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Neuroendocrine and cardiovascular reactions to acute psychological stress are attenuated in smokers.

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Summary

A number of studies have now examined the association between smoking and the magnitude of physiological reactions to acute psychological stress. However, no large-scale study has demonstrated this association incorporating neuroendocrine in addition to cardiovascular reactions to stress. The present study compared neuroendocrine and cardiovascular reactions to acute stress exposure in current smokers, ex-smokers, and those who had never smoked in a large community sample. Salivary cortisol, systolic and diastolic blood pressure, heart rate and frequency components of systolic blood pressure and heart rate variability were measured at rest and during exposure to a battery of three standardised stress tasks in 480 male and female participants from the Dutch Famine Birth Cohort Study. Current smokers had significantly lower cortisol, systolic and diastolic blood pressure, and heart rate reactions to stress. They also exhibited smaller changes in the low frequency band of blood pressure variability compared to ex- and never smokers. There were no group differences in stress related changes in overall heart rate variability as measured by the root mean square of successive interbeat interval differences or in the high frequency band of heart rate variability. In all cases, effects remained significant following statistical adjustment for a host of variables likely to be associated with reactivity and/or smoking. In secondary analyses, there were no significant associations between lifetime cigarette consumption or current consumption and stress reactivity. In conclusion, compared to non-smokers and ex-smokers, current smokers exhibited attenuated neuroendocrine and cardiovascular reactions to acute psychological stress. Among smokers and ex-smokers, there is no evidence that lifetime exposure was associated with physiological reactions to acute stress, nor that current levels of cigarette consumption were associated with reactivity. It is possible, then, that attenuated stress reactivity may be a marker for an increased susceptibility to take up and/or maintain smoking behaviour once initiated.

Keywords Acute psychological stress; cardiovascular activity; cortisol; smoking

1. Introduction

A number of studies have now examined the association between smoking and the magnitude of cortisol and cardiovascular reactions to acute psychological stress. The act of smoking *per se* is associated with increases in cortisol and cardiovascular activity (Benowitz, Porchet, Sheiner, & Jacob, 1988; Grassi et al., 1994; Kirschbaum, Wust, & Strasburger, 1992; Pomerleau, Fertig, Seyler, & Jaffe, 1983). Further, a number of studies have found that smoking and psychological stress have additive activating effects (Davis & Matthews, 1990; Macdougall et al., 1988; Ray et al., 1986). However, although there is some counter evidence (Perkins et al., 1992; Tersman et al. 1991), habitual smokers have generally been found to show diminished salivary and plasma cortisol (al'Absi, Wittmers, Erickson, Hatsukami, & Crouse, 2003; Back et al., 2008; Childs & de Wit, 2009; Kirschbaum, Scherer, & Strasburger, 1994; Kirschbaum, Strasburger, & Langkrar, 1993; Rohleder & Kirschbaum, 2006) and cardiovascular (Girdler, Jamner, Jarvik, Soles, & Shapiro, 1997; Roy, Steptoe, & Kirschbaum, 1994; Straneva, Hinderliter, Wells, Lenahan, & Girdler, 2000) reactions to acute psychological stress. To date, only three large scale population studies have examined this issue and their asssessment was confined to cardiovascular reactivity (Evans et al., 2012; Phillips, Der, Hunt & Carroll, 2009; Sheffield, Smith, Carroll, Shipley, & Marmot, 1997). All found that smokers showed attenuated cardiovascular reactions to acute stress exposure. As yet, no population study has examined the influence of smoking status on cortisol reactions to acute stress. In addition, cardiovascular measurement in previous studies has largely been restricted to blood pressure and heart rate; little is known about the hemodynamic mechanisms underlying blunted cardiovascular reactivity in smokers.

It is unlikely that the effects of smoking status on reactivity reflect temporary abstinence during stress testing and its effects on stress task engagement (Roy et al., 1994). Blunted cardiovascular reactivity has been observed in female smokers regardless of whether they were wearing a nicotine replacement patch or not (Girdler et al., 1997). In addition, cortisol and cardiovascular reactivity has been compared among non-smokers, smokers who abstained from smoking, and smokers who continued to smoke at their usual rate; smokers, irrespective of their assigned condition, showed blunted cortisol and cardiovascular reactions to acute stress (al'Absi et al., 2003; Tsuda et al., 1996). Given that cortisol and cardiovascular stress reactivity are strongly correlated (Bosch et al., 2009; Cacioppo, 1994; Lovallo, Pincomb, Brackett, & Wilson, 1990), it is perhaps unsurprising that attenuated

reactivity in one system is paralleled by the diminished reactions of the other. If smoking is characterised by blunted stress reactivity, the question of direction of effect arises. Might prolonged smoking exposure somehow weaken the autonomic nervous system's ability to react to environmental challenges (Koob & Kreck, 2007) or, alternatively might blunted stress reactivity be a marker for an increased susceptibility to take up and/or maintain smoking behaviour once initiated (Lovallo, 2011, 2006)? Two considerations favour the latter. First, diminished stress reactivity was found to predict relapse among smokers who had quit (al'Absi, Hatsukami, & Davis, 2005), suggesting that blunted reactivity precedes smoking addiction. Second, blunted cortisol and cardiovascular reactions to stress have been associated with other substance addictions, such as alcohol (Lovallo, Dickensheets, Myers, Thomas, & Nixon, 2000; Panknin, Dickensheets, Nixon, & Lovallo, 2002) and, indeed, has been shown to characterise those with non-substance dependencies and problems, such as exercise dependence (Heaney, Ginty, Carroll, & Phillips, 2011), gambling addiction (Paris, Franco, Sodano, Frye, & Wulfert, 2009), bulimia (Ginty, Phillips, Higgs, Heaney, & Carroll, 2012), and repeated self-harm (Kaess, Hille, Parzer, Maser-Gluth, Resch, & Brunner, 2011).

The present study revisited the issue of smoking and physiological reactions to acute stress exposure and implemented a number of novel features: 1) a large community sample with both cortisol and cardiovascular activity recorded at rest and during a battery of acute psychological stress tasks; 2) inclusion of continuous measurements of autonomic reactivity (e.g., low frequency blood pressure variability); 3) self-report assessments of lifetime and current smoking consumption among ex- and current smokers. This is the first large scale study to examine whether dysregulation of the stress response in smokers is characteristic of both branches of the stress effector system: hypothalamic-pituitary-adrenal (HPA) axis and the sympathomedullary (SAM) pathway. Additionally, continuous autonomic measurements will allow for a more comprehensive analysis of the underlying haemodynamics in response to stress. Based on the balance of previous evidence, we hypothesized that current smokers would exhibit attenuated cortisol and cardiovascular stress reactivity relative to ex-smokers and those who had never smoked. In addition, we expected that the underlying haemodynamics of blunted cardiovascular stress reactivity in smokers would reflect sympathetic dysregulation (Phillips et al., 2009) rather than vagal dysregulation. Lastly, examining the relationship between cortisol and cardiovascular stress reactivity and lifetime and current consumption will shed light on the direction of effects. Were smoking causally implicated in neuroendocrine and sympathetic malfunction in the face of challenge we would

expect there to be a negative association between the extent of lifetime exposure and reactivity.

2. Methods

Participants were selected from the Dutch Famine Birth Cohort, which consists of 2,414 men and women who were born in Amsterdam, the Netherlands, between November 1943 and February 1947. The selection procedures and subsequent loss to follow up have been described elsewhere (Painter et al., 2005; Ravelli et al., 1998). All 1,423 members of the cohort who lived in the Netherlands on 1 September 2002 and whose current address was known were invited to the clinic to participate in detailed medical examinations, including stress testing; a total of 740 attended. There were no exclusion criteria. The study was approved by the local Medical Ethics Committee and carried out in accordance with the Declaration of Helsinki and the informed written consent of the participants.

2.1 General Study Parameters

Participants arrived at the hospital at 08:00. They were not given instructions regarding dietary, sleep, or smoking restrictions prior to their visit. After completing consent forms trained research nurses took anthropometric measurements and conducted a standardized interview in which information was obtained about socio-economic status (SES), lifestyle, and use of medication. Height was measured twice using a fixed or portable stadiometer and weight twice using Seca and portable Tefal scales. Body Mass Index (BMI) was computed as weight (kg) / height (m^2) from the averages of the two height and weight measurements. SES was defined according to the International Socio-Economic Index (ISEI)-92, which is based on the participant's or their partner's occupation, whichever has the higher status (Bakker & Sieben, 1997). Values in the ISEI-92 scale ranged from 16 (low status) to 87. The Hopsital Anxiety and Depression Scale (HADS) was used to assess symptoms of depression (Zigmond & Snaith, 1983). Possible caseness for depression was defined by scores ≥ 8. Alcohol consumption was recorded as the number of units consumed per week; one unit was defined as one glass of an alcoholic beverage (wine, beer, or shot).

2.2 Smoking

During the standardized interview participants were asked "Do you currently smoke cigarettes?" They were given the option of answering "Yes, on average 1 or more cigarettes per month," "Yes, but on average less than 1 cigarette a month," "No, I used to smoke

cigarettes, but now I don't anymore," and "No, I never smoked." Participants were then classified as current, ex-, or never smokers. Participants who were current smokers were asked three additional questions: "How many cigarettes per day do/did you currently smoke per day?"; "At what age did you start smoking?" and "Did you ever quit during the period of smoking and for how long?" Ex-smokers were also asked, "At what age did you totally quit smoking?" These answers were then multiplied to create packs per day × years, a self-report consumption variable with one pack containing 20 cigarettes.

2.3 Psychological Stress Protocol

The stress protocol started in the afternoon between the hours of 12:00-14:00 on the day participants visited the hospital (as indicated, arrival time was 08:00), approximately one hour after a light lunch. It began with a 20-minute baseline period after which three psychological tasks were performed in a fixed order: Stroop, mirror tracing, and a speech task. Participants were in a seated position during all phases. Each stress task lasted 5 minutes with 6 minutes in between and 30 minutes of recovery following the final stress task. The Stroop task consisted of a single-trial computerized version of the classic Stroop colour-word conflict challenge. After a short introduction, participants were allowed to practice until they fully understood the requirements of the task. Errors and exceeding the response time limit of 5 seconds triggered a short auditory beep. For the mirror-tracing task, a star had to be traced that could only be seen in a mirror image (Lafayette Instruments Corp, Lafayette, IN, USA). Every divergence from the line triggered an auditory stimulus. Participants were allowed to practice one circuit of tracing. They were instructed to prioritize accuracy over speed and were told that most people could perform five circuits of the star without divergence from the line within the given 5 minutes. Prior to the speech task, participants listened to an audio taped instruction in which they were told to imagine a situation in which they were falsely accused of pick pocketing. They were then given 2 minutes to prepare a 3-minute speech in which they had to respond to the accusation. The speech was videotaped and participants were told that the number of repetitions, eloquence, and persuasiveness of their performance would be assessed by a team of communication experts and psychologists. After completion of each of the three stress tasks, participants completed a 7-point rating scale of stress task impact, including participants' commitment to the tasks. Figure 1 displays a schematic representing the stress protocol.

A total of seven saliva samples were collected using Salivettes (Sarstedt, Rommelsdorf, Germany): at 5 and 20 minutes of the baseline period, at 6 minutes following completion of the Stroop task and the mirror tracing task, and at 10, 20, and 30 minutes after completion of the speech task. Participants were instructed to keep the Salivette in their mouth under their tongue for 2-minutes and not to chew or suck on it. After the 2-minute period participants were asked whether they thought it was wet enough; if it was not, they kept it in their mouth a little longer until they felt it was sufficiently saturated. Salivary cortisol concentrations were measured using a time-resolved immunofluorescent assay (DELFIA) (Wood et al., 1997). The assay had a lower detection limit of 0.4 nmol/l and an inter-assay variance of 9-11% and an intra-assay variance of less than 10%.

Continuous blood pressure (BP) and heart rate (HR) measurements were made using a Finometer or a Portapres Model-2 (Finapres Medical systems, Amsterdam, the Netherlands). There were no differences in reactivity as a function of the two different measuring devices. Six periods of 5 minutes were designated as the key measurement periods: resting baseline (15 minutes into the baseline period), Stroop, mirror-tracing task, speech task (including preparation time), recovery 1 (5 minutes after completing the speech task), recovery 2 (25 minutes after completing the speech task). Mean systolic blood pressure (SBP), diastolic blood pressure (DBP) and HR were calculated for each measuring period.
Data from the Finometer and Portapres were extracted using Beatscope 1.1 (Finapres Medical Systems, Amsterdam, The Netherlands) and imported into MATLAB (The Mathworks, Natick, MA, United States). Three observers, using an automated abnormal heart period (HP; the interval between adjacent heart beats) rejection algorithm as a guide, edited the data to remove heart periods influenced by artifacts or ectopic beats. HP and systolic arterial pressure (SAP) variability were estimated in two frequency bands: low frequency (LF; 0.04-0.15 Hz) and high frequency (HF; 0.15-0.4 Hz) using a standard Fourier-based spectrum analysis (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The cardiovascular parameters we used included LF blood pressure variability (LF BPV) (an indicator of sympathetic nervous system activity; Alex et al., 2013; Lucini et al. 1996; Pagani et al., 1986) and HF heart rate variability (HRV) (an indicator of parasympathetic nervous system activity). Additionally, we calculated time-domain based measures, including the root mean square of successive differences (RMSSD, which is also a measure predominantly influenced by parasympathetic nervous system

activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

2.4 Statistical Analyses

To examine the socio-demographic and physical differences between smoking status groups, χ^2 and ANOVAs were applied. Baseline cortisol was computed as the mean of the first and second cortisol concentration measures during the baseline period. The cortisol concentrations taken 10 min and 20 min following stress exposure, fifth and sixth samples, were used to determine stress phase cortisol. These time lags are characteristic of peak responses in other stress research and were the peak cortisol values in the present study (Dickerson and Kemeny, 2004; Schlotz, Kumsta, Layes, Entringer, Jones, & Wust, 2008). Baseline cardiovascular activity was the average of the values recorded in the 5-minute period 15 minutes into the baseline. Cardiovascular measures were averaged across the three stress tasks to determine the cardiovascular value for each cardiovascular variable. Repeated measures ANOVAs, comparing baseline and post stress task value in the case of cortisol and mean task value in the case of cardiovascular activity, were undertaken to confirm that the stress battery perturbed physiological activity. Prior to testing the association between smoking and stress responses, the relationship between smoking and baseline cortisol concentration and cardiovascular levels were examined using ANOVA; significant relationships were then examined using ANCOVA adjusting for potential confounding variables: age, SES, BMI, gender, depression status, and use of anti-hypertensive medication. Analysis of group differences in cortisol concentrations across the stress testing protocol were conducted using a mixed-between-within ANOVA. To determine if group differences withstood adjustment for potential confounding variables, baseline cortisol concentration, age, SES, BMI, gender, depression status, use of anti-hypertensive medication, alcohol use, and self-reported commitment to the stress tasks, ANCOVA was used with stress phase cortisol as the dependent variable and smoking status as the independent variable. Analyses of cardiovascular stress reactivity were performed by ANCOVA, adjusting for appropriate baseline physiological measures, with stress phase average as the dependent variable and smoking status as the independent variable. Where significant effects emerged, ANCOVAs were again undertaken additionally adjusting for age, SES, BMI, gender, depression status, use of anti-hypertensive medication, alcohol use, and self-reported commitment to the stress tasks. For both ANOVAs and ANCOVAs, partial η^2 is reported as a measure of effect size.

As a sensitivity analysis repeated measures analysis (linear mixed models), using an unstructured variance-covariance matrix were used to check associations between cortisol and cardiovascular temporal profiles and smoking status. These analyses allowed for the inclusion of participants (N = 68) who had missing data on one or more of the measurements and hence were excluded in the ANOVAs and ANCOVAs. The linear mixed models were fully adjusted as above. Since both depression and hypertension have been related to smoking and cardiovascular reactions to stress, sensitivity analyses excluding all participants who met criteria for depression or who were on anti-hypertensive medications were conducted for all group reactivity analyses. The relationship between lifetime consumption and stress phase activity were examined using two-step regressions in the ex- and current smoking groups. The association between the current number of cigarettes smoked per day in the current smoking group and stress phase activity were examined using the same strategy. The dependent variables were the respective average stress values, with baseline values entered at step 1 as covariates and total number of packs per day × years smoked or current number of cigarettes per day was entered at step 2.

3. Results

3.1 Study Population

Of the 740 cohort members who participated in the study, 721 completed the stress protocol. Logistical problems (n = 5) and illness (n = 10) prevented some participants from finishing the stress protocol. Due to technical problems, BP and HR recordings were unavailable for four individuals. Incomplete cardiovascular data for some participants and exclusion of participants with significant arrhythmia, determined during data processing based on resting Finometer/Portapres readings, reduced the effective sample size to 480 participants. No participants were excluded prior to participation based on history of arrhythmia. A total of 106 participants had one or more missing cortisol value as a result of insufficient saliva, and were excluded from the cortisol analyses.

3.2 Smoking status

One hundred and two of the sample were current smokers (21%), 203 were ex-smokers (43%), and 174 (36%) never smoked. All current smokers reported smoking at least two cigarettes per day. The mean (SD) number of cigarettes per day in the smoking group was

15.6 (9.17). There were no significant differences in age between the groups (p = .72). There were, however, significant differences in SES, F(2,472) = 7.02, p = < .001, η^2 = .029, BMI F(2,476) = 3.56, p = .03, η^2 = .015, and weekly alcohol consumption, F(2,467) = 4.62, p = .01, η^2 =.019; those who never smoked came from a significantly higher SES background and consumed less alcohol than ex- and current smokers. In addition, smokers had a lower BMI than ex-smokers. There was a tendency for proportionally more women (57%) than men (43%) to have never smoked (p = .07). There were significant differences between groups on depression status, in that 42% of those with possible depression currently smoked as opposed to only 20% among the non-depressed, χ^2 (2) = 11.84, p = .001. Ex-smokers were more likely to be on anti-hypertensive medication, 52% of those on anti-hypertensive medication were ex-smokers, χ^2 (2) = 6.60, p = .037. The physical characteristics and demographic details of the participants overall and by smoking status are presented in Table 1.

[Insert Table 1 about here]

3.3 Cortisol and cardiovascular reactions to the stress tasks

The stress task battery elicited a significant increase in cortisol concentration, F(1,373) = 81.89, p < .001, η^2 = .180. Stress exposure also provoked significant increases in SBP, F(1,479) = 2287.98, p < .001, η^2 = .827, DBP, F(1,479) = 2799.49, p < .001, η^2 = .854, HR, F(1,479) = 554.52, p < .001, η^2 = .537, and LF BPV, F(1,479) = 237.12, p < .001, η^2 =.331. It caused significant decreases in HF HRV, F(1,479) = 50.80, p < .001, η^2 =.096, and RMSSD, F(1,479) = 94.16, p < .001, η^2 =.164. The summary statistics are presented in Table 2.

[Insert Table 2 about here]

3.4 Smoking and baseline cortisol and cardiovascular activity

Baseline cortisol concentrations and cardiovascular levels according to smoking status are presented in Table 2. Smokers had lower resting SBP and DBP than ex-smokers and individuals who never smoked and ex-smokers had lower resting DBP than individuals who never smoked, F(2,479) = 16.31, p < .001, η^2 = .064, and F(2,478) = 23.12, p < .001, η^2 = .089. There were no significant differences between groups in baseline cortisol (p = .79), HR (p = .46), LF BPV (p = .11), HF HRV (p = .45), or RMSSD (p = .32). In ANCOVA models that adjusted for age, sex, SES, BMI, depression status, use of anti-hypertensive medications,

and alcohol consumption, the group differences at baseline remained statistically significant, SBP, $F(2,437) = 13.57$, $p < .001$, $\eta^2 = .058$, DBP, $F(2,437) = 22.03$, $p < .001$, $\eta^2 = .092$.

3.5 Smoking and cortisol reactivity

In the group (smoking status) x time analysis of cortisol concentrations, there was a significant main effect for time, $F(6, 1710) = 29.70$, $p < .001$, $\eta^2 = .094$, participants had significantly higher cortisol concentrations at time points 5 and 6 compared with all other time points. There was no main effect for group ($p = .17$). Importantly, there was a significant group x time interaction, $F (12, 1710) = 3.58$, $p = .025$, $\eta^2 = .025$. Participants in the ex- and never smokers displayed a significant increase in cortisol in response to the stress battery, whereas cortisol concentrations in the smoking group did not change over time. Figure 2 displays cortisol concentrations across the psychological stress protocol by category of smoker. In ANCOVA adjusting for average baseline cortisol, age, sex, SES, BMI, depression status, use of hypertensive medication, weekly alcohol consumption, and stress task commitment, there was a significant difference between groups on stress phase cortisol levels, $F(2,337) = 8.83$, $p < .001$, $\eta^2 = .050$. Smokers had significantly smaller cortisol reactions than ex- and never smokers. The significant group effects for cortisol reactivity (difference between stress and baseline) are shown in Figure 3.

3.6 Smoking and cardiovascular reactivity

In ANCOVAs adjusting for appropriate baseline cardiovascular values, there were main effects of smoking status for the following stress phase values: SBP, $F(2,475) = 9.89$, $p < .001$, $\eta^2 = .040$, DBP, $F(2,475) = 5.28$, $p = .005$, $\eta^2 = .022$, HR, $F(2,475) = 10.91$, $p < .001$, $\eta^2 = .044$, and LF BPV, $F(2,475) = 10.57$, $p < .001$, $\eta^2 = .043$. In all cases, except DBP, smokers had significantly smaller reactions than ex- and never smokers. In the case of DBP, smokers had significantly smaller reactions than never smokers. There were no significant group differences for HF HRV reactivity ($p = .52$) or RMSSD ($p = .18$). The significant group effects are illustrated as reactivity (difference between stress and baseline) in Figure 3. ANCOVAs were then undertaken adjusting for, in addition to the appropriate cardiovascular baseline level, age, sex, SES, BMI, depression status, use of hypertensive medication, weekly alcohol consumption, and stress task commitment. The group effects identified above were still significant following such adjustment: SBP, $F(2,436) = 8.21$, $p < .001$, $\eta^2 = .035$, DBP, $F(2,436) = 6.02$, $p = .003$, $\eta^2 = .027$, HR, $F(2,436) = 11.51$, $p < .001$, $\eta^2 = .050$, LF BPV, $F(2,436) = 10.55$, $p < .001$, $\eta^2 = .046$.

3.7 Smoking, cortisol and cardiovascular reactivity: sensitivity analyses

The sensitivity analyses, using linear mixed models to account for missing data, revealed virtually identical results. Due to the potential confounds involving the presence of disorders (depression) and medications (anti-hypertensive medication) that could influence the main outcome measures, all analyses in sections 3.5 and 3.6 were conducted removing participants with depression and/or on anti-hypertensive medication (N = 144). Re-analysis with removed participants yielded similar outcomes to those found for the whole sample.

[Insert Figures 1 and 2 about here]

3.8 Self-reported smoking consumption and reactivity

In two-step regressions adjusting for appropriate baseline physiological values, there were no significant associations between average stress phase physiological activity and packs per day x number of years smoked, $p > .15$ in all instances. Similarly, there were no significant associations between current number of cigarettes smoked per day and average stress physiological activity adjusted for baseline, $p > .12$ in all instances.

4. Discussion

The present study compared cortisol and cardiovascular reactions to acute stress exposure in current smokers, ex-smokers, and those who never smoked. The results were in line with our hypothesis; individuals who smoked exhibited blunted salivary cortisol, SBP, DBP, HR, and LF BPV reactions to a battery of stress tasks compared to ex- and never smokers. There were no significant differences between groups in HF HRV or RMSSD reactivity. All these effects remained statistically significant when adjusting for the appropriate baseline physiological level, as well as, age, SES, BMI, gender, depression status, use of anti-hypertensive medication, alcohol consumption, and self-reported commitment to the tasks.

This is the first large scale study we know of to examine the relationship between both neuroendocrine and continuous cardiovascular responses to stress and smoking status. Our results confirm previous findings showing that smokers exhibit blunted cortisol (al'Absi, Wittmers, Erickson, Hatsukami, & Crouse, 2003; Childs & de Wit, 2009; Kirschbaum, Scherer, & Strasburger, 1994; Kirschbaum, Strasburger, & Langkrar, 1993; Rohleder & Kirschbaum, 2006) and cardiovascular (Girdler, Jamner, Jarvik, Soles, & Shapiro, 1997; Phillips, Der, Hunt, & Carroll, 2009; Roy, Steptoe, & Kirschbaum, 1994; Sheffield, Smith,

Carroll, Shipley, & Marmot, 1997; Straneva, Hinderliter, Wells, Lenahan, & Girdler, 2000) reactions to acute psychological stress.

The current study is also able to shed light on the proximal mechanisms underlying the blunted BP and HR stress reactions of smokers. Previous research showed that smokers have a blunted low frequency HRV reaction to orthostatic stress (Lucini et al., 1996). On the other hand, blunted stress induced HF HRV reactions, indicating decreased vagal reactivity, have been reported to relate to less time to initiate smoking following stress exposure (Ashare, et al., 2011). We found, additionally, that smokers were characterized by decreased sympathetic activation to stress, as indicated by blunted LF BPV stress reactivity (Alex et al., 2013; Lucini et al. 1996; Pagani et al., 1986). HF HRV and RMSSD reactivity was unrelated to smoking status. Consequently, it would appear that smokers are predominantly characterized by blunted sympathetic activation during stress, which may be driven by an attenuated β-adrenergic responses. A recent meta-analysis showed that β-adrenergic, but not α-adrenergic, blockade significantly attenuated cardiovascular reactivity, indicating that the sympathetic basis of cardiovascular reactivity is primarily β-adrenergic (Brindle, Ginty, Phillips, & Carroll, in press). In line with this contention, smokers have been shown to have a reduced density and down-regulated function of β-adrenergic receptors (Laustiola et al., 1988). It is not beyond the realms of possibility that some common pathway linking the β-adrenergic system and the hypothalamic-pituitary-adrenocortical axis is responsible for the parallel pattern of findings for both cortisol and cardiovascular stress reactivity in the present study. After all, individual differences in the pre-ejection period (PEP) response to acute stress, considered to be a marker of cardiac β-adrenergic drive, have been found to be highly correlated with the cortisol response to stress (Bosch et al., 2009; Cacioppo, 1994). Indeed, in the first of these studies, variations in cortisol stress reactivity to different intensities of stress exposure were largely explained by variations in PEP reactivity (Bosch et al., 2009).

The present study is observational and cross-sectional, and as such cannot determine causality and the direction of causation (Christenfeld et al., 2004). It is unlikely that blunted stress reactivity in smokers is artefactual, attributable to the temporary abstinence enforced by participation in a laboratory stress testing protocol (Roy et al., 1994). Blunted cortisol and cardiovascular reactivity have been observed in smokers who abstained from smoking during a laboratory stress testing session or were allowed to smoke *ad libitum* (al'Absi et al., 2003) and blunted cardiovascular reactivity was seen in smokers who wore a nicotine patch during

stress (Girdler et al., 1997). It is possible that prolonged smoking results in a dysregulation of the stress response. Such dysregulation might underlie the precipitative effects of stress on smoking behaviour (Schachter et al., 1977) and relapse from abstinence (Childs & deWit, 2009). However analyses examining the association of number of packs per day × number of years smoked with physiological responses to stress revealed no significant relationship between smoking consumption and reactivity. Nor was there a relationship between current number of cigarettes smoked per day and reactivity among current smokers. Alternatively, the direction of causality may be from low reactivity to smoking, such that blunted stress reactivity may reflect a general disengagement of the biological systems that support motivated behaviour (Ginty, Gianaros, Derbyshire, Phillips, & Caroll, 2013; Lovallo, 2011), and that this dysregulation may increase susceptibility to addictive behaviour. The direction of causation has implications for intervention particularly given evidence that blunted cortisol reactors are more likely to relapse following smoking cessation (al' Absi, 2006; al'Absi et al., 2005). Thus, stress reactivity status could act as a useful screening tool to identify those most likely to benefit from standard cessation programmes and those for whom more rigorous approaches will be required. It is also important to note that blunted cortisol and cardiovascular stress reactivity would not only appear to be associated with smoking addiction, but also with alcohol dependence and the risk of alcohol dependence (Brenner & Beauchaine, 2011; Lovallo, 2006; Lovallo, 2007). Indeed, we have recently reported that blunted cortisol and cardiovascular stress reactions are characteristic of young people who appear to be addicted to exercise (Heaney, Ginty, Phillips, & Carroll, 2011), as well as typical of individuals with bulimia (Ginty, Phillips, Higgs, Heaney, & Carroll, 2012). Further, pathological gamblers have been shown to exhibit dampened cortisol stress reactivity relative to recreational gamblers (Paris, Franco, Sodano, Frye, & Wulfert, 2009). Finally, blunted stress reactions have also been reported to be characteristic of those who repeatedly engage in self-harm (Kaess et al., 2012).

Areas within the greater amygdala system that converge at the striatum and ventromedial prefrontal cortex are not only implicated in the regulation of the stress response but also shape our feelings and the motivation of our behaviour (Lovallo, 2005) . There is at least preliminary evidence from imaging studies that areas within this system exhibit blunted activation to varying stimuli in those at high risk of alcoholism (Glahn, Lovallo, & Fox, 2007). There is also evidence from animal research of extensive alteration of neurochemical communication among these areas when experimental animals are exposed to increasing

amounts of self-administered drugs of abuse (Koob, 2003). Additionally, there is also some evidence that individuals who show blunted cardiovascular reactions to an acute psychological stress task show blunted neural reactions in the greater amydala to the same stress task (Gianaros, May, Siegle, & Jennings, 2005; Gianaros, et al., 2008; Ginty et al., 2013).

Aside from its cross-sectional nature, the present study has other limitations. First, it was not possible to derive performance scores for all of the stress tasks used in this study. Nevertheless, we do have a measure of commitment to the task and have included this as a covariate in the fully adjusted analyses. Second, this is a unique population and it has been suggested that early life adversity may predispose individuals to life-long vulnerability to stress. However, a previous study using this population showed that individuals who experienced prenatal exposure to the Dutch Famine did not differ in cortisol stress reactivity from those who did not (de Rooij, Painter, Phillips, Osmond, Tanck, Bossuyt, & Roseboom, 2006). Third, our main measure of smoking behaviour was unsophisticated. For example, it has been argued that in terms of the total exposure to the toxic smoke components of tobacco, the way in which cigarettes are smoked may be as important as whether people smoke (Jarvis and Russell, 1980). In the present study no account was taken for the extent of inhalation. However, subjective measures of inhalation have proved unsatisfactory (Stepney, 1982) and most previous studies, including those on smoking and stress reactivity, have relied on simple categorizations of the sort used here. Although smoking is sometimes underestimated in self-reports, particularly among ex-smokers (Lewis et al, 2003), there is evidence that smokers' reports can be reasonably reliable and agree with objective measures such as carbon monoxide exhalation (Mak et al., 2005).

In conclusion, compared to non-smokers and ex-smokers, current smokers exhibited blunted cortisol and cardiovascular reactions to acute psychological stress. Reduced β-adrenergic activation would appear to underlie the diminished cardiovascular stress reactions that characterize smokers. There was no evidence that smoking status was associated with differences in vagal activation during stress exposure. Among smokers and ex-smokers, there was no evidence that lifetime exposure was associated with physiological reactions to acute stress, nor evidence that current levels of cigarette consumption was associated with reactivity. It is possible, then, that blunted stress reactivity may be a marker for an increased susceptibility to take up and/or maintain smoking behaviour once initiated.

References

Al' absi, M., 2006. Hypothalamic–pituitary–adrenocortical responses to psychological stress and risk for smoking relapse. Int. J. Psychophysiol. 59, 218–227.

Al' absi, M., Hatsukami, D., Davis, G.L., 2005. Attenuated adrenocorticotropic responses to psychological stress are associated with early smoking relapse. Psychopharmacology (Berl.) 181, 107–117.

Al' absi, M., Wittmers, L.E., Erickson, J., Hatsukami, D., Crouse, B., 2003. Attenuated adrenocortical and blood pressure responses to psychological stress in ad libitum and abstinent smokers. Pharmacol. Biochem. Behav. 74, 401–410.

Alex, C., Lindren, M., Shapiro, P.A., McKinley, P.S., Brondolo, E.N., Myers, M.M., Zhao, Y., Sloan, R.P., 2013. Aerobic exercise and strength training effects on cardiovascular sympathetic function in healthy adults: a randomized control trial. Psychosom. Med., 75, 375-381.

Ashare, R.L., Sinha, R., Lampert, R., Weinberger, A.H., Anderson, G.M., Lavery, M.E., Yanagisawa, K., McKee, S.A., 2012. Blunted vagal reactivity predicts stress-precipitated tobacco smoking. Psychopharmacology (Berl)., 220, 259-268.

Back, S.E., Waldrop, A.E., Saladin, M.E., Yeatts, S.D., Simpson, A., McRae, A.L., Upadhyaya, H.P., Contini Sisson, R., Spratt, E.G., Allen, J., Kreek, M.J., Brady, K.T. 2008. Effects of gender and cigarette smoking on reactivity to psychological and pharmacological stress provocation. Psychoneuroendocrinology, 33, 560-568.

Bakker, B., Sieben, I., 1997. Maten voor prestige, social-economische status en sociale klasse voor de standard beroepenclassificatie. Sociale Wetenschappen 40, 1-22.

Benowitz, N.L, Porchet, H., Sheiner, L., Jacob, P. 1988. Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum. Clinical Pharmacology and Therapy, 44, 23-28.

Bosch, J.A., de Geus, E.J., Carroll, D., Goedhart, A.D., Anane, L.A., van Zanten, J.J., Helmerhorst, E.J., Edwards, K.M., 2009. A general enhancement of autonomic cortisol response during social evaluative threat. Psychosom. Med. 71, 877-885.

Brindle, R.C., Ginty, A.T., Phillips, A.C., & Carroll, D. in press. A tale of two mechanisms: A meta-analysis of the autonomic basis of cardiovascular reactivity to acute psychological stress. Psychophysiology

Brenner, S.L., Beauchaine, T.P. , 2011. Pre-ejection period reactivity and psychiatric comorbidity prospectively predict substance use initiation among middle-schoolers: A pilot study. Psychophysiology 48, 1588-1596.

Christenfeld, N.J., Sloan, R.P., Carroll, D., Greenland, S., 2004. Risk factors, confounding, and the illusion of statistical control. Psychosom. Med. 66, 868–875.

Cacioppo, J.T., 1994. Social neuroscience: autonomic, neuroendocrine, and immune responses to stress. Psychophysiology 31, 113-128.

Childs, E., de Wit, H., 2009. Hormonal, cardiovascular, and subjective responses to acute stress in smokers. Psychopharmacology (Berl.) 1, 1-12.

Davis, M.C., Matthews, K.A., 1990. Cigarette smoking and oral contraceptive use influence women's lipid, lipoprotein, and cardiovascular responses during stress. Health Psychol. 9, 717–736.

Evans, B.E., Greaves-Lord, K., Euser, A.S., Tulen, J.H., Franken, I.H., Franken, I.H., Huizink, A.C., 2012. Alcohol and tobacco use and heart rate reactivity to a psychosocial stressor in an adolescent population. Drug Alcohol Depend. 126, 296-303.

Gianaros, P.J., May, J.C., Siegle, G.J., & Jennings, J.R., 2005. Is there a functional neural correlate of individual differences in cardiovascular reactivity? Psychosom. Med. 67, 31-39.

Gianaros, P.J., Sheu, L.K., Matthews, K.A., Jennings, J.R., Manuck, S.B., Hariri, A.R., 2008. Individual differences in stressor-evoked blood pressure reactivity vary with activation, volume, and functional connectivity of the amygdale. J. Neurosci. 28, 990-999.

Ginty, A.T., Gianaros, P.J., Derbyshire, S.W., Phillips, A.C., & Carroll, D., 2013. Blunted cardiac stress reactivity relates to neural hypoactivation. Psychophysiolgy 50, 219-229.

Ginty, A.T., Phillips, A.C., Higgs, S., Heaney, J.L.J., Carroll, D., 2012. Disordered eating behaviour is associated with blunted cortisol and cardiovascular reactions to acute psychological stress. Psychoneuroendocrinology 37, 715-724.

Girdler, S.S., Jamner, L.D., Jarvik, M., Soles, J.R., Shapiro, D., 1997. Smoking status and nicotine administration differentially modify hemodynamic stress reactivity in men and women. Psychosom. Med. 59, 294–306.

Glahn, D.C., Lovallo, W.R., Fox, P.T., 2007. Reduced amygdale activation in young adults at high risk of alcoholism: studies from the Oklahoma family health patterns project. Biol. Psychiatry 11, 1306-1309.

Grassi, G., Seravalle, G., Calhoun, D.A., Bolla, G.B., Giannattasio, C., Marabini, M., Del Bo, A., Mancia, G. 1994. Mechanisms responsible for sympathetic activation by cigarette smoking in humans. Circulation, 90, 248-253.

Heaney, J.L.J., Ginty, A.T., Carroll, D., Phillips, A.C., 2011. Preliminary evidence that exercise dependence is associated with blunted cardiac and cortisol reactiosn to acute psychological stress. Int. J. Psychophysiol. 79, 323-329.

Jarvis, M.J., Russell, M.A., 1980. Comment on the hunter committee's second report. Br.Med. J. 280, 994–995.

Kaess, M., Hille, M., Parzer, P., Maser-Gluth, C., Resch, F., & Brunner, R. 2012. Alterations in the neuroendocrinological stress response to acute psychosocial stress in adolescents engaging in nonsuicidal self-injury. Psychoneuroendocrinology 37, 157-161.

Kirschbaum, C., Scherer, G., Strasburger, C.J., 1994. Pituitary and adrenal hormoneresponses to pharmacological, physical, and psychological stimulation in habitual smokers and nonsmokers. Clin. Investig. 72, 804–810.

Kirschbaum, C., Strasburger, C.J., Langkrar, J., 1993. Attenuated cortisol response to psychological stress but not to CRH or ergometry in young habitual smokers. Pharmacol. Biochem. Behav. 44, 527–531.

Kirschbaum, C., Wust, S., Strasburger, C.J., 1992. 'Normal' cigarette smoking increases free cortisol in habitual smokers. Life Sci. 50, 435-442.

Koob, G.F., 2003. Neuroadaptive mechanisms of addiction: studies on the extended amygdala. Eur. Neuropsychopharmacol. 13, 442-452.

Koob, G.F., Kreek, M.J. 2007. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. Am. J. Psychiatry 164, 1149-1159.

Laustiola, K.E., Kotamaki, M., Lassila, R., Kallioniemi, O.P., Manninen, V., 1991. Cigarette smoking alters sympathoadrenal regulation by decreasing the density of beta 2-adrenoceptors. A study of monitored smoking cessation. J. Cardiovasc. Pharmacol. 17, 923–928.

Laustiola, K.E., Lassila, R., Kaprio, J., Koskenvuo, M., 1988. Decreased beta-adrenergic receptor density and catecholamine response in male cigarette smokers. A study of monozygotic twin pairs discordant for smoking. Circulation 78, 1234–1240.

Lewis, S.J., Cherry, N.M., Mc, L.N.R., Barber, P.V., Wilde, K., Povey, A.C., 2003. Cotinine levels and self-reported smoking status in patients attending a bronchoscopy clinic. Biomarkers 8, 218–228.

Lovallo, W.R., 2005. Cardiovascular reactivity: Mechanisms and pathways to cardiovascular disease. Int. J. Psychophysiol. 58, 119-132.

Lovallo, W.R., 2006. Cortisol secretion patters in addiction and addiction risk. Int. J. Psychophysiol. 59, 195-202.

Lovallo, W.R., 2007. Individual differences in response to stress and risk for addiction. In: al'Absi, M. (Ed.), Stress and Addiction: Biological and Psychological Mechanisms. 1st ed. Academic Press, Burlington, MA, pp. 227–248.

Lovallo, W.R., 2011. Do low levels of stress reactivtiy signal poor states of health? Biol. Psychol. 86, 121-8.

Lovallo, W.R., Dickensheets, S.L., Myers, D.A., Thomas, T.L., & Nixon, S.J. 2000. Blunted stress cortisol responses in abstinent alcoholic and polysubstance-abusing men. Alcohol Clin. Exp. Res. 24, 651-658.

Lovallo, W.R., Pincomb, G.A., Brackett, D.J., Wilson, M.F., 1990. Heart rate reactivity as a predictor of neuroendocrine responses to aversive and appetitive challenges. Psychosom. Med. 52, 17-26.

Lucini, D., Bertocchi, F., Malliani, A., Pagani, M., 1996. A controlled study of the autonomic changes produced by habitual cigarette smoking in healthy subjects. Cardiovasc. Res. 31, 633-639.

Macdougall, J.M., Musante, L., Castillo, S., Acevedo, M.C., 1988. Smoking, caffeine, and stress: effects on blood pressure and heart rate in male and female college students. Health Psychol. 7, 461–478.

Mak, Y.W., Loke, A.Y., Lam, T.H., Abdullah, A.S., 2005. Validity of self-reports and reliability of spousal proxy reports on the smoking behavior of Chinese parents with young children. Addict. Behav. 30, 841–845.

Painter, R.C., Roseboom, T.J., Bossuyt, P.M., Osmond, C., Barker, D.J., Bleker, O.P., 2005. Adult mortality at age 57 after prenatal exposure to the Dutch famine. Eur. J. Epidemiol. 20, 673-676.

Pagani, M., Lombardi, F., Guzzetti, S., Rimoldi, O., Furlan, R., Pizzinelli, P., Sandrone, G., Malfatto, G., Dell'Orto, S., & Piccaluga, E. 1986. Power spectral analysis of heart rate and arterial pressure variability as a marker for sympatho-vagal interaction in man and conscious dog. Circ. Res. 59, 178-193.

Panknin, T.L., Dickensheets, S.L., Nixon, S.J., Lovallo, W.R. 2002. Attenuated heart rate responses to public speaking in individuals with alcohol dependence. Alcohol Clin. Exp. Res. 26, 841-847.

Paris, J.J., Franco, C., Sodano, R., Frye, C.A., Wulfert, E., 2010. Gambling pathology is associated with dampened cortisol response among men and women. Physiol. Behav. 99, 230-233.

Perkins, K.A., Grobe, J.E., Fonte, C., Breus, M., 1992. "Paradoxical" effects of smoking on subjective stress versus cardiovascular arousal in males and females. Pharmacol. Biochem. Behav. 42, 301–311.

Phillips, A.C., Der, G., Hunt, K., Carroll, D., 2009. Haemodynamic reactions to acute psychological stress and smoking status in a large community sample. Int. J. Psychophysiol. 73, 273-278.

Pomerleau, O.F., Fertig, J.B., Seyler, L.E., Jaffe, J., 1983. Neuroendocrine reactivity to nicotine in smokers. Psychopharmacology (Berl.) 81, 61–67.

Ravelli, A.C., van der Meulen, J.H., Michels, R.P., Osmond, C., Barker, D.J., Hales, C.N., et al., 1998. Glucose tolerance in adults after prenatal exposure to famine. Lancet 351, 173-177.

Ray, R.L., Nellis, M.J., Brady, J.V., Foltin, R.W., 1986. Nicotine and caffeine effects on the task-elicited blood pressure response. Addict. Behav. 11, 31–36.

de Rooij, S.R., Painter, R.C., Phillips, D.I., Osmond, C., Tanck, M.W., Bossuyt, P.M., et al., 2006. Cortisol responses to psychological stress in adults after prenatal exposure to the Dutch famine. Psychoneuroendocrinology 31, 1257-1265.

Schachter, S., Silverstein, B., Kozlowski, L.T., Perlick, D., Herman, C.P., Liebling, B., 1977. Studies of the interaction of psychological and pharmacological determinants of smoking. J. Exp. Psychol. Gen. 106, 3-12.

Schlotz, W., Kumsta, R, Layes, I., Entringer, S., Jones, A., Wust, S., 2008. Covariance between psychological and endocrine responses to pharmacological challenge and psychosocial stress: a question of timing. Psychosom. Med. 2008, 787-796.

Sheffield, D., Smith, G.D., Carroll, D., Shipley, M.J., Marmot, M.G., 1997. The effects of recent food, alcohol, and tobacco intake and the temporal scheduling of testing on cardiovascular activity at rest and during psychological stress. Psychophysiology 34, 204–212.

Stepney, R., 1982. Are smokers' self-reports of inhalation a useful measure of smoke exposure. J. Epidemiol. Community Health 36, 109–112.

Swan, G.E., Ward, M.M., Jack, L.M., Javitz, H.S., 1993. Cardiovascular reactivity as a predictor of relapse in male and female smokers. Health Psychol. 12, 451-458.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology., 1996. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. Eur. Heart. J., 17, 354-381.

Tsuda, A., Steptoe, A., West, R., Fieldman, G., & Kirschbaum, C. 1996. Cigarette smoking and psychophysiological stress responsiveness: effects of recent smoking and temporary abstinence. Psychopharmacology, 126, 226-233.

Rohleder, N., Kirschbaum, C., 2006. The hypothalamic–pituitary–adrenal (HPA) axis in habitual smokers. Int. J. Psychophysiol. 59, 236–243.

Roy, M.P., Steptoe, A., Kirschbaum, C., 1994. Association between smoking status and cardiovascular and cortisol stress responsivity in healthy young men. Int. J. Behav. Med. 1, 264–283.

Straneva, P., Hinderliter, A., Wells, E., Lenahan, H., Girdler, S., 2000. Smoking, oral contraceptives, and cardiovascular reactivity to stress. Obstet. Gynecol. 95, 78–83.

Tersman, Z., Collins, A., Eneroth, P., 1991. Cardiovascular responses to psychological and physiological stressors during the menstrual cycle. Psychosom. Med. 53, 185–197.

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Conflict of interest

The authors have no conflicts of interest.

Contributions

Annie T. Ginty helped conceive the study, conducted the primary analysis, and co-produced the initial and revised draft.

Alexander Jones processed the autonomic data, assisted with the statistical analysis, and contributed to the final version of the paper.

Douglas Carroll helped conceive the study and co-produced the initial draft of the paper.

Tessa J. Roseboom set-up the Dutch Famine Birth Cohort Study, made the data available to the first author, and contributed to the final version of the paper.

Anna C. Phillips helped conceive the study and contributed to the final version of the paper.

Rebecca Painter contributed to data collection, the analysis and the final version of the paper.

Susanne R. de Rooij helped conceive the study, contributed to data collection and the analysis, and contributed to the final version of the paper.

Table 1. Age, sex, SES based on ISEI-92 score, BMI, weekly alcohol consumption, depression, and anti-hypertensive medication by smoking status.

	Current	**Ex-**	**Never**	**Overall**
Number of participants	102	203	174	479
Mean Age (SD) years	58.4 (0.91)	58.4 (0.88)	58.3 (0.94)	58.3 (0.91)
Sex				
Male n (%)	47 (46%)	103 (55%)	75 (43%)	233
Female n (%)	55 (54%)	92 (45%)	95 (57%)	246
Mean SES (SD) ISEI-92	46.1 (13.49)	49.6 (13.33)	52.4 (13.77)	49.8 (13.70)
Mean BMI (SD) kg/m^2	27.7 (5.33)	29.1 (4.64)	28.3 (3.97)	28.5 (4.59)
Alcohol (SD) units per week	12.4 (18.9)	10.8 (11.48)	7.5 (13.2)	10.0 (14.06)
Depression				
Depressed n (%)	16 (16%)	15 (8%)	7 (4%)	38
Non-depressed n (%)	84 (84%)	182 (92%)	161 (96%)	427
Anti-hypertensive medication n (%)				
Medication (%)	16 (16%)	57 (28%)	36 (21%)	109
No medication (%)	86 (84%)	146 (72%)	138 (79%)	370

Table 2. Mean (SD) Cortisol, SBP, DBP, HR, LF BPV, HF HRV, RMSSD during baseline and in the response to the stress tasks by smoking status.

	Current	Ex-	Never	Overall
SBP (mmHg)				
Baseline	118.4 (21.50)	129.3 (19.16)	132.3 (20.13)	128.1 (20.66)
Task average	146.3 (26.71)	162.8 (21.50)	167.7 (24.69)	161.0 (25.11)
DBP (mmHg)				
Baseline	74.6 (10.91)	73.3 (9.73)	74.4 (11.18)	74.0 (10.52)
Task average	74.5 (12.42)	82.2 (11.76)	86.1 (12.87)	82.0 (13.00)
HR (bpm)				
Baseline	60.6 (12.26)	66.9 (10.64)	70.1 (11.19)	66.8 (11.71)
Task average	79.0 (11.82)	80.8 (12.27)	82.1 (12.60)	80.9 (12.33)
LF BPV ($mmHg^2$)				
Baseline	14.1 (9.55)	15.1 (12.06)	16.4 (13.31)	15.4 (12.07)
Task average	18.4 (11.82)	24.9 (16.04)	26.8 (18.23)	24.2 (16.38)
HF HRV (ms^2)				
Baseline	222.4 (396.32)	205.4 (570.78)	177.4 (253.21)	198.8 (440.88)
Task average	197.0 (354.92)	152.6 (236.74)	139.3 (180.45)	157.2 (250.11)
RMSSD (ms)				
Baseline	23.1 (12.15)	21.5 (11.93)	21.1 (10.86)	21.7 (11.60)
Task average	21.6 (12.14)	19.0 (9.69)	18.7 (9.03)	19.4 (10.08)
Cortisol (nmol/L)				
Baseline	4.5 (2.68)	4.3 (2.50)	4.5 (3.74)	4.44 (3.05)
Task average	4.7 (2.91)	6.1 (4.02)	5.7 (3.39)	5.6 (3.59)

Figure captions

Figure 1. Schematic representation of the acute psychological stress protocol.

Figure 2. Mean (SE) salivary cortisol concentrations across the laboratory protocol by smoking status.

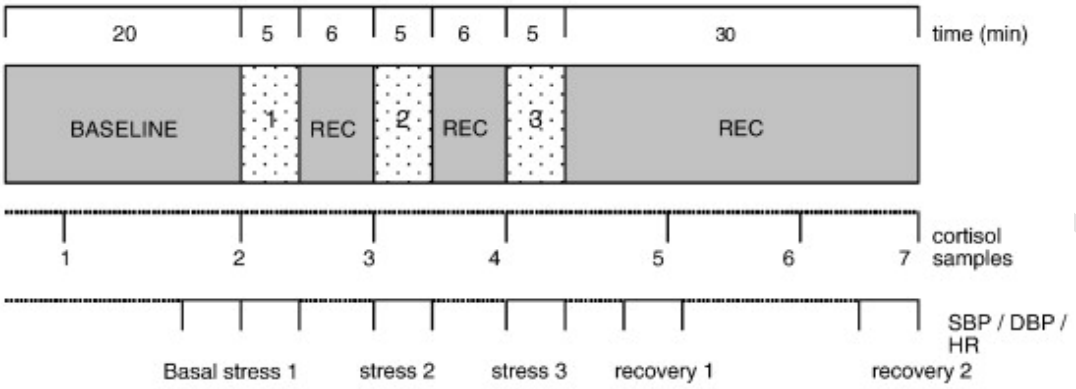
Figure 3. Unadjusted mean (SE) salivary cortisol reactivity by smoking status. a = significantly different from ex-smokers, b = significantly different than never smokers, * = $p < .001$

Figure 4a. Unadjusted mean (SE) systolic blood pressure (SBP) reactivity by smoking status. a = significantly different from ex-smokers, b = significantly different than never smokers, * = $p < .005$

Figure 4b. Unadjusted mean (SE) diastolic blood pressure (DBP) reactivity by smoking status. a = significantly different from never smokers, * = $p < .05$

Figure 4c. Unadjusted mean (SE) low frequency blood pressure variability (LF BPV) reactivity by smoking status. a = significantly different from ex-smokers, b = significantly different than never smokers, * = $p < .005$

Figure 4d. Unadjusted mean (SE) heart rate (HR) reactivity by smoking status. a = significantly different from ex-current, b = significantly different than never smokers, * = $p < .005$



Stress 1 = Stroop test
Stress 2 = Mirror drawing test
Stress 3 = Speech test
REC = Recovery period