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Impact of early life adversity on the stress biobehavioral response during nicotine withdrawal

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Abstract

Exposure to early life adversity (ELA) is associated with increased subsequent risk for addiction and relapse. We examined changes in psychobiological responses to stress in dependent smokers and nonsmoking controls and evaluated how history of early adversity may exacerbate acute changes during nicotine withdrawal and acute stress. Smokers were randomly assigned to one of two conditions; 24 h withdrawal (66 smokers) from smoking and all nicotine-containing products or smoking ad libitum (46 smokers) prior to an acute laboratory stress induction session; and 44 nonsmokers provided normal referencing. The laboratory session included a baseline rest, stress and recovery periods. Plasma and saliva samples for the measurement stress hormones and cardiovascular and self-report mood measures were collected multiple times during the session. Multivariate analysis confirmed that all groups showed stress-related increases in negative mood, cardiovascular measures and stress hormones, particularly smokers in the withdrawal condition. Individuals with high ELA showed greater adrenocorticotrophic hormone (ACTH), but lower plasma and salivary cortisol levels, than those with low ELA. Cortisol differences were abolished during tobacco withdrawal. These findings demonstrate that ELA moderates the effects of withdrawal on stress-related biobehavioral changes.

Keywords

Life adversity; Stress; HPA; Addiction; Tobacco

1. Introduction

Accumulating evidence demonstrates an association between exposure to early life adversity (ELA), including emotional, physical and sexual abuse, with subsequent risk for addiction (Harrison, Fulkerson et al. 1997; Bensley, Spieker et al. 1999; Widom, Weiler et al. 1999; Lovallo, 2013). ELA also predicts acceleration and maintenance of tobacco, marijuana and alcohol use (Kaplan and Johnson, 1992; Wills, Vaccaro et al. 1996; Harrison, Fulkerson et al. 1997; Anda, Croft et al. 1999; Edwards, Anda et al. 2007; Ford, Anda et al. 2011; Lovallo, 2013). ELA likely moderates the link between multiple biological and social

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Conflict of interest

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processes with risk for and maintenance of addiction (al'Absi, 2007; Sinha, 2008). We report here an attempt to replicate previous demonstrations of withdrawal-associated HPA and SNS blunting response to stress and extend this research by examining the influence of ELA on nicotine withdrawal and acute psychobiological responses to stress. Our goal was to do this in a study design that allows for the examination of ELA and sex as moderators of the stress response, withdrawal symptoms, and craving associations, as well as the role of withdrawal as an amplifier of the stress response.

Exposure to ELA has been shown to lead to an enhanced reactivity to environmental and psychosocial stressors that contribute to chronic or frequent experiences of negative affective states (Morris, Rao et al. 2014; Suzuki, Poon et al. 2014). This exaggerated and ongoing reactivity may, in the long-term, lead to disruptions of the HPA axis activity. Animals reared under various stressful conditions exhibit significant alterations in the HPA stress response throughout development and adult life (Plotsky and Meaney, 1993; Coplan, Andrews et al. 1996; Arborelius, Owens et al. 1999). These conditions have been associated with chronic abnormalities of the stress response systems including the HPA axis (Klaassens, Giltay et al. 2010; McCrory, De Brito et al. 2010; Carpenter, Shattuck et al. 2011; Gonzalez, 2013) and sympathetic nervous system (Winzeler, Voellmin et al. 2016). For example, high ELA exposure leads exaggerated cortisol and sympathetic responses to acute, experimentally induced socio-evaluative stress consisting of standardized public speaking and mental arithmetic challenges in front of an audience (Kuras, McInnis et al. 2017; Mielock, Morris et al. 2017). This sympathetic and HPA dysregulation, in turn, has been related to increased vulnerability to drug use and enhanced drug reinforcement (De Bellis, 2002; Picetti, Schlussman et al. 2013) as well as risk for psychopathology (Coplan, Andrews et al. 1996; Novak, Fan et al. 2013). This work is notable and important as it includes direct manipulation of the stress state of the individual rather than relying on cross-sectional, self-report study of stressful life circumstances. It also suggests that ELA-associated sympathetic and HPA dysregulation may account for past findings of increased risk of addictive behaviors in individuals with heightened ELA.

Chronic exposure to substances of abuse produces an acute stress response profile that is similar to chronic stress (Lovallo, Dickensheets et al. 2000; Sorocco, Lovallo et al. 2006; al'Absi, Lemieux et al. 2017; Cuttler, Spradlin et al. 2017). For example, we have shown that chronic exposure to tobacco has been associated with blunting in multiple stress-response systems (al'Absi et al., 2002, 2003). This blunting is especially pronounced after a period of acute withdrawal (deprivation) from tobacco versus periods of regular (ad libitum) smoking (al'Absi et al., 2002). These changes in the stress response has been associated with enhanced perception of withdrawal related symptoms, distress and pain, and this has, in turn, been shown to contribute to early relapse (Kreek and Koob, 1998, Piazza and Le Moal, 1998, al'Absi et al., 2004, al'Absi et al., 2013).

Beyond the manipulation of withdrawal, examining alterations in the stress response by chronic tobacco use greatly benefits from comparing smokers with nonsmokers. Earlier studies have indicated certain disruptions of the stress response in smokers as shown by smaller salivary cortisol response to acute stressors (Kirschbaum, Strasburger et al. 1993; Roy, Steptoe et al. 1994; Tsuda, Steptoe et al. 1996), but this was not consistently observed

across studies (Tersman, Collins et al. 1991; Baron, Comi et al. 1995). Later research applying more careful selection of participants, accounting for comorbidity and controlling for acute tobacco use, has confirmed a blunted stress response in smokers (al'Absi et al., 2003; Rohleder and Kirschbaum, 2006). The ability to apply terms such as “blunted” and “disrupted” could not be possible without this nonsmoker reference group.

The literature examining the moderating effect of ELA on various addictive processes is small but growing. Acute socio-evaluative stress has resulted in blunted sympathetic activity in those who abuse alcohol, though in this context there was no effect of ELA (Muehlhan, Hocker et al. 2017). Acute stress in a controlled setting (socio-evaluative stress) increases craving for prescription opioids (Back, Gros et al. 2015) and tobacco (al'Absi et al., 2017). We have found in an earlier study with smokers interested in cessation that salivary cortisol negatively predicted craving in those with low ELA but not those with high ELA and a high level of ACTH stress response was associated with relapse in high ELA smokers (al'Absi et al., 2017). Though seemingly contradictory, it is important to distinguish the drug of abuse studied (alcohol versus nicotine) and the outcome (SNS versus HPA). Until both SNS and HPA factors can be evaluated within the same sample, determining whether the current study of nicotine dependence contradicts or complements the work of Muehlhan et al. with alcohol dependence (2017) remains unclear.

We attempted to replicate previous demonstrations of withdrawal-associated HPA and SNS blunting and extend this research by examining the influence of ELA and nicotine withdrawal on acute psychobiological responses to stress. Using an independent sample, participants (dependent smokers and nonsmokers) attended a laboratory session during which they were exposed to acute stressful challenges. In addition to assessing HPA axis, as was done in a previous study related to smoking relapse (al'Absi et al., 2017), we expand the evaluations of stress to include sympathetic reactivity and pain responding. To address the causal nature of changes under withdrawal, smokers were randomly assigned to one of two conditions; 24 h withdrawal from smoking and all nicotine-containing products or smoking ad libitum prior to the lab session. Based on earlier research (cf., (Lavallo, 2013)), we predicted that exposure to ELA would be associated with blunted biological response to stress and enhanced distress and withdrawal symptoms, and that these differences would be particularly pronounced in smokers who were going through withdrawal relative to ad libitum smokers and nonsmokers with similar ELA. Finally, sex is an important moderator of relapse and withdrawal. Low HPA reactivity and high nicotine craving predicts relapse in men but high HPA reactivity predicts relapse in women (al'Absi et al., 2015). Further, relapsing women report a higher adversity than abstinent women (Lemieux, Olson et al. 2016). Thus, sex may also be an important moderator of both stress reactivity and smoking related outcomes in the context of ELA. We predicted that ELA would interact with both smoking condition and sex such that the blunting of the HPA would occur in men with high ELA, but not women, assigned to the withdrawal condition.

2. Methods

2.1. Participants

Participants were recruited from community flyers and advertisements in both print and online. Interested participants called the laboratory and were screened using initial criteria. Participants who were within $\pm 30\%$ of ideal weight as indicated by Metropolitan Life Insurance norms, had a regular sleep cycle and a low risk drinking style (≤ 2 drinks/day) were invited to an on-site medical screening for further eligibility assessment and consenting. Smokers (smoke at least 5 cigarettes per day for the past 2 years) and nonsmokers were included in the study. Nonsmokers had not smoked for the last 5 years and had smoked less than 100 cigarettes in their lifetime. Participants were excluded if they had current or recent history of: (1) chronic medical conditions (e.g., cardiac disease, hypertension, renal or hepatic disease); (2) major psychiatric disorder (e.g., psychotic or bipolar disorders, depression, anxiety disorders, alcohol and drug abuse), or (3) opiate dependence. Females who were currently pregnant were excluded from the study as well. Eligible participants read and signed the consent form approved by the Institutional Review Board of the University of Minnesota. After that, participants were asked to complete demographic, smoking history and nicotine dependence measures. Smokers were randomized to either a withdrawal condition (forced withdrawal for 24 h) or ad libitum condition (smoking as usual). Randomization of smoking conditions took place at the end of this session. Participants were provided with instructions and scheduled for subsequent laboratory sessions at rate of 3 (withdrawal) to 2 (ad libitum). The different ratio was used to maximize the representation of the withdrawal condition, which has previously shown to have a higher attrition rate (al'Absi et al., 2008). Data from 46 smokers (18 females) randomized into an ad libitum smoking condition, 66 smokers (26 females) randomized into a withdrawal condition and 44 nonsmokers (23 females) were included in this study.

2.2. Procedure

Before each laboratory session, participants were instructed to abstain from any medications for 72 h and alcohol for 24 h prior to the session. Smokers assigned to withdrawal condition were required to refrain from any tobacco product for at least 24 h before the lab session. Abstinence was objectively verified through expired carbon monoxide (CO) at the start of the session. Those failing to meet abstinence verification (CO ≥ 8 ppm) were re-scheduled for testing. Participants were tested individually. At the beginning of each session the participant was greeted, brought to a testing room and provided with a standard lunch. The results reported here were obtained from a placebo condition of a double blind pharmacological opioid blockade study.

All laboratory sessions started early afternoon to control for diurnal changes in hormones. Following CO testing and lunch, the laboratory session began with catheterization of the non-dominant antecubital vein. The first period within the lab was a baseline rest period (20 min) during which they watched Planet Earth. Following the baseline rest period, each participant ingested a capsule containing either drug or placebo. The participants and research staff were blinded to the order of drug/placebo administration. This was followed by two 20 min resting periods (40 min total). After the second resting period, participants in

the ad libitum condition were asked to smoke one cigarette of their preferred brand. For all participants the stress tasks were completed after the baseline and second rest periods and included public speaking, mental arithmetic and the cold pressor test (CPT) in fixed order (a total of approximately 30 min). The order of stressors was fixed to maximize their effects on psychobiological responses. In the speech task participants were given a topic, 4 min for preparation and 4 min for delivery. In the math task, participants were given a three-digit number and asked to sum the three digits of that number and then add the sum to the original three-digit number. The CPT was administered using a 1-gallon container filled with an ice-water slurry. Participants were asked to immerse their hand into the container up to the wrist and rate pain every 15 s during the 90-second exposure and 90-second during recovery. The McGill Pain Questionnaire (MPQ; (Melzack, 1987)) was administered immediately after CPT. After all stress procedures the participants rested for a total of 80 min. Plasma and saliva samples as well as self-report measures were collected 7 times during the session: after the initial baseline, 40-minute rest, 30-minute rest, stress, 30 min post-stress 1, 30 min post-stress 2, and 20 min post-stress 3. This schedule allowed for three extended baseline measures and 3 post-stress recovery measures. Heart rate (HR) and blood pressure (SBP: systolic and DBP: diastolic) were collected every 5 min during pre-stress baseline, during acute stress tasks and post-stress recovery periods. As seen in the depiction of the protocol below (see Fig. 1), the cardiovascular measures coincided with the final post-stress recovery period. We did not collect saliva and plasma every 5 min to create an exact time match for the cardiovascular measures, because such a frequent sampling was not needed to capture changes in the targeted hormonal measures.

2.3. Measures

2.3.1. Biological, physiological and biochemical measures—Blood samples were collected via intravenous catheter and plasma samples were stored in -80°C freezers until assays. Plasma cortisol and ACTH assays were conducted at a local hospital lab (Fairview Hospital, Minneapolis, MN) using chemiluminescent immunoassays using the ADVIA Centaur[®] cortisol kit and the Immulite[®] 2000 ACTH kit (Siemens Medical Solutions USA, Malvern, PA). Salivette[®] tubes (Sarstedt, Numbrecht, Germany) were used throughout the lab to collect ~1 ml of saliva. Salivary cortisol was assayed using time-resolved fluorescence immunoassay (cortisol-biotin conjugation). The assay kits (IBL International) had a sensitivity of 0.4 nmol/L and inter- and intra-assay coefficients of variation less than 10% and 12%, respectively. Cotinine was quantified using enzyme linked immunoassays (DRG Diagnostics; inter- and intra-assay variability < 12%). HR, SBP and DBP as indices of sympathetic activity were measured by a Dinamap oscillometric monitor (Critikon, Tampa, Florida). Expired carbon monoxide (CO) level was measured by a Bedfont Micro + monitor (coVita, Haddonfield, NJ).

2.3.2. Adverse life experiences—The Adverse Childhood Experiences (ACE) questionnaire (Felitti, Anda et al. 1998) was used to measure ELA. Items related to psychological abuse (n = 2), physical abuse (n = 2), sexual abuse (n = 4), household member substance abuse (n = 2), household member mental illness (n = 1), household member suicide attempt (n = 1), mother treated violently (n = 4) and household member incarceration (n = 1) were included. Participants were defined to be exposed to a category if

he/she reported “yes” to one or more individual items in a category. The sum score of seven categories were calculated with 0 being not exposed to any ELA and 8 being exposed to all categories. A dichotomized index was also created (Low: scores between 0–2; High: scores 3 and above) as it has been shown that individuals with 3+ ACEs have elevated risk of smoking (Felitti et al., 1998). Our instructions did not specify “prior to age 18”, but because many of the items refer to parents or stepparents in the household, it can be assumed that these scores were reflective of early life adversity, though adversities beyond age 18 (divorce, incarceration, household mental illness) cannot be ruled out.

2.3.3. Self-report mood and tobacco withdrawal symptoms—Subjective distress and positive affect were assessed by a modified version of Subjective States Questionnaire (SSQ; (Lundberg and Frankenhaeuser, 1980)). Distress consisted of items of anxiety, irritability, impatience and restlessness. Positive affect included items of cheerfulness, contentedness, calmness, controllability and interest. Withdrawal symptoms were assessed using Minnesota Nicotine Withdrawal Scale (Hughes and Hatsukami, 1986, 1998). The item craving was analyzed separately from the rest of items (Hughes and Hatsukami, 1986). Smoking urges were assessed by a shortened version of the Questionnaire on Smoking Urges (QSU-B; (Tiffany and Drobes, 1991; Cox, Tiffany et al. 2001)). The QSU-B measures two dimensions of smoking urges (Factor 1: smoking for rewarding effects, Factor 2: smoking to reduce aversive effects). MPQ (Melzack, 1987) was administered to assess the pain experience following the CPT.

During the on-site screening, participants were asked to complete forms on demographics (e.g., age, education) and the ELA questionnaire. Smokers were asked about their smoking history (e.g., cigarettes per day, duration of smoking, etc.) and dependence levels using the Fagerstrom Test of Nicotine Dependence (FTND; (Heatherton, Kozlowski et al. 1991)).

2.4. Data analysis

All dependent measures were transformed to meet normality assumption. To remain consistent with the existing stress and smoking relapse literature, we administered and tested the stress manipulation as one block. We did not attempt to parse out response to each manipulation with the exception of pain self-report, which arguably applied only to the cold pressor test only. Hormonal (ACTH and plasma and saliva cortisol) and subjective mood measures were analyzed using 3 smoking group (nonsmoking control, ad libitum condition smokers, withdrawal condition smokers) x 2 sex (female, male) x 7 time (baseline, rest 1, rest 2, stress, post-stress 1, post-stress 2, post-stress 3) multivariate analysis of variance (MANOVAs). Smoking withdrawal symptoms and urges were analyzed using above models but restricted to only the two smoking conditions (ad lib and withdrawal). Blood pressure (SBP, DBP) and HR variables were averaged in each period prior to analysis but were kept relatively consistent in overall time with the 7 time points used for the endocrine outcomes. These were analyzed using a series of 3 smoking group x 2 sex x 7 time (baseline, rest 1, rest 2, speech prep, speech delivery, math, post-stress recovery) MANOVAs. Analysis confirmed that such averaging faithfully captured the baseline and recovery periods (data not shown; see Results section for discussion of cardiovascular measures across stress manipulations). In all models ELA score was included as a continuous predictor. That is, all

possible interactions among independent variables and main effects of smoking, sex, ELA and time were tested. When there was a statistically significant main effect of time, multiple comparisons with Bonferroni correction was conducted. Since our focus was on the stress response, we compared with pre- to post-stress periods. When there was a significant interaction, post-hoc analyses were conducted with following conditions. If an interaction included time (e.g., smoking group by time effect), a change score representing stress response (difference between rest and stress period) was calculated and group differences were tested using ANOVA. When there was an interaction including groups (e.g., smoking group x ELA effect), ANOVA was conducted to test ELA effect in each smoking condition (Bonferroni corrected). To simplify the depiction of significant ELA interactions we split the ELA continuous measure by using a cut-off score of 3 to represent low (0–2) and high (3 and above) ELA. Demographic and smoking-related variables were analyzed by smoking group x sex ANOVAs for continuous variables and chi-square tests for categorical variables. SPSS v24 was used for the data analysis. Reported degrees of freedom slightly varied due to occasional missing data.

3. Results

3.1. Sample characteristics

Thirty smokers (31% of the total recruited pool of 96) in the smoking withdrawal condition, eight smokers in the ad lib smoking condition (15% of the total recruited pool of 54), and 14 non-smokers (24% of the total recruited pool of 58) did not complete the study. Major reasons for discontinuation included a failure of abstinence, illness, time constraints and no response after multiple contacts. There were no differences between those who completed or withdrew from the study on age, BMI, daily cigarette consumption, years of smoking, age of onset of smoking or FTND scores.

Demographic and smoking history variables of participants who completed the study are presented in Table 1. The mean age of the sample was 34.8 years ($SD=12.4$) and the majority of the participants were unmarried Caucasians. Smokers (ad libitum, withdrawal conditions) had lower years of education than nonsmokers ($F(2, 148) = 10.4, p < .001; \eta^2 = 0.12$). There were no smoking group or sex differences in racial distribution, age, or BMI. Average age of onset of smoking was 15.9 years ($SD = 4.6$). The smokers had smoked on average 14.8 ($SD = 5.7$) cigarettes per day for 11.0 years ($SD = 10.5$). As expected, smokers in the withdrawal condition had lower CO at this lab session than ad libitum smokers ($F(1, 108) = 106, p < .001; \eta^2 = 0.50$). Smokers in the withdrawal condition also had a significantly lower cotinine levels than ad libitum smokers ($F(1, 105) = 10.5, p = .002; \eta^2 = 0.09$) corresponding with the length of smoking abstinence (24 h, see Table 1).

3.2. Manipulation check of smoking withdrawal and acute stress

There was evidence that the withdrawal condition and stress manipulation aroused psychobiological outcomes, though the interaction with ELA was specific to some, but not all, outcomes. All participants showed increased ACTH in response to stress (time effect: ($F(6, 92) = 12.3, p < .001, \eta^2 = 0.45$; stress period was greater than prestress rest 1 and 2 and post-stress recovery: $p < .001$; see Fig. 2b) but the magnitude of response was more

pronounced in men than women (sex by time interaction: $F(6, 125) = 2.27, p = .04, \eta^2 = 0.10$). Both plasma cortisol ($F(6, 118) = 34.3, p < .001, \eta^2 = 0.64$) and salivary cortisol levels ($F(6, 125) = 21.1, p < .001, \eta^2 = 0.50$) increased in response to stress (stress period was greater than post-stress recovery: $p < .001$; Fig. 2b). Time effects found in cardiovascular measures indicated expected increases in response to stress (SBP: ($F(6, 131) = 37.5, p < .001, \eta^2 = 0.63$); DBP: ($F(6, 131) = 43.2, p < .001, \eta^2 = 0.66$; HR: ($F(6, 131) = 41.8, p < .001, \eta^2 = 0.66$; in all measures, stress periods were greater than pre- and post-stress rest periods: $p < .001$). Furthermore, HR responses to stress were smaller among smokers in the withdrawal condition relative to other two groups (smoking group by time interaction: $F(12, 262) = 2.09, p = .02, \eta^2 = 0.09$) (Table 2).

Many psychological and behavioral outcomes (mood, withdrawal, and pain) also changed in response to withdrawal and stress manipulations. Pain ratings increased over time during the CPT ($F(5, 134) = 65, p < .001, \eta^2 = 0.71$) and decreased across time post-CPT exposure ($F(5, 134) = 82.6, p < .001, \eta^2 = 0.76$). Smokers in the withdrawal condition showed a consistently low positive affect throughout the session while the nonsmoking group showed a stress-related decrease in positive affect (smoking group by time interaction ($F(12, 248) = 2.17, p = .01, \eta^2 = 0.10$). Distress increased in response to stress in all groups (time effect: $F(6, 129) = 6.66, p < .001, \eta^2 = 0.24$; stress period was greater than pre- and post-stress rest periods). Smokers in the withdrawal condition had greater levels of distress than other two groups (group effect: $F(2, 134) = 4.20, p = .02, \eta^2 = 0.06$). Withdrawal symptoms, craving, and smoking urges remained stable and high in smokers in the withdrawal condition, while these measures changed in response to stress in ad libitum smokers, as indicated by smoking condition x time interaction in MNWS ($F(6, 93) = 2.91, p = .01, \eta^2 = 0.16$), craving ($F(6, 94) = 3.20, p = .007, \eta^2 = 0.17$), and QSUB F1 ($F(6, 90) = 3.58, p = .003, \eta^2 = 0.19$).

3.3. Smoking status, ELA and the stress response

There was a positive association between ACTH and ELA ($F(1, 97) = 6.09, p = .02, \eta^2 = 0.06$; see Fig. 3a). A main effect of ELA revealed a negative association between ELA and both plasma and saliva cortisol ($F_s(1, 123) > 6.26, p = .01, \eta^2 = 0.05$; see Fig. 3a). To further examine the different pattern of ACTH and cortisol findings across groups, we conducted an additional analysis using ACTH to cortisol ratios. As shown in Fig. 3b, these analyses revealed positive associations between ELA and the ACTH to salivary cortisol ratio ($F(1, 92) = 16.5, p < .001, \eta^2 = 0.15$) and the ACTH to plasma cortisol ratio ($F(1, 97) = 8.49, p = .004, \eta^2 = 0.08$). The main effect of ELA in salivary cortisol was further qualified by a smoking group by ELA interaction ($F(2, 130) = 5.40, p = .006, \eta^2 = 0.08$). Follow-up analysis conducted in each smoking group (adjusted p-value = $0.5/3 = .017$) revealed a negative association between ELA and salivary cortisol among nonsmoking ($F(1, 38) = 7.96, p = .008, \eta^2 = 0.17$) and the ad libitum smoking condition ($F(1, 36) = 7.07, p = .012, \eta^2 = 0.16$), but not in the withdrawal condition ($p = .43$; see Fig. 4). Neither sex nor ELA was associated with distress. There were no main effect or interactions for ELA or sex on the tobacco withdrawal and smoking urge measures. Women with higher ELA scores had lower positive affect than women with low ELA scores whereas this difference was not found among men (sex by ELA interaction ($F(1, 129) = 4.44, p = .04, \eta^2 = 0.03$).

With respect to pain ratings during CPT, greater pain report was associated with greater ELA scores in nonsmokers and smokers in the withdrawal condition but this relationship was reversed in ad libitum smokers (smoking group by ELA by time interaction ($F(10, 268) = 2.03$, $p = .03$, $\eta^2 = 0.07$; see Fig. 5). The impact of ELA on pain report was particularly evident in women as indicated by an ELA by sex by time interaction ($F(5, 134) = 4.02$, $p = .002$, $\eta^2 = 0.13$). Unlike the males, as the cold pressor proceeded women with higher ELA had higher pain ratings than women with low ELA. In contrast, no main effect or interactions for pain ratings post-CPT were evident. Regarding MPQ, female smokers in the ad libitum condition had greater pain than those in the smoking withdrawal condition; however, this difference was not found in men (group x sex interaction ($F(2, 137) = 4.09$, $p = .02$, $\eta^2 = 0.06$).

4. Discussion

This study provides novel findings related to early life adversity and its impact on the association between tobacco withdrawal and multiple measures of the stress response. Although ACTH, plasma cortisol and salivary cortisol showed the expected increase in response to stress in all groups, the high ELA group showed greater ACTH, lower plasma and salivary cortisol levels, greater ACTH to cortisol ratio, and lower positive affect. The reduced cortisol levels were particularly noted in nonsmoking and ad libitum high ELA smoking groups, suggesting a disruptive influence of ELA on the withdrawal-HPA association. In addition to an effect of ELA on pain perception, particularly in women, we also found that greater pain report associated with high ELA for those without nicotine in their system. High ELA nonsmokers and participants going through tobacco withdrawal reported more pain than those with low ELA, but for smokers assigned to smoke as usual, pain responding in the participants with high ELA was attenuated or the positions for high versus low ELA were reversed.

Our HR and withdrawal symptom results confirms the effects of abstinence and provides internal validation of the withdrawal manipulation but this is in contrast to others who have shown that those with more ELA have lower HR response to acute stress manipulation (Lovallo, Enoch et al. 2017). Our lack of ELA findings for any of the cardiovascular measures may be due to our use of same day baseline rather than a non-stress control session scheduled on a different day.

Findings from this study address the role of ELA as a moderator of the stress response and pain perception during tobacco withdrawal and the challenges smokers with high ELA face when attempting to quit. Recent research from a prospective study with smokers interested in cessation and examining predictors of relapse demonstrated that high early life adversity was associated with higher distress and smoking withdrawal symptoms and with elevated HPA activity, particularly ACTH (al'Absi et al., 2017). That study demonstrated that this link between high life adversity and high ACTH concentrations was associated with increased risk for smoking relapse. Using a population approach, we have also found through prospective observations an overall association between life adversity and risk for smoking relapse (Lemieux, Olson et al. 2016) where early relapse was associated with high

level of early life adversity. This association seemed to be more significant in women than in men.

Our demonstration of lower positive affect in women with high ELA than men with high ELA is consistent with neuroimaging studies showing that maltreated females tend to show lower prefrontal activation to positive emotion words than males (van Harmelen, van Tol et al. 2014). We found that women with high ELA had higher pain sensitivity during the CPT than women with low ELA. This too is consistent with previous findings that female nonsmokers, but not smokers, have been found to have lower pain tolerance than male nonsmokers (al'Absi et al., 2013), but the paucity of research on ELA as a moderator of experimental pain manipulation in the context of smoking precludes any firm conclusion. The fact that there was no sex differences for post-CPT pain ratings suggests that while women with high ELA are more sensitive to pain, they are not impaired in their recovery from pain. No studies of smokers using the CPT recovery have been identified, though studies of cold detection and cold pain threshold have failed to demonstrate differences among types of childhood adversities and pain free controls (Tesarz, Eich et al. 2016). Two other studies utilizing heat induced pain showed a positive association between some ELAs and pain sensitivity, decreased tolerance, and decay but not intensity (Pieritz, Rief et al. 2015; You and Meagher, 2016), though comparing heat and cold pain induction is problematic due to differences in mechanisms (Girdler, Maixner et al. 2005). Additionally, the Tesarz study (Tesarz, Eich et al., 2016) focused on chronic back pain patients and the smoking status was unreported, which limits the comparability to the current study. The lack of ELA main effects or interactions for the global measure of pain (MPQ) also suggest that the difference demonstrated here is unique to the immediate rating of pain and not in ELA-associated ability to subjectively describe the sensory and affective components of pain. Thus, we are the first to report this ELA moderation of pain perception in the context of nicotine dependence. Further research to characterize the subjective experience more fully and to assess mediating factors such as endogenous opioid functioning are warranted. The sex differences in MPQ related to smoking status are, however, consistent with the existing literature (al'Absi et al., 2013; Nakajima and al'Absi, 2014).

One intriguing finding in this study is the dissociation between ELA and HPA hormones: while levels of ACTH were higher in individuals with high ELA, both plasma and salivary cortisol levels were lower in this group. These patterns are consistent with the post-traumatic stress disorder literature, which suggests that lower cortisol concentrations and higher ACTH to cortisol ratios in PTSD (Yehuda, 2002; Vythilingam, Gill et al. 2010). The ACTH results are similar to those obtained from another sample of smokers interested in cessation (al'Absi, Lemieux et al. 2017) and previous results examining CRF and ACTH stimulation challenges in individuals with high level of childhood adversity but no psychiatric comorbidity (Heim, Newport et al. 2001). It is worth noting that the present study also included those who had no active psychiatric dysfunction or other substance use problems. The previous studies have reported lower cortisol concentrations in PTSD subjects relative to normal comparison groups and other psychiatric patients (Yehuda, Boisoineau et al. 1995; Heim, Ehler et al. 1998; Yehuda, 2000). As such, our findings indicate the possibility that ELA in this population may produce long-term effects similar to those produced by exposure to other traumatic experiences. It was interesting to note that, for cortisol levels,

this pattern was abolished by the acute withdrawal, indicating that acute changes resulting from withdrawal may blur the signal related to the chronic effects of ELA. Indeed, it is possible that mood and motivational changes that are induced by tobacco withdrawal could confound any background effects of ELA. Further research would be needed to test this hypothesis.

It is worth noting that chronic tobacco use, like other psychostimulant use, reduces cortisol responses observed with acute drug intake (Lee and Rivier, 1997; Sinha, 2001; Koob and Le Moal, 2008; Richardson, Lee et al. 2008) and acute stress (al'Absi et al., 2003). Preclinical research has demonstrated that chronic, uncontrollable stress leads to dysregulation of the HPA activity as well as GC gene expression changes (McEwen, 2007; Lupien, McEwen et al. 2009) leading to greater vulnerability to the effects of stress in risk for addictive behaviors (Perez-Rubio, Lopez-Flores et al. 2017). This dysregulated pattern in ELA has also been shown to contribute directly or indirectly to initiation and maintenance of tobacco use and addiction possibly through its impact on blunting rewards and dopamine transmission (Volkow, Wang et al. 2012). It is therefore possible that the impact of ELA on HPA activity and other stress response systems mediate the effects of ELA on risk for tobacco use and dependence and other psychiatric risks (McEwen, 2013; Ridout et al., 2015; Ridout, Carpenter et al. 2016).

While specific neurobiological mechanisms relate to effects of ELA on HPA changes observed in this study are not clear, it is reasonable to speculate that repeated stimulation of the HPA by stress or acute tobacco use (Bruijnzeel and Gold, 2005; Coplan, Abdallah et al. 2011) may heighten CRF release contributing to increased ACTH hyper-secretion in this population. The lower cortisol levels in the high ELA group likely reflects an adaptive process in the HPA activity due to repeated exposure and/or enhanced sensitivity to stress (Heim, Ehlert et al. 2000; McEwen, 2007; Doom, Cicchetti et al. 2014). This in turn can lead to counter-regulation contributing to reduced adrenocortical activity (Heim, Ehlert et al. 2000; Fries, Hesse et al. 2005; McEwen, 2007; Tyrka, Parade et al. 2016). Enhanced ACTH and blunted cortisol levels has also been related to alternations in the availability and sensitivity of the glucocorticoid receptors numbers (Anacker, Zunszain et al. 2011; van Zuiden, Geuze et al. 2011). Furthermore, it is possible that ELA, through epigenetic modifications such as methylation of various HPA related genes, influences cortisol related expression of different genes (McEwen, Bowles et al. 2015; Lovallo, Enoch et al. 2016; Tyrka, Parade et al. 2016) and moderates HPA response to stress and risk for substance use.

The ELA findings raise several important questions that we cannot adequately address due to limitations in the current study. For example, without further quantification of cortisol binding globulin, the saliva-to-plasma cortisol dissociation seen here will remain uncertain. Saliva cortisol references free cortisol and plasma reflects total cortisol, which can dissociate under challenge due to differential stress effects on corticosteroid-binding globulin (CBG) (Hellhammer, Wust et al. 2009). Our choice to assess saliva and blood following a fixed battery of stress manipulations, rather than following each component, also limits our ability to determine whether those with heightened ELA experiences are differentially sensitive to socio-evaluative, mental or pain manipulations. While these results are consistent with the PTSD neuroendocrine literature as discussed earlier, our choice to index sympathetic

activation via cardiac outcomes precludes our ability to assess directly other outcomes (e.g. the urine cortisol/norepinephrine ratio) so critical to the development of that literature (Daskalakis, Lehrner et al. 2013; Yehuda, Hoge et al. 2015). While we have demonstrated here and elsewhere (Lemieux, Olson et al. 2016; al'Absi et al., 2017) that smoking and ELA have a combined effect on saliva cortisol and that ELA is associated with relapse risk, as of yet it is unclear what the role of ELA in experimentation with tobacco and progression to dependency. Notwithstanding additional limitations related to the sample size and the potential bias inherent in retrospective recall of abuse, this study includes multiple methodological strengths. The study examined effects of tobacco using two approaches, a cross-sectional comparison between smokers and nonsmoking control and a quasi-experimental approach comparing smokers who were randomly assigned to continue to smoke ad libitum with those who were assigned to the withdrawal condition. This withdrawal-stress manipulation is a clinically relevant approach to tease out the effects of stress on reward under conditions of high motivation for use and could provide insight into how acute stress contributes to the alteration of the rewarding properties of nicotine (Piazza and Le Moal, 1998). We have previously examined the stress response profile after period of abstinence (al'Absi et al., 2003) and used the results to develop a stress-related relapse prediction model (al'Absi, Hatsukami et al. 2005; al'Absi, 2006; al'Absi, Nakajima et al. 2015). The use of a cross-sectional design limited us from drawing any causal conclusions regarding ELA, smoking, and psychobiological stress response. The approaches used in this study, combined with the multiple biological, physiological and psychological measures, provide a robust means to evaluate the impact of tobacco use and acute withdrawal and yield interpretable findings on the collected measures, though our choice to use this rarely utilized approach is not without its downsides. Due to its static nature, we sacrificed the ability to carefully examine time-dependent features of the withdrawal in the name of being able to make firmer causal conclusions. In addition, while earlier results were obtained from healthy individuals, the current study is more clinically relevant given its focus on smoking, which is already known to be associated with high ELA (Felitti, Anda et al. 1998; Anda, Croft et al. 1999; Fuller-Thomson, Filippelli et al. 2013; Bellis, Hughes et al. 2014; Elliott, Stohl et al. 2014).

In summary, this study provides novel findings indicating dysregulation of the HPA axis in the impact of withdrawal and stress among high ELA participants. Individuals with high ELA had greater ACTH, but lower plasma and salivary cortisol levels and higher ACTH/cortisol ratio than low ELA. Cortisol differences were abolished during tobacco withdrawal. The effects of ELA are particularly evident in women with high ELAs. These findings are in support of mechanisms proposed to date such as chronic HPA activation leading to blunted cortisol responding and subsequent blunting of drug reward/dopaminergic transmission, possibly through epigenetic modifications. Studies such as ours highlight the potential for programmatic or individualized cessation treatment modifications. A deeper understanding of ELA-associated neurobehavioral dysregulations in the context of smoking and withdrawal may inform future studies of pharmacologic management of nicotine addiction.

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X = Plasma, saliva and mood measures collected

Fig. 1.

Study time line depicting cardiovascular, plasma, saliva and mood measures across the stress protocol.

Note. The stress protocol was presented in fixed order and consisted of a battery of public speaking, mental arithmetic, and the cold pressor test.

Fig. 2.

Note. Cardiovascular measures before, during and after acute stress (smoking by gender) (Fig. 2a) and hormonal levels before, during and after stress (smoking by ELA groups) (Fig. 2b).

Fig. 3.

a Associations between ELA and ACTH, plasma cortisol and salivary cortisol. All ELA group differences were statistically significant.

Note. ACTH (68 individuals in Low ELA group; 48 individuals in High ELA group).

Plasma cortisol (86 individuals in Low ELA group; 49 individuals in High ELA group).

Salivary cortisol (91 individuals in Low ELA group; 51 individuals in High ELA group).

b Relationships between ELA and stress hormones using ratio scores. Individuals with High ELA exhibited greater scores on ACTH/plasma cortisol ($p = .01$) and ACTH/salivary cortisol ($p < .01$). Note. ACTH/plasma cortisol (68 individuals in Low ELA group; 41 individuals in High ELA group). ACTH/salivary cortisol (66 individuals in Low ELA group; 38 individuals in High ELA group).

Fig. 4. Smoking group by ELA interaction in salivary cortisol. Note. Low ELA group (29 nonsmokers, 28 ad libitum smokers and 34 withdrawal smokers). High ELA group (13 nonsmokers, 12 ad libitum smokers and 26 withdrawal smokers).

Fig. 5. Smoking by ELA by time interaction in pain during the cold pressor test. Low ELA group (30 nonsmokers, 30 ad libitum smokers and 35 withdrawal smokers). High ELA group (13 nonsmokers, 12 ad libitum smokers and 30 withdrawal smokers).

Table 1

Sample characteristics.

	Nonsmoker (n = 44)		Smoker (ad libitum) (n = 46)		Smoker (withdrawal) (n = 66)	
	Female (n = 23)	Male (n = 21)	Female (n = 18)	Male (n = 28)	Female (n = 26)	Male (n = 40)
Age (yrs)	34.4 (2.6)	39.1 (2.7)	31.4 (2.9)	34.8 (2.3)	32.9 (2.4)	35.5 (2.0)
BMI	26.3 (1.2)	26.8 (1.2)	28.2 (1.3)	27.4 (1.1)	29.4 (1.1)	25.0 (0.9)
Education ^a (yrs)	15.9 (0.5)	14.7 (0.6)	13.3 (0.6)	12.6 (0.5)	13.2 (0.5)	13.5 (0.4)
% Single ^a	56.5	61.9	77.8	71.4	76.9	82.5
% White	81.8	71.4	70.6	77.8	56.0	75.0
Adversity score ^b	1.7 (0.4)	1.3 (0.4)	2.2 (0.5)	1.3 (0.4)	2.5 (0.4)	2.3 (0.3)
Age of smoking onset	n/a	n/a	15.0 (1.1)	17.1 (0.9)	15.6 (0.9)	15.7 (0.7)
Cigarettes/day ^{b,c}	n/a	n/a	15.0 (1.3)	15.1 (1.1)	11.8 (1.1)	16.4 (0.9)
Duration (yrs)	n/a	n/a	9.2 (2.5)	9.9 (2.0)	10.2 (2.2)	12.9 (1.7)
FTND	n/a	n/a	5.6 (0.5)	5.4 (0.4)	4.5 (0.4)	5.4 (0.3)
CO (ppm) ^a	n/a	n/a	17.3 (1.6)	15.6 (1.3)	2.4 (1.3)	2.7 (1.1)
Cotinine ^a (ng/mL)	n/a	n/a	248.6 (43.5)	200.5 (32.9)	97.3 (34.8)	124.0 (27.5)

^aMain effect of smoking was significant.^bMain effect of sex was significant.^cSmoking by sex interaction was significant.

Table 2

Changes in subjective measures in response to stress.

	Nonsmoker (n = 44)		Smoker (ad libitum) (n = 46)		Smoker (withdrawal) (n = 66)	
	Female (n = 23)	Male (n = 21)	Female (n = 18)	Male (n = 28)	Female (n = 26)	Male (n = 40)
Positive affect ^{a,b,c}						
baseline	14.1 (1.4)	21.5 (1.6)	17.5 (1.7)	19.0 (1.4)	12.9 (1.3)	16.3 (1.1)
rest 1	14.4 (1.4)	20.5 (1.6)	19.0 (1.7)	19.1 (1.4)	12.2 (1.3)	16.5 (1.1)
rest 2	13.7 (1.4)	20.8 (1.6)	17.5 (1.7)	18.5 (1.4)	11.4 (1.3)	16.2 (1.1)
stress	9.9 (1.5)	14.9 (1.7)	15.2 (1.8)	16.0 (1.5)	10.2 (1.3)	15.1 (1.1)
post-str 1	11.6 (1.4)	16.6 (1.6)	15.6 (1.7)	16.5 (1.5)	9.3 (1.3)	15.0 (1.1)
post-str 2	12.4 (1.5)	17.5 (1.7)	14.3 (1.8)	15.3 (1.5)	11.2 (1.4)	15.9 (1.2)
post-str 3	14.4 (1.5)	18.3 (1.7)	15.0 (1.8)	17.5 (1.5)	12.5 (1.4)	16.3 (1.2)
Distress ^{a,d}						
baseline	3.0 (1.0)	2.1 (1.1)	4.5 (1.2)	2.8 (1.0)	7.2 (0.9)	6.3 (0.8)
rest 1	2.7 (0.9)	1.5 (1.0)	2.7 (1.1)	2.8 (0.9)	6.5 (0.8)	5.9 (0.7)
rest 2	3.3 (1.0)	2.2 (1.0)	4.1 (1.1)	3.4 (1.0)	6.4 (0.9)	6.0 (0.7)
stress	5.3 (1.2)	6.3 (1.2)	6.4 (1.4)	5.2 (1.2)	8.3 (1.1)	7.6 (0.9)
post-str 1	4.7 (1.3)	4.8 (1.3)	5.3 (1.5)	4.6 (1.2)	8.5 (1.1)	7.6 (0.9)
post-str 2	4.4 (1.3)	4.7 (1.3)	6.1 (1.5)	6.1 (1.3)	7.8 (1.2)	6.8 (1.0)
post-str 3	2.9 (1.2)	3.4 (1.3)	5.5 (1.4)	4.5 (1.2)	8.2 (1.1)	7.5 (0.9)
MNWS ^{a,b}						
baseline	N/A	N/A	3.8 (1.4)	3.3 (1.3)	7.5 (1.1)	7.1 (0.9)
rest 1	N/A	N/A	3.0 (1.4)	3.2 (1.2)	7.2 (1.1)	6.7 (0.9)
rest 2	N/A	N/A	4.0 (1.5)	4.4 (1.4)	7.2 (1.2)	6.9 (0.9)
stress	N/A	N/A	7.3 (1.8)	5.8 (1.6)	9.3 (1.4)	9.1 (1.1)
post-str 1	N/A	N/A	5.9 (1.7)	5.2 (1.6)	8.9 (1.3)	9.1 (1.1)
post-str 2	N/A	N/A	7.6 (1.8)	6.6 (1.6)	8.0 (1.4)	8.5 (1.1)
post-str 3	N/A	N/A	7.5 (1.6)	5.4 (1.5)	8.7 (1.3)	9.2 (1.0)
Craving ^{a,b}						
baseline	N/A	N/A	4.7 (0.5)	3.4 (0.4)	5.0 (0.4)	4.5 (0.3)

	Nonsmoker (n = 44)		Smoker (ad libitum) (n = 46)		Smoker (withdrawal) (n = 66)	
	Female (n = 23)	Male (n = 21)	Female (n = 18)	Male (n = 28)	Female (n = 26)	Male (n = 40)
rest 1	N/A	N/A	4.6 (0.5)	4.5 (0.5)	5.3 (0.4)	4.6 (0.3)
rest 2	N/A	N/A	5.1 (0.5)	5.1 (0.5)	5.2 (0.4)	4.4 (0.3)
stress	N/A	N/A	3.1 (0.6)	3.0 (0.5)	4.8 (0.4)	4.6 (0.3)
post-str 1	N/A	N/A	3.7 (0.5)	4.0 (0.5)	5.1 (0.4)	4.6 (0.3)
post-str 2	N/A	N/A	4.9 (0.5)	4.5 (0.5)	5.3 (0.4)	4.7 (0.3)
post-str 3	N/A	N/A	4.7 (0.5)	4.5 (0.5)	5.4 (0.4)	4.7 (0.3)
QSUB F1 ^{a,b}						
baseline	N/A	N/A	36.7 (3.5)	32.0 (3.1)	36.7 (2.6)	35.9 (2.1)
rest 1	N/A	N/A	37.8 (3.4)	36.7 (3.1)	35.9 (2.6)	35.2 (2.1)
rest 2	N/A	N/A	40.8 (3.2)	37.6 (2.9)	34.9 (2.4)	34.0 (2.0)
stress	N/A	N/A	25.7 (3.5)	25.8 (3.2)	35.7 (2.6)	34.6 (2.1)
post-str 1	N/A	N/A	30.9 (3.5)	31.6 (3.1)	35.0 (2.6)	35.1 (2.1)
post-str 2	N/A	N/A	35.1 (3.5)	34.3 (3.2)	35.5 (2.7)	34.3 (2.2)
post-str 3	N/A	N/A	36.0 (3.5)	32.3 (3.2)	35.8 (2.7)	34.8 (2.2)
QSUB F2 ^a						
baseline	N/A	N/A	19.6 (2.9)	15.4 (2.6)	19.7 (2.2)	15.9 (1.8)
rest 1	N/A	N/A	20.6 (3.1)	17.0 (2.8)	18.6 (2.3)	16.1 (1.9)
rest 2	N/A	N/A	21.7 (3.1)	16.8 (2.8)	16.7 (2.4)	15.1 (1.9)
stress	N/A	N/A	16.8 (2.9)	12.4 (2.7)	17.7 (2.2)	15.2 (1.8)
post-str 1	N/A	N/A	18.8 (3.3)	15.3 (3.0)	18.1 (2.5)	16.1 (2.0)
post-str 2	N/A	N/A	19.0 (3.3)	16.2 (3.0)	18.3 (2.5)	15.2 (2.0)
post-str 3	N/A	N/A	22.6 (3.4)	16.4 (3.1)	18.8 (2.6)	16.0 (2.1)

Note. Entries are shown in mean with standard error of the mean. Log-transformed scores were used in statistical analysis.

^aMain effect of time was significant.

^bSmoking group by time interaction was significant.

^cELA by sex interaction was significant.

^dMain effect of smoking group was significant.