MD, Scates SM, Ji Z, Bowman ER. Nicotine's opioid and anti-opioid interactions: proposed role in smoking behavior. *European Journal of Pharmacology*. 1993; 248:333–335. [PubMed: 8181540]
- Akil H, Watson SJ, Young E, Lewis ME, Khachaturian H, Walker JM. Endogenous opioids: biology and function. *Annual Review of Neuroscience*. 1984; 7:223–255.
- al'Absi M, Buchanan T, Lovallo WR. Pain perception and cardiovascular responses in men with positive parental history for hypertension. *Psychophysiology*. 1996; 33:655–661. [PubMed: 8961787]
- al'Absi M, Lovallo WR, McKey B, Sung BH, Whitsett TL, Wilson MF. Hypothalamic-pituitary-adrenocortical responses to psychological stress and caffeine in men at high and low risk for hypertension. *Psychosomatic Medicine*. 1998; 60:521–527. [PubMed: 9710300]
- al'Absi M, Lovallo WR, McKey BS, Pincomb GA. Borderline hypertensives produce exaggerated adrenocortical responses to mental stress. *Psychosomatic Medicine*. 1994; 56:245–250. [PubMed: 8084971]
- al'Absi M, Petersen KL. Blood pressure but not cortisol mediates stress effects on subsequent pain perception in healthy men and women. *Pain*. 2003; 106:285–295. [PubMed: 14659511]
- al'Absi M, Petersen KL, Wittmers LE. Blood pressure but not parental history for hypertension predicts pain perception in women. *Pain*. 2000; 88:61–68. [PubMed: 11098100]
- al'Absi M, Petersen KL, Wittmers LE. Adrenocortical and hemodynamic predictors of pain perception in men and women. *Pain*. 2002; 96:197–204. [PubMed: 11932075]

- al'Absi M, Rokke PD. Can anxiety help us tolerate pain? *Pain*. 1991; 46:43–51. [PubMed: 1896207]
- al'Absi M, Wittmers LE, Ellestad D, Nordehn G, Kim SW, Kirschbaum C, Grant JE. Sex differences in pain and hypothalamic-pituitary-adrenocortical responses to opioid blockade. *Psychosomatic Medicine*. 2004; 66:198–206. [PubMed: 15039504]
- al'Absi M, Wittmers LE, Erickson J, Hatsukami DK, Crouse B. Attenuated adrenocortical and blood pressure responses to psychological stress in ad libitum and abstinent smokers. *Pharmacology Biochemistry and Behavior*. 2003; 74:401–410.
- al'Absi M, Wittmers LE, Hatsukami D, Westra R. Blunted opiate modulation of hypothalamic-pituitary-adrenocortical activity in men and women who smoke. *Psychosomatic Medicine*. 2008; 70:928–935. [PubMed: 18799426]
- al'Absi M, Wittmers LE Jr. Enhanced adrenocortical responses to stress in hypertension-prone men and women. *Annals of Behavioral Medicine*. 2003; 25:25–33. [PubMed: 12581933]
- Alfven G, de la Torre B, Uvnas-Moberg K. Depressed concentrations of oxytocin and cortisol in children with recurrent abdominal pain of non-organic origin. *Acta Paediatrica*. 1994; 83:1076–1080. [PubMed: 7841708]
- Bannon AW, Decker MW, Holladay MW, Curzon P, Donnelly-Roberts D, Puttfarcken PS, Bitner RS, Diaz A, Dickenson AH, Porsolt RD, Williams M, Arneric SP. Broad-spectrum, non-opioid analgesic activity by selective modulation of neuronal nicotinic acetylcholine receptors. *Science*. 1998; 279:77–81. [PubMed: 9417028]
- Bansevicius D, Westgaard RH, Jensen C. Mental stress of long duration: EMG activity, perceived tension, fatigue, and pain development in pain-free subjects. *Headache*. 1997; 37:499–510. [PubMed: 9329233]
- Benwell ME, Balfour DJ, Anderson JM. Smoking-associated changes in the serotonergic systems of discrete regions of human brain. *Psychopharmacology*. 1990; 102:68–72. [PubMed: 1697418]
- Caceres C, Burns JW. Cardiovascular reactivity to psychological stress may enhance subsequent pain sensitivity. *Pain*. 1997; 69:237–244. [PubMed: 9085297]
- Caggiula AR, Epstein LH, Perkins KA, Saylor S. Different methods of assessing nicotine-induced antinociception may engage different neural mechanisms. *Psychopharmacology*. 1995; 122:301–306. [PubMed: 8748400]
- Chikanza IC, Petrou P, Kingsley G, Chrousos G, Panayi GS. Defective hypothalamic response to immune and inflammatory stimuli in patients with rheumatoid arthritis. *Arthritis and Rheumatism*. 1992; 35:1281–1288. [PubMed: 1445443]
- Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *Journal of Health and Social Behavior*. 1983; 24:385–396. [PubMed: 6668417]
- Cox LS, Tiffany ST, Christen AG. Evaluation of the brief questionnaire of smoking urges (QSU-brief) in laboratory and clinical settings. *Nicotine and Tobacco Research*. 2001; 3:7–16. [PubMed: 11260806]
- Crofford LJ, Pillemer SR, Kalogeras KT, Cash JM, Michelson D, Kling MA, Sternberg EM, Gold PW, Chrousos GP, Wilder RL. Hypothalamic-pituitary-adrenal axis perturbations in patients with fibromyalgia. *Arthritis and Rheumatism*. 1994; 37:1583–1592. [PubMed: 7980669]
- Demitrack MA, Crofford LJ. Evidence for and pathophysiologic implications of hypothalamic-pituitary-adrenal axis dysregulation in fibromyalgia and chronic fatigue syndrome. *Annals of the New York Academy of Sciences*. 1998; 840:684–697. [PubMed: 9629295]
- Demitrack MA, Dale JK, Straus SE, Laue L, Listwak SJ, Kruesi MJ, Chrousos GP, Gold PW. Evidence for impaired activation of the hypothalamic-pituitary-adrenal axis in patients with chronic fatigue syndrome. *Journal of Clinical Endocrinology and Metabolism*. 1991; 73:1224–1234. [PubMed: 1659582]
- Ditre JW, Brandon TH, Zale EL, Meagher MM. Pain, nicotine, and smoking: research findings and mechanistic considerations. *Psychological Bulletin*. 2011; 137:1065–1093. [PubMed: 21967450]
- Edwards S, Koob GF. Neurobiology of dysregulated motivational systems in drug addiction. *Future neurology*. 2010; 5:393–401. [PubMed: 20563312]
- Elwan O, Abdella M, el Bayad AB, Hamdy S. Hormonal changes in headache patients. *Journal of the Neurological Sciences*. 1991; 106:75–81. [PubMed: 1779242]

- Filligim R, Maixner W. The influence of resting blood pressure and gender on pain responses. *Psychosomatic Medicine*. 1996; 58:326–332. [PubMed: 8827795]
- Filligim RB, Maixner W, Kincaid S, Silva S. Sex differences in temporal summation but not sensory-discriminative processing of thermal pain. *Pain*. 1998; 75:121–127. [PubMed: 9539681]
- France CR. Decreased pain perception and risk for hypertension: considering a common physiological mechanism. *Psychophysiology*. 1999; 36:683–692. [PubMed: 10554582]
- France CR, Stewart KM. Parental history of hypertension and enhanced cardiovascular reactivity are associated with decreased pain ratings. *Psychophysiology*. 1995; 52:571–578. [PubMed: 8524991]
- Fu Y, Matta SG, Brower VG, Sharp BM. Norepinephrine secretion in the hypothalamic paraventricular nucleus of rats during unlimited access to self-administered nicotine: an in vivo microdialysis study. *Journal of Neuroscience*. 2001; 21:8979–8989. [PubMed: 11698608]
- Fung YK, Schmid MJ, Anderson TM, Lau YS. Effects of nicotine withdrawal on central dopaminergic systems. *Pharmacology Biochemistry and Behavior*. 1996; 53:635–640.
- Geraciotti TD Jr, West SA, Baker DG, Hill KK, Ekhaton NN, Wortman MD, Keck PE Jr, Norman AB. Low CSF concentration of a dopamine metabolite in tobacco smokers. *American Journal of Psychiatry*. 1999; 156:130–132. [PubMed: 9892309]
- Gerhardt U, Vorneweg P, Riedasch M, Hohage H. Acute and persistent effects of smoking on the baroreceptor function. *Journal of Autonomic Pharmacology*. 1999; 18:105–108. [PubMed: 10466943]
- Ghione S. Hypertension-associated hypalgesia. Evidence in experimental animals and humans, pathophysiological mechanisms, and potential clinical consequences. *Hypertension*. 1996; 28:494–504.
- Gilbert DG, McClemon FJ, Rabinovich NE, Dibb WD, Plath LC, Hiyane S, Jensen RA, Meliska CJ, Estes SL, Gehlbach BA. EEG, physiology, and task-related mood fail to resolve across 31 days of smoking abstinence: relations to depressive traits, nicotine exposure, and dependence. *Experimental and Clinical Psychopharmacology*. 1999; 7:427–443. [PubMed: 10609977]
- Girdler SS, Maixner W, Naftel HA, Stewart PW, Moretz RL, Light KC. Cigarette smoking, stress-induced analgesia and pain perception in men and women. *Pain*. 2005; 114:372–385. [PubMed: 15777863]
- Griep EN, Boersma JW, de Kloet ER. Altered reactivity of the hypothalamic-pituitary-adrenal axis in the primary fibromyalgia syndrome. *Journal of Rheumatology*. 1993; 20:469–474. [PubMed: 8386766]
- Guasti L, Zannotta D, Mainardi LT, Petrozzino MR, Grimoldi P, Garganico D, Diolisi A, Gaudio G, Klersy C, Grandi AM, Simoni C, Cerutti S. Hypertension-related hypoalgesia, autonomic function and spontaneous baroreflex sensitivity. *Autonomic Neuroscience*. 2002; 99:127–133. [PubMed: 12241087]
- Hargreaves KM, Mueller GP, Dubner R, Goldstein D, Dionne RA. Corticotropin-releasing factor (CRF) produces analgesia in humans and rats. *Brain Research*. 1987; 422:154–157. [PubMed: 3315118]
- Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom test for nicotine dependence: a revision of the Fagerstrom tolerance questionnaire. *British Journal of Addiction*. 1991; 86:1119–1127. [PubMed: 1932883]
- Hedman M, Nilsson E, de la Torre B. Low blood and synovial fluid levels of sulpho-conjugated steroids in rheumatoid arthritis. *Clinical and Experimental Rheumatology*. 1992; 10:25–30. [PubMed: 1532354]
- Heim C, Ehler U, Hanker JP, Hellhammer DH. Abuse-related posttraumatic stress disorder and alterations of the hypothalamic-pituitary-adrenal axis in women with chronic pelvic pain. *Psychosomatic Medicine*. 1998; 60:309–318. [PubMed: 9625218]
- Hughes JR, Hatsukami D. Signs and symptoms of tobacco withdrawal. *Archives of General Psychiatry*. 1986; 43:289–294. [PubMed: 3954551]
- Janssen SA. Negative affect and sensitization to pain. *Scandinavian Journal of Psychology*. 2002; 43:131–137. [PubMed: 12004950]
- Janssen SA, Spinhoven P, Brosschot JF. Experimentally induced anger, cardiovascular reactivity, and pain sensitivity. *Journal of Psychosomatic Research*. 2001; 51:479–485. [PubMed: 11602217]

- Khan IM, Buerkle H, Taylor P, Yaksh TL. Nociceptive and antinociceptive responses to intrathecally administered nicotinic agonists. *Neuropharmacology*. 1998; 37:1515–1525. [PubMed: 9886674]
- Kirschbaum C, Wust S, Strasburger CJ. Normal cigarette smoking increases free cortisol in habitual smokers. *Life Sciences*. 1992; 50:435–442. [PubMed: 1734161]
- Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *American Journal of Psychiatry*. 2007; 164:1149–1159. [PubMed: 17671276]
- Lariviere WR, Melzack R. The role of corticotropin-releasing factor in pain and analgesia. *Pain*. 2000; 84:1–12. [PubMed: 10601667]
- Lautenbacher S, Roscher S, Kohl G, Vedder H, Krieg J. Corticotropin-releasing-hormone lacks analgesic properties: an experimental study in humans, using non-inflammatory pain. *Pain*. 1999; 83:1–7. [PubMed: 10506666]
- Lewis ME, Khachaturian H, Schafer MK, Watson SJ. Anatomical approaches to the study of neuropeptides and related mRNA in the central nervous system. Research Publications: Association for Research in Nervous and Mental Disease. 1986; 64:79–109. [PubMed: 3088692]
- Lovallo, W. Individual differences in response to stress and risk for addiction. In: al'Absi, M., editor. *Stress and Addiction: Biological and Psychological Mechanisms*. Academic Press/Elsevier; London: 2007. p. 265-284.
- Lundberg U. Stress responses in low-status jobs and their relationship to health risks: musculoskeletal disorders. *Annals of the New York Academy of Sciences*. 1999; 896:162–172. [PubMed: 10681896]
- Lundberg U, Frankenhaeuser M. Pituitary-adrenal and sympathetic-adrenal correlates of distress and effort. *Journal of Psychosomatic Research*. 1980; 24:125–130. [PubMed: 7441580]
- Maixner W, Touw DB, Brody MJ, Gebhart GF, Long JP. Factors influencing the altered pain perception in the spontaneously hypertensive rat. *Brain Research*. 1982; 237:137–145. [PubMed: 6122488]
- Mandillo S, Kanarek RB. Chronic sucrose intake enhances nicotine-induced antinociception in female but not male Long-Evans rats. *Pharmacology Biochemistry and Behavior*. 2001; 68:211–219.
- McNair, DM.; Lorr, M.; Droppleman, LF. *POMS Manual-Profile of Mood Questionnaire*. Edits; San Diego: 1992.
- Melzack R. The McGill pain questionnaire: major properties and scoring methods. *Pain*. 1975; 1:277–299. [PubMed: 1235985]
- Morrison MF, Redei E, TenHave T, Parmelee P, Boyce AA, Sinha PS, Katz IR. Dehydroepiandrosterone sulfate and psychiatric measures in a frail, elderly residential care population. *Biological Psychiatry*. 2000; 47:144–150. [PubMed: 10664831]
- Perkins K, Epstein L, Grobe J, Fonte C. Tobacco abstinence, smoking cues, and the reinforcing value of smoking. *Pharmacology Biochemistry and Behavior*. 1994; 47:107–112.
- Pomerleau OF, Turk DC, Fertig JB. The effects of cigarette smoking on pain and anxiety. *Addictive Behaviors*. 1984; 9:265–271. [PubMed: 6496202]
- Randich A, Maixner W. [D-Ala2]-methionine enkephalinamide reflexively induces antinociception by activating vagal afferents. *Pharmacology Biochemistry and Behavior*. 1984; 21:441–448.
- Randich A, Maixner W. The role of sinoaortic and cardiopulmonary baroreceptor reflex arcs in nociception and stress-induced analgesia. *Annals of the New York Academy of Sciences*. 1986; 467:385–401. [PubMed: 3524386]
- Robledo P, Koob GF. Two discrete nucleus accumbens projection areas differentially mediate cocaine self-administration in the rat. *Behavioural Brain Research*. 1993; 55:159–166. [PubMed: 8395179]
- Rose JE, Corrigan WA. Nicotine self-administration in animals and humans: similarities and differences. *Psychopharmacology*. 1997; 130:28–40. [PubMed: 9089846]
- Rossier J, Battenberg E, Pittman Q, Bayon A, Koda L, Miller R, Guillemin R, Bloom F. Hypothalamic enkephalin neurones may regulate the neurohypophysis. *Nature*. 1979; 277:653–655. [PubMed: 34105]
- Salokangas RK, Vilkinen H, Ilonen T, Taiminen T, Bergman J, Haaparanta M, Solin O, Alanen A, Syvalahti E, Hietala J. High levels of dopamine activity in the basal ganglia of cigarette smokers. *American Journal of Psychiatry*. 2000; 157:632–634. [PubMed: 10739427]

- Seyler LE Jr, Fertig J, Pomerleau O, Hunt D, Parker K. The effects of smoking on ACTH and cortisol secretion. *Life Sciences*. 1984; 34:57–65. [PubMed: 6319934]
- Shaw D, al'Absi M. Blunted opiate modulation of prolactin response in smoking men and women. *Pharmacology Biochemistry and Behavior*. 2010; 95:1–5.
- Sheps DS, Maixner W, Hinderliter AL, Herbst MC, Bragdon EE, Herdt J, Kropp S, Adams KF, Koch G, Ekelund LG. Relationship between systolic blood pressure, ventricular volume and ischemic pain perception in patients with angina pectoris: a potential role for baroreceptors. *Israel Journal of Medical Sciences*. 1989; 25:482–487. [PubMed: 2807864]
- Silverstein B. Cigarette smoking, nicotine addiction, and relaxation. *Journal of Personality and Social Psychology*. 1982; 42:946–950. [PubMed: 7086634]
- Simons CT, Cuellar JM, Moore JA, Pinkerton KE, Uyeminami D, Carstens MI, Carstens E. Nicotinic receptor involvement in antinociception induced by exposure to cigarette smoke. *Neuroscience Letters*. 2005; 389:71–76. [PubMed: 16095820]
- Theorell T, Hasselhorn HM, Vingrd E, Andersson B. Interleukin 6 and cortisol in acute musculoskeletal disorders: results from a case-referent study in Sweden. *Stress Medicine*. 2000; 16:27–35.
- Tiffany ST, Drobes DJ. The development and initial validation of a questionnaire on smoking urges. *British Journal of Addiction*. 1991; 86:1467–1476. [PubMed: 1777741]
- Tripathi HL, Martin BR, Aceto MD. Nicotine-induced antinociception in rats and mice: correlation with nicotine brain levels. *Journal of Pharmacology and Experimental Therapeutics*. 1982; 221:91–96. [PubMed: 7062295]
- Valdes M, Garcia L, Treserra J, de Pablo J, de Flores T. Psychogenic pain and depressive disorders: an empirical study. *Journal of Affective Disorders*. 1989; 16:21–25. [PubMed: 2521646]
- Wise RA, Munn E. Withdrawal from chronic amphetamine elevates baseline intracranial self-stimulation thresholds. *Psychopharmacology*. 1995; 117:130–136. [PubMed: 7753958]

Fig. 1.

Graphic representation of the study protocol. Note: pain assessment was conducted twice in each session, after rest and after stress. The order of condition (rest–pain vs. stress–pain) was counterbalanced across subjects.

Fig. 2.

Cardiovascular response to and recovery from acute laboratory stress. Bars indicate mean and line bars indicate standard error. Note: reactivity was defined as the difference between measures during rest and measures during stress. Recovery was defined as the difference between stress and the recovery period. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig. 3. Changes in salivary cortisol concentrations in response to stress. Line bars indicate standard error. Note: There was a significant smoking group $\times$ time interaction due to attenuated cortisol response to stress in smokers. $*p < 0.05$.

Fig. 4.

Group differences in pain measures. Note: (A) significant smoking group $\times$ condition interaction in thermal heat pain threshold was due to stress induced analgesia (SIA) among nonsmokers. (B) Significant smoking group $\times$ condition interaction in thermal heat tolerance was due to SIA among nonsmokers. (C) Significant smoking group $\times$ sex interaction in thermal pain tolerance due to pronounced gender difference in nonsmokers. * $p < 0.05$, ** $p < 0.01$.

Table 1

Participant characteristics, smoking variables, and biochemical measures.

	Nonsmokers (n = 38)		Smokers (n = 116)	
	Men (n = 19)	Women (n = 19)	Men (n = 64)	Women (n = 52)
Age (years) ^{a,b}	26.5 (2.7)	33.9 (2.7)	33.9(1.5)	35.4(1.6)
BMI	24.5 (1.0)	24.1 (1.0)	25.9 (0.6)	24.7 (0.6)
Education (years) ^a	16.1 (0.7)	15.4 (0.6)	14.0 (0.4)	14.3 (0.4)
Caffeine (drinks/day) ^a	0.6 (0.4)	0.7 (0.4)	1.6 (0.2)	1.7 (0.2)
Sleep (hours per day)	7.5 (0.2)	7.1 (0.2)	7.1 (0.1)	7.1 (0.1)
Single (%) ^b	83.3	57.9	63.5	51.9
Caucasian (%) ^a	57.9	68.4	84.4	84.3
Past psychiatric history (%) ^a	10.5	5.3	35.9	48.1
Mood disturbance (POMS) ^a	8.4 (6.5)	16.8 (6.5)	28.6 (3.6)	27.5 (4.0)
Perceived stress (PSS) ^a	16.2 (1.1)	17.5 (1.1)	19.1 (0.6)	20.0 (0.6)
ACTH first sample (pg/ml) ^b	20.7 (2.3)	18.0 (2.4)	20.4 (1.3)	14.3 (1.5)
Plasma cortisol first sample (ng/ml)	72.3 (8.2)	73.4 (8.7)	76.0 (4.7)	74.6 (5.3)
Salivary cortisol (log) first sample (nmol/L)	2.2 (0.2)	1.8 (0.2)	2.1 (0.1)	2.1 (0.1)
Cigarettes (per day)	n/a	n/a	18.6 (0.8)	17.9 (0.9)
Duration (years)	n/a	n/a	11.9 (1.2)	10.5 (1.3)
Previous quit attempts	n/a	n/a	5.4 (1.2)	6.6 (1.3)
Motivation to quit (1 not at all - 7 very strong)	n/a	n/a	5.8 (0.1)	5.7 (0.1)
FTND	n/a	n/a	5.6 (0.3)	5.2 (0.3)
CO (ppm)	n/a	n/a	24.4 (1.5)	25.2 (1.7)
Cotinine (ng/ml)	n/a	n/a	151.6 (18.6)	156.6 (21.6)

Entries show mean and standard error. *Note:* BMI: body mass index; POMS: profile of mood state questionnaire; PSS: perceived stress scale; FTND: Fagerström test of nicotine dependence; CO: carbon monoxide.

^aSmoking group effect was significant.

^bSex effect was significant.

Table 2

Mood and withdrawal measures.

	<u>Nonsmokers</u>		<u>Smokers</u>	
	Men	Women	Men	Women
Distress ^{a,b}				
Baseline	3.4 (1.0)	4.6 (1.0)	5.4 (0.6)	4.9 (0.6)
Stress	5.4 (1.3)	6.3 (1.3)	8.3 (0.7)	7.9 (0.8)
Recovery	3.9 (1.1)	2.8 (1.1)	5.1 (0.6)	5.0 (0.7)
Positive affect ^{a,b,c}				
Baseline	20.4 (1.6)	17.9 (1.6)	17.3 (0.9)	15.9 (1.0)
Stress	16.8 (1.5)	12.7 (1.5)	13.4 (0.8)	10.5 (0.9)
Recovery	17.8 (1.6)	18.2 (1.6)	16.6 (0.8)	15.9 (1.0)
MNWS ^a				
Baseline	n/a	n/a	7.9 (0.8)	6.5 (0.9)
Stress	n/a	n/a	12.7 (0.9)	11.1 (1.1)
Recovery	n/a	n/a	8.9 (0.8)	7.0 (0.9)
Craving (MNWS) ^a				
Baseline	n/a	n/a	3.1 (0.2)	2.3 (0.2)
Stress	n/a	n/a	3.4 (0.3)	3.3 (0.3)
Recovery	n/a	n/a	3.2 (0.2)	2.7 (0.3)
QSU-B F1 ^a				
Baseline	n/a	n/a	27.5 (1.5)	26.6 (1.8)
Stress	n/a	n/a	29.8 (1.7)	28.3 (2.0)
Recovery	n/a	n/a	26.9 (1.5)	25.2 (1.8)
QSU-B F2 ^a				
Baseline	n/a	n/a	10.5 (0.8)	11.6 (1.0)
Stress	n/a	n/a	12.6 (1.1)	13.2 (1.3)
Recovery	n/a	n/a	11.0 (0.8)	9.5 (1.0)

Entries show mean and standard error of mood and withdrawal measures in response to stress, adjusting for site site and task order. *Note:* MNWS: Minnesota nicotine withdrawal scale; Craving: this item was analyzed separately from MNWS; QSU-B: questionnaire of smoking urges (factor 1: appetitive urges; factor 2: aversive urges).

^aTime effect was significant.

^bSmoking group effect was significant.

^cSex × time interaction was significant.

Table 3

Cardiovascular and hormonal measures

	Nonsmokers		Smokers	
	Men	Women	Men	Women
ACTH (pg/ml) ^{a,b}				
Rest	17.8 (2.1)	10.9 (2.0)	17.1 (1.1)	10.6 (1.4)
Stress	24.4 (2.7)	16.5 (2.7)	22.5 (1.5)	14.4 (1.8)
Pain	22.1 (2.8)	19.1 (2.7)	23.2 (1.5)	16.8 (1.8)
Recovery	18.2 (2.0)	10.1 (1.9)	17.5 (1.1)	12.1 (1.3)
Plasma Cortisol (ng/ml) ^a				
Rest	60.4 (7.0)	53.7 (7.2)	57.5 (3.9)	56.3 (4.6)
Stress	75.7 (7.2)	76.3 (7.5)	75.0 (4.0)	63.9 (4.7)
Pain	76.4 (7.9)	75.4 (8.1)	72.6 (4.3)	67.3 (5.2)
Recovery	54.7 (7.1)	64.7 (7.3)	57.1 (3.9)	59.0 (4.7)
Saliva Cortisol (nmol/l) ^{a,b,c}				
Rest	1.7 (0.1)	1.4 (0.1)	1.6 (0.1)	1.7 (0.1)
Stress	2.2 (0.1)	1.8 (0.1)	2.0 (0.1)	1.8 (0.1)
Pain	2.2 (0.2)	1.9 (0.2)	1.8 (0.1)	1.7 (0.1)
Recovery	2.0 (0.1)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)
SBP (mmHg) ^{a,b,c,d}				
Baseline	115.5 (2.3)	105.9 (2.3)	116.1 (1.2)	106.3 (1.4)
Stress	133.4 (2.8)	122.6 (2.8)	133.0 (1.5)	118.8 (1.8)
Recovery	112.1 (2.4)	106.9 (2.4)	117.8 (1.3)	108.6 (1.5)
DBP (mmHg) ^{a,c,d}				
Baseline	63.3 (1.6)	65.2 (1.6)	67.6 (0.9)	65.6 (1.0)
Stress	76.0 (1.8)	75.5 (1.8)	78.3 (1.0)	73.6 (1.6)
Recovery	64.0 (1.8)	65.5 (1.9)	70.0 (1.0)	67.5 (1.2)
HR (bpm) ^{a,b,c,d,e,f}				
Baseline	66.0 (2.0)	71.4 (2.1)	74.8 (1.1)	76.2 (1.3)
Stress	71.6 (2.4)	83.3 (2.4)	78.8 (1.3)	80.6 (1.5)
Recovery	59.0 (2.0)	67.5 (2.0)	66.7 (1.1)	72.1 (1.3)

Entries show mean and standard error of cardiovascular and hormonal measures in response to stress, adjusting for site and task order. *Note:* ACTH: adrenocorticotropic hormone; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; bpm: beats per minute.

^aTime effect was significant.

^bSex effect was significant.

^cSmoking group $\times$ time interaction was significant.

^dSex $\times$ time interaction was significant.

^eSmoking group effect was significant.

^fSmoking group $\times$ sex $\times$ time interaction was significant.

Table 4

Pain measures.

	Nonsmokers		Smokers	
	Men	Women	Men	Women
CPT				
Pain tolerance (sec) ^{a,b}				
Rest–pain	115.5 (16.2)	99.4 (16.3)	101.1 (9.0)	59.6 (10.3)
Stress–pain	133.5 (16.6)	95.1 (16.7)	102.1 (9.2)	64.7 (10.5)
MPQ				
Rest–pain	15.0 (1.8)	15.9 (1.8)	16.0 (1.0)	16.2 (1.1)
Stress–pain	15.8 (1.8)	16.3 (1.8)	15.1 (1.0)	16.3 (1.1)
Thermal heat pain				
Pain threshold (°C) ^c				
Rest–pain	43.8 (1.0)	43.8 (0.9)	44.9 (0.6)	45.2 (0.5)
Stress–pain	45.9 (1.0)	44.1 (0.9)	44.7 (0.6)	45.3 (0.5)
Pain tolerance (°C) ^{b,c,d,e}				
Rest–pain	48.9 (0.5)	47.9 (0.5)	49.3 (0.3)	48.8 (0.3)
Stress–pain	49.8 (0.5)	48.3 (0.4)	49.5 (0.3)	48.9 (0.2)
MPQ				
Rest–pain	14.9 (2.5)	16.0 (2.3)	14.4 (1.4)	15.6 (1.3)
Stress–pain	14.5 (2.4)	16.1 (2.2)	14.3 (1.4)	16.7 (1.3)

Entries show mean and standard error of pain measures after stress or rest, adjusting for site and task order. *Note:* CPT: cold pressor test; MPQ: McGill pain questionnaire.

^aSmoking group effect was significant.

^bSex effect was significant.

^cGroup × pain condition interaction was significant when additional covariates were included in the model.

^dPain condition effect was significant.

^eGroup × sex interaction was significant when additional covariates were included in the model.