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Contribution of Hypothalamic–Pituitary–Adrenal Activity and Environmental Stress to Vulnerability for Smoking in Adolescents

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Although tobacco smoking, which has been linked to depression, is a major public health problem, little is known about the neurobiological factors that confer vulnerability to smoking in youngsters and the effects of adolescent smoking on the course of depression. This study examined whether hypothalamic–pituitary–adrenal (HPA) activity and stressful life experiences are related to smoking behavior in depressed and non-depressed adolescents, and whether smoking predicts a worsening course of depression. Smoking history and stressful experiences were assessed in 151 adolescents (48 with no personal or family history of psychiatric disorder, 48 with no psychiatric history, but at high risk for depression by virtue of parental depression, and 55 with current major depressive disorder). Evening salivary cortisol and nocturnal urinary-free cortisol were measured for three consecutive evenings. The participants were then followed at regular intervals for up to 5 years to assess smoking history, clinical course of depression and stressful experiences during the follow-up period. Increased evening/night-time cortisol levels were associated with both initiation and persistence of smoking during follow-up. Stressful life experiences further increased the risk for smoking in depressed as well as non-depressed youth. Smoking was also associated with a higher frequency of depressive episodes during follow-up. A model that included stressful experiences and cortisol levels reduced the contribution of smoking *per se* to depression. High evening/night-time cortisol level appears to be a vulnerability marker for smoking in adolescents, with stressful experiences further increasing the risk for smoking in vulnerable youth. High evening/night-time cortisol levels and stressful experiences accounted, at least partially, for the association between depressive illness and smoking behavior.

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INTRODUCTION

Cigarette smoking is a substantial source of morbidity and premature mortality (Centers for Disease Control and Prevention, 2002; Lopez *et al*, 2006; Max, 2001). Almost all tobacco use begins during adolescence, and despite decades of public health efforts, millions of adolescents initiate and continue to smoke (Botello-Harbaum *et al*, 2009; Centers for Disease Control and Prevention, 2002, 2006; Johnston *et al*, 2009). Generating knowledge of the factors that promote smoking in adolescents, therefore, is a high priority from a public health perspective.

Prospective studies in adolescents and adults have shown that depression increases the risk for smoking, and vice versa (Breslau *et al*, 1998; Brown *et al*, 1996; Choi *et al*, 1997; Fergusson *et al*, 2003; Klungsøyr *et al*, 2006; Munafò *et al*, 2008; Patton *et al*, 1998; Steuber and Danner, 2006; Windle and Windle, 2001). In some studies, adult smokers with a history of depression were less likely to succeed in smoking cessation, and were more likely to experience severe withdrawal symptoms during attempts to quit, compared with non-depressed smokers (Anda *et al*, 1990; Berlin *et al*, 1997; Breslau *et al*, 1992; Covey *et al*, 1990; Ginsberg *et al*, 1995; Glassman *et al*, 2001; John *et al*, 2004). Smoking cessation also might trigger relapse of depressive episodes in smokers with a history of depression (Borrelli *et al*, 1996; Breslau and Johnson, 2000; Covey *et al*, 1997; Khaled *et al*, 2009; Tsoh *et al*, 2000).

This study was undertaken to examine biopsychosocial factors associated with initiation and persistence of smoking in depressed and non-depressed adolescents. Environmental stress and hypothalamic–pituitary–adrenal (HPA) activity were selected as study variables for the following reasons. Stress has been associated with smoking initiation (Byrne and Mazanov, 2003; Koval *et al*, 2000; Nichter *et al*, 1997), as well as the transition to regular smoking (Orlando

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et al, 2001; Siqueira *et al*, 2000). Research findings suggest that high responsivity of the brain stress systems (including the HPA axis) moderates the effects of exposure to stress on drug-seeking behavior (Koob and Kreek, 2007; Rao and Chen, 2008; Sinha, 2008; Zislis *et al*, 2007). There is also an extensive literature demonstrating a relationship between HPA status and nicotine administration in animals and adult humans (al'Absi, 2006; Berlin, 2009; Lovallo, 2006; Mendelson *et al*, 2008; Rohleder and Kirschbaum, 2006; Sidhartha *et al*, 2009). Stressful life experiences and abnormalities in the HPA system also have been noted in numerous investigations of depression (Hammen, 2005; Holsboer and Ising, 2008; Meyer *et al*, 2001). We, therefore, posed the following hypotheses: (1) that HPA activity measured at baseline would be positively correlated with likelihood of smoking initiation and persistent smoking during prospective follow-up, (2) that recent stressful life experiences would further increase the risk for smoking in individuals who have high HPA activity, and (3) that the association between depressive disorder and smoking might be explained partly by stressful life experiences and high HPA activity.

MATERIALS AND METHODS

The data reported here are part of a larger study on the development and course of depression in adolescents, as well as the relationship between depression and substance use disorders (Rao *et al*, 2009a, b).

Participants

Subjects were recruited from the outpatient clinics at university-affiliated medical centers and through advertisements in local newspapers. After receiving a complete explanation of the procedures, all adolescents signed a written assent form, and parents gave written informed consent to participate in the research protocol, which was approved by institutional review boards at the University of California at Los Angeles and associated medical institutions. The participants for the study included 55 adolescents with depression, 48 adolescents at high risk for developing depression, and 48 normal controls.

The depressed subjects met criteria for major depressive disorder according to DSM-IV criteria (American Psychiatric Association, 1994), with a minimum duration of 4 weeks and a score of $\geqslant$15 on the first 17 items of the Hamilton Depression Rating Scale (HDRS; Hamilton, 1960). Adolescents with a current or prior history of mania, hypomania, substance use disorder symptoms, schizophrenia, schizoaffective disorder, or autism were excluded from the study. Subjects were also excluded if there was a family history of bipolar disorder. All subjects were free from antidepressant drugs and other psychotropic agents for at least 4 weeks (8 weeks for fluoxetine). The normal and high-risk control subjects were free from any psychopathology, including depression, over their lifetime. In addition, the high-risk control subjects had at least one biological parent with a history of unipolar major depressive disorder that required treatment. Subjects were excluded from the normal control group if any first-degree relative had history of a psychiatric disorder. All participants were medically healthy and free from alcohol or illicit drug use, as determined by physical examination, full chemistry panel, thyroid function tests, electrocardiogram, and urine drug screens.

Diagnostic Evaluation

The diagnosis of major depressive disorder and other psychiatric disorders was done using the Schedule for Affective Disorders and Schizophrenia for School-Age Children—the Present and Lifetime Version (K-SADS-PL; Kaufman *et al*, 1996). The K-SADS-PL is a semistructured interview designed to ascertain present and lifetime history of psychiatric illness according to DSM-IV criteria. Probes and objective criteria are provided for individual symptoms at both diagnostic and subthreshold levels. Inter-rater and test–retest reliability have been established, as well as convergent and discriminant validity (Kaufman *et al*, 1997). The K-SADS-PL was administered separately to the parent and the adolescent, and both were re-interviewed to resolve any discrepancies. Summary scores were tabulated on the basis of the information obtained from both informants. The children's global assessment scale (CGAS), a global psychosocial functioning measure, was also completed (Shaffer *et al*, 1983). The adolescents completed the beck depression inventory (BDI) for self-assessment of depression severity (Beck *et al*, 1961).

Family Psychopathology

The Family History-Research Diagnostic Criteria (FH-RDC), a semistructured instrument, was used for the evaluation of psychiatric disorders in family members (Andreasen *et al*, 1977). One parent was interviewed regarding major psychiatric disorders over lifetime in all first-degree relatives of the adolescent subject. The FH-RDC is sensitive for obtaining information from knowledgeable relatives (Thompson *et al*, 1982).

Smoking Behavior

Information was obtained on current and past smoking history, including number of cigarettes, frequency, as well as number of attempt(s) to quit and duration of smoke-free period(s). Other forms of nicotine use were also noted. At the time of recruitment, to specifically identify non-smokers, any subject who reported smoking one or more cigarettes over his/her lifetime was considered a smoker. Smokers were administered the adolescent version of the Fagerstrõm tolerance questionnaire (FTQ) (Fagerström, 1978), which measures behavioral responses that are suggestive of nicotine dependence. The adolescent version of the FTQ has 7 items, and scores range from 0 to 9 (Prokhorov *et al*, 1996). A score $\leqslant$2 suggests no evidence of nicotine dependence, and values ranging from 3 to 5 are considered to reflect moderate dependence. A score of $\geqslant$6 on the FTQ indicates a high level of nicotine dependence. In earlier research, nicotine dependence symptoms on the FTQ correlated with cotinine (a metabolite of nicotine) levels (Prokhorov *et al*, 2000).

Stressful Life Experiences

To obtain information on recent stressful life experiences, a semistructured interview developed by a member of our team was used (Hammen *et al*, 1995). This interview is based on the contextual threat assessment (Brown and Harris, 1978). Inter-rater reliability has been established for this instrument (Hammen and Brennan, 2001; Hammen *et al*, 1995). Using the list developed by Paykel and Mangen (1980), and adapted for an adolescent population, the participants were systematically probed about the occurrence and timing of particular life events specifically to obtain objective features of the events and circumstances. For the baseline assessment, the time frame for events included the earlier 6 months. Narrative summaries of the event and the surrounding context were presented to a group of trained raters on the instrument. To obtain ratings of severity of stressors that were not distorted by participants' depressive symptoms or misattribution of the meaning of the stressors, the raters were blind to the subject's diagnostic status and perception of stress. Consensus group ratings were given for the degree of stress for each event ($1 =$ not at all stressful, and $5 =$ extremely stressful), and whether the event was a positive, neutral or negative experience under the given circumstances. To be included in the analysis, an event was required to have an impact for at least 1 day and to involve at least mild stress. Only events that were considered negative were included in the analyses. Symptom-related events were not scored.

HPA Measures

Each subject participated in a 3-night, sleep-neuroendocrine study in the laboratory. Before these studies, sleep–wake schedules were regulated for at least 1 week, with participants going to bed between 10:00 to 11:00 p.m. and waking between 6:30 to 7:30 a.m. The sleep–wake schedule was confirmed through diary and actigraphy. The participants were free from alcohol/illicit drug use at the time of HPA studies (confirmed by urine drug screens). Only evening and night-time cortisol values were used to measure HPA activity. Subjects arrived in the laboratory by 8:00 p.m., and they were instructed to have dinner at least an hour before arrival in the laboratory. Immediately after arriving in the laboratory, the participants were asked to lie down and were allowed only non-caloric fluids before and during the saliva collection. Saliva samples were obtained at half-hour intervals from 8:30 to 9:30 p.m. Subjects were asked to void urine before switching off the lights. After lights out, all urine voided during the night (including the sample obtained immediately upon awakening) was collected.

The choice of evening and nocturnal cortisol samples as study measures was based on prior research in youngsters, indicating that cortisol sampling during this time period, when the HPA axis is normally quiescent, was most robust in discriminating depressed adolescents from controls and also in predicting the longitudinal clinical course (Dahl *et al*, 1991; Goodyer *et al*, 1997, 2001; Rao *et al*, 1996, 1999). To prevent potential sleep disruption, salivary and urinary samples, instead of plasma samples, were collected to assess HPA activity. A radioimmunoassay procedure was used for the cortisol assays (McCracken and Poland, 1989; Poland and Rubin, 1982). Urinary-free cortisol values were expressed as concentration and total amount excreted. All samples from any individual subject were analyzed in the same assay. Low, medium, and high cortisol pools were re-analyzed in each assay to assess intra- and inter-assay variability. The intra- and inter-assay coefficients of variation for the assays were $<10\%$.

Follow-up Evaluation

After the initial assessments, the participants were followed longitudinally at 6-month intervals in the first year and yearly thereafter, for up to 5 years, to obtain information on smoking history and the clinical course of depression and other psychiatric disorders. Any youngster who smoked at least 4 days/week for >1 week during follow-up was considered a smoker. This definition is consistent with a regular smoker; smoking often but still not frequent enough to be considered a daily/established smoker (Mayhew *et al*, 2000). Development of a depressive episode was defined as a rating of 5 on the psychiatric status rating (PSR) component of the longitudinal interval follow-up evaluation (LIFE) for a minimum 4-week duration. Remission from a depressive episode was defined as a rating of 2 for $\geqslant 12$ weeks on the PSR. Recurrence was defined as a PSR rating of $\geqslant 5$ for 4 or more weeks with a minimum duration of 12 weeks between episodes. The conventional criterion for remission is 8 weeks, and the minimum duration for recurrent depressive episode is 2 weeks (Frank *et al*, 1991). In this study, stringent criteria were used for remission and recurrence to ensure stability of symptoms because depressive symptoms in youngsters tend to be more variable, with greater heterogeneity in clinical response and course, than adults (Kaufman *et al*, 2001; Rao and Chen, 2009). The LIFE is a semistructured instrument used for charting the clinical course of depression and other psychiatric disorders during longitudinal follow-up (Shapiro and Keller, 1979). The PSR is a 6-point scale providing information on the severity of depressive symptom profile.

Information from the diagnostic assessments was presented to an independent clinician (UR) 'blind' to the diagnostic status, stressful life events, and HPA status. Final diagnosis was based on consensus ratings. Assessment of stressful experiences during the follow-up period was similar to the method used during initial evaluation. Time frame for the events included the period since the last interview. Ratings for the magnitude of stress occurred blind to information from the diagnostic and HPA assessment.

Primary Dependent and Independent Variables

The primary outcome measure was onset of smoking during follow-up. The primary independent variables included HPA activity (evening salivary and nocturnal urinary-free cortisol levels) and stressful experiences. For salivary cortisol, average values of the three samples on each night were determined and then a single mean value was obtained across the 3 nights. For nocturnal urinary-free cortisol samples, a single mean value was obtained across the 3 nights. Timelines were generated, charting the onset of

smoking behavior and stressful life events. For each participant who began smoking during follow-up, the total episodic stress score for the 3 months preceding smoking initiation was computed. Each non-smoker was paired with a smoker based on demographic and clinical information, and the episodic stress that he/she experienced in the corresponding 3 months was tabulated. This method was adopted instead of using a random 3-month period because events were not evenly distributed across the 5 years due to developmentally expected events (eg high school graduation and transition to college occurs in older adolescents). Secondary outcome measures included the onset of a depressive episode during follow-up (in normal and high-risk controls), and recurrence of a depressive episode (in the depressed group). Smoking status during follow-up, HPA activity (evening salivary and nocturnal urinary-free cortisol levels) at baseline and stressful experiences during follow-up were the independent variables.

Statistical Analysis

For all summary variables, data were examined for normality using the Shapiro–Wilk's W statistic (Shapiro and Wilk, 1965). Cortisol variables were log-transformed and the psychosocial measures were standardized before the application of statistical tests for significance. For group comparisons, the χ^2 was used to analyze categorical variables and analysis of variance for continuous variables. Correlation procedures were used to examine associations between variables. Cox regression, with appropriate covariates, was used to compute the probability of smoking during follow-up. Cortisol measures obtained at baseline, stress score in the 3 months preceding the onset of smoking and the interaction term (HPA $\times$ stress) were used as independent variables. The relationships among depression, smoking, HPA activity and stress were examined through the logistic regression procedure. All analyses were two-tailed, and the α was set at 0.05.

RESULTS

Demographic, Clinical, and Biological Parameters at Baseline

Demographic, clinical, and biological features of the three groups of adolescents are outlined in Table 1. The groups did not differ significantly with respect to age, gender, or ethnicity. High-risk and depressed groups had significantly lower socioeconomic score than normal controls. Depressed adolescents scored significantly higher on the BDI, HDRS, and stressful experiences, but lower on CGAS, than both comparison groups. The groups did not differ significantly with respect to smoking status or salivary cortisol, but depressed youth had higher nocturnal urinary-free cortisol than normal controls, whereas the high-risk subjects had intermediate levels.

Follow-up Information

Three initially normal controls, four high-risk subjects and four depressed adolescents only were assessed at intake and did not complete any follow-up evaluations. Subjects who did not participate in follow-up assessments did not differ significantly from those with follow-up information on any demographic or clinical characteristics. Recruitment did not occur simultaneously and, therefore, not all subjects were studied longitudinally for the same period of time. Of the 140 adolescents who had follow-up information, 9.3% were followed for 2 years, 19.3% for 3 years, 31.4% for 4 years, and 40.0% for 5 years. The three groups were comparable on the mean follow-up interval (mean follow-up interval = 3.6 years, SD = 1.0).

Initiation of Smoking

Of a total of 140 adolescents who had follow-up information, 109 (77.9%) had no prior smoking history at intake. Of these 109 participants, 21 (19.3%) initiated smoking during the study. The mean FTQ score in the smokers was 3.7 (SD = 1.3; range, 2.0–6.5). On the basis of the FTQ score, four (19.0%) showed no evidence of nicotine dependence, 15 (71.4%) had moderate level of dependence and two (9.5%) manifested high level of dependence. Severity of nicotine dependence was comparable among the three diagnostic groups. Demographic and clinical characteristics of subjects who initiated smoking and those who never smoked are provided in Table 2. The groups did not differ significantly on any demographic or clinical variables but there was a trend for adolescents who initiated smoking to have a longer follow-up period compared with non-smokers.

Of a total of 37 depressed youth with no prior smoking history at intake, 6/13 (46.2%) participants with comorbid anxiety disorder initiated smoking compared with 3/24 (12.5%) subjects without anxiety disorder (FET, $p = 0.04$). Of 5 youth with comorbid disruptive disorder, 1 (20%) initiated smoking compared with 8/32 (25.0%) without disruptive disorder (FET, NS).

Effects of HPA Activity and Stress on Smoking Initiation

There was a high correlation among the three cortisol measures ($r = 0.66$ between salivary cortisol and urinary-free cortisol concentration; $r = 0.62$ between salivary cortisol and total urinary cortisol; and $r = 0.94$ between the two urinary cortisol measures). Hence, a composite measure of HPA activity was derived by taking a mean of the three measures.

Among adolescents who had no prior smoking history at intake, after accounting for differential follow-up period, higher evening/night-time cortisol levels predicted initiation of smoking (see Table 3). Stressful experiences also made an independent contribution to the initiation of smoking. The analyses were run separately in depressed adolescents and control subjects (combining normal and high-risk groups), and the same pattern emerged even after controlling for comorbid disorders in the depressed cohort.

For the purpose of graphical representation, the sample was stratified into four groups based on a median split of HPA activity (evening/night-time cortisol levels) and stress at follow-up: low HPA activity-low stress ($n = 39$); low HPA activity-high stress ($n = 22$); high HPA activity-low stress ($n = 22$); and high HPA activity-high stress ($n = 26$). These groups were then compared on the probability of smoking

Table 1 Baseline Demographic, Clinical, and Biological Parameters by Diagnosis

	Normal ($n=48$)	High risk ($n=48$)	Depressed ($n=55$)	Statistic	*p*
Age (years)	15.2 (1.4)	15.0 (1.5)	15.3 (1.4)	0.38	NS
Gender				0.17	NS
Male	19 (39.6)	21 (43.7)	23 (41.8)		
Female	29 (60.4)	27 (56.3)	32 (58.2)		
Ethnicity				0.84	NS
African-American	6 (12.5)	7 (14.6)	6 (10.9)		
Asian-American	10 (20.8)	8 (16.7)	9 (16.4)		
Caucasian	23 (47.9)	23 (47.9)	29 (52.7)		
Hispanic	9 (18.8)	10 (20.8)	11 (20.0)		
Socioeconomic status[a]	49.2 (9.4)[a]	43.5 (10.1)[b]	40.9 (11.2)[b]	8.38	0.0001
Beck depression inventory	1.7 (2.4)[a]	3.5 (3.1)[b]	18.4 (7.9)[c]	160.11	0.0001
Hamilton depression scale	0.9 (1.4)[a]	1.0 (1.4)[a]	19.8 (4.0)[b]	896.06	0.0001
CGAS score	83.2 (9.7)[a]	78.9 (10.0)[a]	50.7 (7.4)[b]	202.37	0.0001
Smoked cigarettes ever (%)	8 (16.7)	9 (18.8)	14 (25.5)	1.35	NS
Current smokers (%)	4 (8.3)	4 (8.3)	7 (12.7)	0.76	NS
FTQ score[b]	1.4 (0.5)	1.1 (0.5)	1.3 (0.6)	0.31	NS
Stress score	3.4 (2.9)[a]	3.7 (3.0)[a]	6.0 (5.5)[b]	6.54	0.002
Salivary cortisol (ng/ml)[c]	0.4 (0.2)	0.5 (0.3)	0.5 (0.3)	0.75	NS
NUFC (ng/ml)[c]	15.6 (7.9)[a]	21.2 (13.1)[a,b]	21.9 (11.4)[b]	3.72	0.03
Total NUFC[c]	8.3 (4.1)[a]	10.8 (6.1)[a,b]	11.7 (5.8)[b]	4.57	0.02
Anxiety disorder	—	—	19 (34.5)	—	—
Disruptive disorder	—	—	7 (12.7)	—	—

Abbreviations: CGAS, children's global assessment scale; FTQ, Fagerström Tolerance Questionnaire; NUFC, nocturnal urinary free cortisol.
[a]Higher score is associated with higher socioeconomic status (Hollingshead scale).
[b]Includes only current smokers.
[c]Analysis was performed on transformed data.
Data are presented as means or raw numbers; data in parentheses reflect standard deviations or percentages.
Different subscripts denote significant differences among groups.

initiation (see Figure 1). Among adolescents who experienced high stress levels in combination with elevated HPA activity, 53.3% were likely to initiate smoking in comparison with 3.0% of youngsters in the low HPA activity-low stress category (Mantel–Cox $\chi^2=20.35$, df $=3$, $p=0.0001$). Among youth in the low HPA activity-high stress group, 35.0% were likely to smoke during the follow-up period compared with 24.1% in the high HPA activity-low stress group.

When HPA activity and stressful experiences were tested as predictors of nicotine dependence symptoms, only HPA activity was significant (std. $\beta=0.70$, CI $=0.54$–1.67, $t=4.11$, $p=0.001$; adjusted $R^2=0.43$, $F_{2,18}=8.43$, $p=0.003$).

Persistence of Smoking

At intake, 31 adolescents reported prior smoking history. Of these, 19 (61.3%) youth also reported smoking during follow-up (persistent smokers). Adolescents who persisted with smoking during follow-up did not differ significantly from abstainers on any demographic or clinical variables (see Table 4). There was, however, a trend for persistent smokers to experience higher stress at baseline compared with abstainers. The mean FTQ score in the smokers was 4.2 (SD $=1.5$; range, 1.5–7.0). On the basis of the FTQ score, 1 (5.3%) persistent smoker showed no evidence of nicotine dependence, 14 (73.7%) had moderate level of dependence and four (21.1%) manifested high level of dependence.

Of a total of 14 depressed youth with prior smoking history at intake, 4/5 (80.0%) adolescents with comorbid anxiety disorder persisted with smoking during follow-up in comparison with 5/9 (55.6%) participants without anxiety disorder (FET, NS). Also, 2/2 (100.0%) youth with comorbid disruptive disorder persisted with smoking compared with 7/12 (58.3%) without disruptive disorder (FET, NS).

Effects of HPA Activity and Stress on Persistent Smoking

There was a trend for adolescents who reported smoking at the time of intake (current smokers; $n=15$) to have higher HPA activity compared with past smokers (0.8 ± 1.1 *vs* 0.2 ± 0.9, $t_{29}=1.72$, $p=0.10$). After controlling for smoking status and magnitude of stress at the time intake, elevated HPA activity (higher evening/night-time cortisol levels) was

Table 2 Demographic and Clinical Parameters in Adolescents Who Reported no Prior Smoking History at Intake, Stratified on Smoking Initiation During Follow-up

	Never smoked ($n=88$)	Initiated smoking ($n=21$)	Statistic	p
Age	14.9 (1.3)	14.8 (1.5)	0.49	NS
Gender			0.01	NS
Male	37 (42.0)	9 (42.9)		
Female	51 (58.0)	12 (57.1)		
Ethnicity			0.33	NS
Caucasian	40 (45.5)	11 (52.4)		
Non-Caucasian	48 (54.5)	10 (47.6)		
Socioeconomic score[a]	44.5 (10.7)	44.0 (12.6)	0.18	NS
Follow-up interval (years)	3.5 (1.1)	4.0 (0.9)	1.84	0.07
Diagnosis at baseline			1.41	NS
Normal	32 (36.4)	5 (23.8)		
High risk	28 (31.8)	7 (33.3)		
Depression	28 (31.8)	9 (42.9)		
Hamilton depression rating scale	7.0 (9.3)	9.0 (9.7)	0.88	NS
Beck depression inventory	8.4 (10.2)	7.6 (8.7)	0.30	NS
Children's global assessment scale	71.7 (16.8)	70.1 (18.9)	0.38	NS
Stressful life experiences	3.9 (3.6)	5.1 (5.5)	1.20	0.09

[a]Higher score is associated with higher socioeconomic status (Hollingshead scale).
Data are presented as means or raw numbers; data in parentheses reflect standard deviations or percentages.

Table 3 Cox Regression Model Predicting Initiation of Smoking in Adolescents Who had no Prior Smoking History at Intake

	β (SE)	Wald	OR (CI)	p
HPA activity[a]	0.70 (0.29)	5.92	2.01 (1.15–3.54)	0.02
Stress at follow-up	1.26 (0.26)	23.78	3.54 (2.15–5.84)	0.0001
HPA activity × stress at follow-up	0.09 (0.25)	0.02	1.09 (0.67–1.78)	NS

Abbreviations: SE, standard error; OR, odds ratio; CI, confidence interval; HPA, hypothalamic–pituitary–adrenal.
[a]HPA activity (a composite measure of evening/night-time cortisol levels).
$\chi^2=37.79$, df = 3, $p=9.0001$.

associated with persistence of smoking during follow-up (see Table 5). Stressful experiences did not significantly influence persistent smoking. After accounting for the effects of smoking status and magnitude of stress at intake, HPA activity (evening/night-time cortisol levels) made a significant contribution to nicotine dependence symptoms in persistent smokers (R^2 change = 0.31, $F_{3,15}=3.84$, $p=0.03$). The analyses were run separately in depressed adolescents, and the same pattern emerged even after controlling for comorbid disorders. Among controls, stressful experiences moderated the effect of HPA activity (evening/night-time cortisol levels) on persistent smoking in such a way that the effect of elevated HPA activity (higher evening/night-time cortisol levels) on the likelihood of persistent smoking was reduced under conditions of low stress ($\beta_e=0.19$, CI = 0.05–0.71, $p=0.02$).

Relationship between Smoking and Depression during Follow-up

Among control subjects, 6/22 (27.3%) smokers developed a major depressive episode during follow-up compared with 8/67 (11.9%) non-smokers (Fisher's exact test, $p=0.10$). Of the six participants with both depression and smoking history, five subjects initiated smoking first and a depressive episode preceded smoking in one participant. Within the depressed group, 12/18 (61.1%) adolescents who reported smoking during follow-up developed a recurrent depressive episode compared with 9/33 (27.3%) youth with no smoking history ($\chi^2=5.60$, $p=0.02$). Of the 12 youngsters with both depression and smoking history, smoking preceded recurrent depressive episode in seven subjects and smoking initiation followed a recurrent depressive episode in five.

To examine whether the relationship between smoking and depression was accounted, in part, by HPA activity (evening/night-time cortisol levels) and stressful experiences, all three diagnostic groups were combined. Smoking during follow-up predicted depressive disorder ($\beta_e=3.61$, CI = 1.60–8.16, $p=0.002$; $\chi^2=9.49$, $p=0.002$). When HPA activity (evening/night-time cortisol levels) and stressful experiences were included in the model, the effect of smoking on depression was reduced ($\beta_e=1.28$, CI = 0.46–3.56, NS; $\delta\chi^2=14.47$, $p=0.001$).

DISCUSSION

To the best of our knowledge, this is the first study to integrate neurobiological and psychosocial factors in evaluating vulnerability factors for cigarette smoking in depressed and non-depressed adolescents. The findings indicate that both evening/night-time cortisol values and stressful life experiences are associated with increased risk for smoking in adolescents. It is noteworthy that higher evening/night-time cortisol values were observed before the onset of smoking in a subset of adolescents, and that stressful life experiences showed additive effects on the risk for smoking. In addition, higher evening/night-time cortisol values were associated with persistent smoking in youth who had prior smoking history. Smoking was also associated with increased frequency of depressive episodes.

Prior research has revealed that nicotine affects the HPA axis, possibly through interactions with nicotinic acetylcholine receptors (Berlin, 2009; Brenner *et al*, 1986; Chen *et al*, 2008; Markou, 2008; Rohleder and Kirschbaum, 2006; Sidhartha *et al*, 2009). In addition, studies in animals have shown that corticotropin-releasing factor and corticosteroids increase self-administration of nicotine, possibly by reducing sensitivity of the brain to the effects of nicotine, and thereby increasing the quantities of nicotine needed to elicit a particular response (Bruijnzeel *et al*, 2007; Caggiula *et al*, 1998; O'Dell and Khroyan, 2009; Pauly *et al*, 1990a; Zislis *et al*, 2007). Stress also increases the onset and relapse

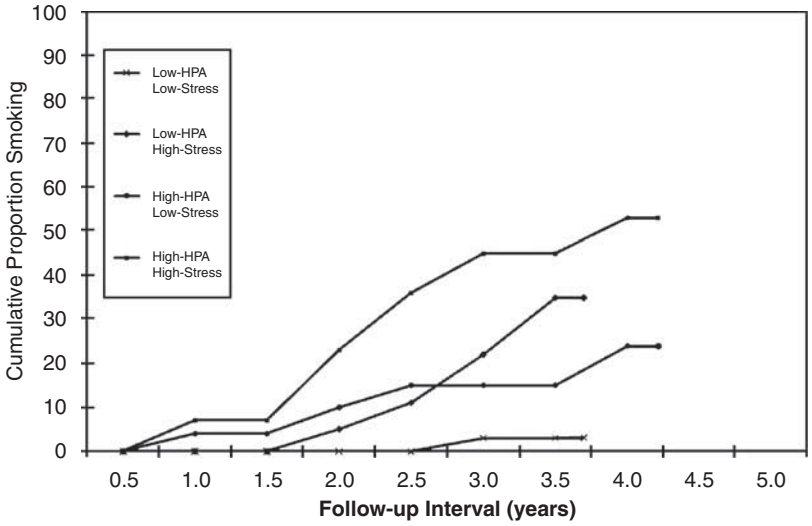

Figure 1 Probability of smoking during follow-up in adolescents who reported no prior smoking history at intake, stratified on HPA activity (a composite measure of evening/night-time cortisol levels) measured at baseline and stressful experiences at follow-up.

Table 4 Demographic and Clinical Parameters in Adolescents who Reported Prior Smoking History at Intake, Stratified on Smoking Status During Follow-up

	Abstain from smoking ($n=12$)	Persist with smoking ($n=19$)	Statistic	p
Age	15.4 (1.6)	15.5 (1.5)	0.19	NS
Gender			0.10	NS
Male	5 (41.7)	9 (47.4)		
Female	7 (58.3)	10 (52.6)		
Ethnicity			0.78	NS
Caucasian	7 (58.3)	8 (42.1)		
Non-Caucasian	5 (41.7)	11 (57.9)		
Socioeconomic score[a]	45.3 (12.0)	42.0 (11.1)	0.77	NS
Follow-up interval (years)	3.7 (0.8)	3.7 (1.1)	0.05	NS
Diagnosis at baseline			0.59	NS
Normal	4 (33.3)	4 (21.1)		
High risk	3 (25.0)	6 (31.6)		
Depression	5 (41.7)	9 (47.4)		
Hamilton depression rating scale	8.8 (9.5)	9.2 (9.4)	0.11	NS
Beck depression inventory	9.1 (8.1)	9.5 (8.4)	0.13	NS
Children's global assessment scale	68.1 (16.1)	62.9 (15.6)	0.89	NS
Stressful life experiences	3.1 (4.1)	5.3 (2.9)	1.77	0.09

[a]Higher score is associated with higher socioeconomic status (Hollingshead scale).
Data are presented as means or raw numbers; data in parentheses reflect standard deviations or percentages.

Table 5 Cox Regression Model Predicting Persistence of Smoking During Follow-up in Adolescents Who had Prior Smoking History at Intake

	β (SE)	Wald	OR (CI)	p
Model 1				
Smoking at intake (current smoker)	−0.32 (0.48)	0.44	0.73 (0.29–1.86)	NS
Stress at intake	0.09 (0.06)	2.24	1.09 (0.97–1.22)	NS
Model 2				
Smoking at intake (current smoker)	0.40 (0.57)	0.50	1.49 (0.49–4.53	NS
Stress at baseline	0.09 (0.08)	1.27	1.09 (0.94–1.27)	NS
HPA activity[a]	0.85 (0.33)	6.59	2.34 (1.22–4.48)	0.01
Stress at follow-up	0.20 (0.18)	1.25	1.23 (0.86–1.75)	NS
HPA activity × stress at follow-up	0.10 (0.18)	0.33	1.11 (0.78–1.56)	NS

Abbreviations: SE, standard error; OR, odds ratio; CI, confidence interval; HPA, hypothalamic–pituitary–adrenal.
[a]HPA activity (a composite measure of evening/night-time cortisol levels).
Model 1: $\chi^2=2.45$, df = 2, NS; $\delta\chi^2=2.42$, df = 2, NS.
Model 2: $\chi^2=15.53$, df = 5, $p=0.008$; $\delta\chi^2=10.80$, df = 3, $p=0.01$.

of smoking behavior in humans (Anda *et al*, 1999; Bruijnzeel and Gold, 2005; Byrne and Mazanov, 2003; Byrne *et al*, 1995; Cohen and Lichtenstein, 1990; McCarthy *et al*, 2006; Park *et al*, 2009; Parrott, 1995; Orlando *et al*, 2001; Perkins and Grobe, 1992; Shapiro *et al*, 2002). There are, however, individual differences in the magnitude of HPA response to stress, and in the ability of the HPA axis to modulate self-administration of nicotine (Frederick *et al*, 1998; Kudielka *et al*, 2009; Pauly *et al*, 1990b; Piazza *et al*, 1989; Pomerleau and Pomerleau, 1990; Sarnyai *et al*, 1998). Our data in adolescents are congruent with these observations in that they showed individual differences in evening/night-time cortisol levels and their relationship with smoking.

Consistent with earlier reports in adolescents and adults (Breslau *et al*, 1998; Brown *et al*, 1996; Choi *et al*, 1997; Fergusson *et al*, 2003; Goodman and Capitman, 2000; Patton

et al, 1998; Windle and Windle, 2001), a high proportion of depressed adolescents exhibited smoking behavior (although not significantly higher than control subjects), and smoking reciprocally increased the risk for development/recurrence of depressive episodes. Evidence suggests that the two conditions might have shared neurobiological substrates (Dursun and Kutcher, 1999; Gamberino and Gold, 1999; Kendler *et al*, 1993; Markou *et al*, 1998; Rao and Chen, 2008; Volkow, 2004). In addition to their association with smoking, numerous studies have demonstrated a role of HPA dysregulation and stressful life experiences in relation to depression (Hammen, 2005; Holsboer and Ising, 2008; Meyer *et al*, 2001; Kendler *et al*, 1995). In this study, higher evening/night-time cortisol values measured at baseline and stressful experiences during follow-up partially mediated the association between smoking and subsequent depressive episodes. Hence, reports of associations between depression and smoking behaviors might reflect, in part, shared neurobiological and psychosocial factors (Rao *et al*, 1999, 2000, 2009a).

Limitations

Several methodological issues should be considered in interpreting these results. The sample sizes were modest, and it is important to replicate these findings in larger cohorts. The participants in the depressed group had moderate to severe depression, and the study findings might not be generalizable to the population of depressed youth in the community. Moreover, there was insufficient power to detect the effect of comorbid disorders on vulnerability to smoking in the depressed cohort. Other studies reported that anxiety and disruptive behavior disorders increase the likelihood of smoking (Breslau *et al*, 1991; Brown *et al*, 1996; Johnson *et al*, 2000; Kendler *et al*, 1993; Pomerleau *et al*, 1995; Riggs *et al*, 1999). Some adolescents already initiated smoking before the cortisol measures were obtained. It is possible that prior nicotine exposure had an influence on cortisol values at least in some youngsters (Frederick *et al*, 1998; Friedman *et al*, 1987; Matta *et al*, 1998; Pomerleau and Pomerleau, 1990; Wilkins *et al*, 1982). Adolescents who did not manifest smoking behavior at intake, but subsequently initiated smoking, showed higher evening/night-time cortisol values before the onset of smoking compared with non-smokers, suggesting that higher evening/night-time cortisol values might serve as vulnerability markers for smoking behavior.

Careful assessment of the various stages of smoking, also including nicotine dependence, was not performed (Mayhew *et al*, 2000). In addition, objective measures of nicotine intake were not obtained. Nevertheless, subjective reports indicated that a substantial proportion of smokers manifested at least moderate nicotine dependence. Severity of nicotine dependence symptoms in the current sample was compared with what was observed in other studies (Colby *et al*, 2000; Prokhorov *et al*, 1996, 2001). Moreover, other investigations in adolescents demonstrated that frequency and quantity of smoking correlate well with both nicotine dependence symptoms and cotinine levels (Caraballo *et al*, 2004, 2009; O'Loughlin *et al*, 2003; Prokhorov *et al*, 2000; Rubinstein *et al*, 2007). Regardless, the magnitude of association between HPA activity/stress and different stages of smoking cannot be delineated from this study.

Only evening/night-time cortisol levels were measured as a reflection of HPA activity. Furthermore, the effect of cortisol values on smoking behavior was only modest. The inclusion of other adrenal steroids as well as precursors of cortisol would be a better reflection of the overall HPA activity, and possibly would have contributed a greater variance to vulnerability for smoking (Rasmusson *et al*, 2006).

Other important variables, such as genetic polymorphisms, temperament, early-life adversity, peer and family influences, and susceptibility to tobacco advertising, which influence the onset and progression of smoking, as well as HPA activity, were not measured (Davies and Soundy, 2009; Kudielka *et al*, 2009; Mayhew *et al*, 2000; Nilsson *et al*, 2009; Moolchan *et al*, 2000; Pallonen *et al*, 1998; Rao *et al*, 2008; Schepis and Rao, 2005). Despite these limitations, the study has several strengths. In addition to the neurobiological and psychosocial assessments, smoking initiation was documented prospectively at least in most of the subjects. Rigorous procedures were used for medical and psychiatric evaluations, and multiple saliva and urinary samples were obtained for HPA status. Moreover, the approach for assessment and coding of stressful life experiences was thorough.

Clinical Implications

If the finding that higher evening/night-time cortisol levels are associated with increased vulnerability for smoking and/or development of nicotine dependence is replicated, it will have clinical implications for developing more specific interventions. For example, metyrapone, an inhibitor of corticosteroid synthesis, and antagonists of the corticotropin-releasing hormone (CRH) reduce self-administration of nicotine and other addictive drugs in animals (Bruijnzeel *et al*, 2007; Fahlke *et al*, 1994; Piazza *et al*, 1994; Zislis *et al*, 2007). Antiglucocorticoid agents and CRH antagonists might have antidepressant properties, and have been tested in humans for the treatment of depression and other psychiatric disorders (Gallagher *et al*, 2008; Holsboer and Ising, 2008; Seymour *et al*, 2003). Moreover, bupropion and other antidepressant drugs are effective in reducing smoking in addition to alleviating depressive symptoms (Hajek *et al*, 2009; Schepis and Rao, 2008; Tonstad, 2002). Data from both clinical and preclinical studies suggest that treatment with most antidepressant agents reduces responsiveness to stress (Duman *et al*, 1999; Holsboer, 2001).

It will be important to examine whether HPA activity measured before initiating treatment predicts clinical response (Rao *et al*, 2005). If smokers with increased HPA activity benefit most from treatment with antidepressant drugs, this particular subgroup could be targeted for pharmacological intervention, and thus minimize the unnecessary exposure of other young smokers to psychotropic medications. In addition to HPA activity, stressful experiences contributed to the vulnerability for smoking. Adolescent smokers frequently report stress reduction as a motive for smoking (Dozois *et al*, 1995; Nichter *et al*, 1997). The data from this study suggest that such persons might benefit from adjunctive psychosocial interventions, such as

assertiveness training and cognitive-behavior therapy, aimed to reduce and/or cope with stressful experiences (Schepis and Rao, 2008). Future studies should evaluate the efficacy of pharmacotherapy and various psychosocial interventions, singly and in combination, in smokers stratified on levels of stress.

Several factors influence the onset and developmental progression of smoking. The fact that individuals might begin on the same developmental pathway, but experience different patterns of tobacco use, leaves open the possibility that risk and resilience factors exert their influence on individuals over time (Mayhew *et al*, 2000). Therefore, to better understand the risk/resiliency factors associated with adolescent smoking, we need to move from simplistic designs to integrated, transactional models. Such approaches will be helpful not only in developing more effective preventive and treatment strategies for youngsters, but also for the treatment of nicotine addiction in adults.

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DISCLOSURE

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