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Research report

Cortisol responses to psychosocial stress predict depression trajectories: Social-evaluative threat and prior depressive episodes as moderators

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ABSTRACT

Background: Alterations of hypothalamic-pituitary-adrenal (HPA) function are well-established in adults with current depression. HPA alterations may persist into remission and confer increased risk for recurrence.
Methods: A modified version of the Trier Social Stress Test (TSST) was administered at baseline to 32 young adults with remitted major depressive disorder and 36 never-depressed controls. Participants were randomly assigned to either a 'high-stress' condition involving social evaluation or a 'low-stress' control condition. Cortisol concentrations were measured in saliva samples throughout the TSST. Participants were assessed again after 6 months for the occurrence of stressful life events and depressive symptoms/disorders during the follow-up period.
Results: Participants who exhibited enhanced cortisol reactivity in the low-stress condition showed increases in depressive symptoms over follow-up, after controlling for stressful life events during the follow-up period. Anticipatory stress cortisol and cortisol reactivity each interacted with history of depressive episodes to predict depression trajectories.
Limitations: The single TSST administration limits conclusions about whether alterations of cortisol reactivity represent trait-like vulnerability factors or consequences ("scars') of past depression.
Conclusions: These results extend previous findings on stress sensitivity in depression and suggest that altered HPA function during remission could reflect an endophenotype for vulnerability to depression recurrence. Findings support interactive models of risk for depression recurrence implicating HPA function, depression history, and sensitivity to minor stressors. Results may have implications for interventions that match treatment approaches to profiles of HPA function.

1. Introduction

Major depressive disorder (MDD) is a common (Kessler et al., 2005) and highly debilitating psychiatric condition (The World Bank, 2006) linked to a host of psychosocial difficulties and negative health outcomes for the individual (Frasure-Smith and Lesperance, 2005; Lewinsohn et al., 2003; Rao et al., 1995) and a significant economic burden on society (Greenberg and Birnbaum, 2005). Risk for recurrence increases with each successive major depressive episode (MDE): 60% of individuals with one MDE will experience a second episode; 70% of those with two MDEs will experience a third episode; and up to 90% of those with three or more MDEs will experience further recurrent episodes (American Psychiatric Association, 2000; Solomon et al., 2000). Identifying factors associated with increased risk for recurrence is critical for the development and refinement of effective interventions.

Depression is often conceptualized as a stress-related disorder. The relation between stressful life events and depression is well-established (Kendler et al., 1998) and appears to strengthen over successive MDEs (Morris et al., 2010). According to stress sensitization models that seek to explain changes in risk over time, minor stressors become increasingly capable of triggering MDEs with each recurrence possibly due to neurobiological sensitization. That is, individuals at greater risk for depression recurrence will exhibit increased sensitivity to the depressogenic effects of lower level stressors. In contrast, stress autonomy models propose that the association of stressors and MDE onsets weakens with each recurrence, such that MDEs eventually may emerge autonomously, without an apparent trigger (for a review of stress sensitization and autonomy models, see Monroe and Harkness, 2005). Stress

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sensitivity may be indexed by changes in reactivity of stress response systems, including the hypothalamic-pituitary-adrenal (HPA) axis, which has been implicated as a mediator of stress sensitization and vulnerability to depressive episodes (Oldehinkel and Bouma, 2011).

Altered HPA function is well-documented in depressed individuals (Burke et al., 2005; Holsboer, 2000), but whether, and to what extent, these alterations persist into remission and recovery is not well understood. Several converging lines of evidence suggest that persistent HPA alterations during remission are associated with increased risk for recurrence (Appelhof et al., 2006; Chopra et al., 2008; Zobel et al., 1999). Additionally, cortisol levels may interact with previous MDEs (Chopra et al., 2008) and acute stress levels (Rao et al., 2010) to predict recurrence. Finally, individuals with recurrent depression are more likely, with each additional MDE, to exhibit elevated cortisol levels during remission (Bos et al., 2005; Gurguis et al., 1990).

The present study extends the literature on stress sensitization by examining cortisol responses to an experimental stressor as a predictor of depressive symptom trajectories and probability of MDE recurrence. Prior research examining cortisol responses to tasks with manipulation of stress levels (e.g., success versus failure, control over an aversive stimulus, degree of difficulty of a cognitive task) has been cross-sectional. These studies have found impaired neuroendocrine function in currently depressed (CD) as compared to never depressed (ND) individuals (Croes et al., 1993; Netter et al., 1991), elevated cortisol responses in a high-stress condition in CD versus ND individuals but no between-group differences in a low-stress condition (Breier, 1989), or no differences between CD and ND groups in cortisol responses to either high- or low-stress conditions (Ravindran et al., 1996). Inconsistencies in this literature may be attributed to differences in psychosocial stress paradigms. Studies employing modifications of stress tasks known to elicit robust cortisol responses (Dickerson and Kemeny, 2004) have shown greater cortisol reactivity in high compared to low social-evaluative threat conditions in healthy controls (Balodis et al., 2010; Gruenewald et al., 2004; Het et al., 2009; Way and Taylor, 2010). In the current study, we randomly assigned participants to either a high-stress condition involving social-evaluative threat or a low-stress control condition without a social evaluation component. Whereas transient cortisol increases in the high-stress condition would be considered adaptive, elevated cortisol levels in the low-stress condition could reflect a pattern of maladaptive responding (McEwen, 1998) consistent with increased stress sensitivity and depression vulnerability. In line with stress sensitization models, we hypothesized that higher cortisol reactivity to a minimal laboratory stressor would predict significant increases in depressive symptoms over a six-month follow-up.

The present study also contributes to the literature on HPA predictors of depression trajectories and recurrence by examining moderators of risk in a sample of remitted depressed (RD) and ND young adults. We hypothesized that the relation between anticipatory stress cortisol levels or cortisol reactivity and depressive symptom trajectories would be stronger for those with a greater number of prior MDEs after controlling for stressful events during the follow-up.

2. Methods

2.1. Participants

Participants were 68 individuals (32 RD, and 36 ND), ages 18 to 31 years (mean age=23.39, *SD*=3.88); 43 were female (63%); mean SES level was 54.05 (*SD*=12.10). Baseline demographic, clinical and HPA data are presented in Table 1. Participants were recruited primarily from undergraduate and graduate programs at Vanderbilt University and consisted of young adults with either remitted MDD or no history of MDD. Of 32 participants with a history of depressive episodes, 13 had experienced one prior MDE, 10 had experienced two episodes, and 9 had experienced three or more episodes. All participants were randomly assigned (based on history of depression and sex) to either a social evaluation (high-stress) condition (n=34) or a no-evaluation (low-stress) condition (n=34), and were part of a larger study examining cortisol reactivity to a psychosocial stressor in remitted depressed and never depressed young adults.

Exclusion criteria included the following: current MDD, current or past bipolar disorder or post-traumatic stress disorder or health conditions (Cushing's disease, Addison's disease, hyperthyroidism, severe kidney or liver disease, pregnancy, hypoglycemia, diabetes) known to influence HPA function. Individuals using prescription (corticosteroids, amphetamines) and non-prescription (e.g., marijuana) drugs that might affect cortisol levels also were excluded. Participants using antidepressants (n=9) or birth control (n=26) were not excluded, however.

Individuals who screened for either (a) a history of MDD but were not currently in a depressive episode (i.e., remitted depressed; RD) or (b) no current or prior history of MDD (i.e., never depressed; ND) were scheduled for the clinical assessment and laboratory tasks. Inclusion in the RD group required a past diagnosis of MDD in full remission according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition (American Psychiatric Association, 2000) criteria as assessed with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I; First et al., 1997). Full remission was defined as an absence of

Table 1
Means and standard deviations of study variables for remitted depressed and never depressed participants.

	Remitted depressed (n=32) *M* (*SD*)	Never depressed (n=36) *M* (*SD*)	RD vs. ND X^2/t
Age (years)	23.9 (3.9)	23.0 (3.9)	0.98
Body mass index (BMI)	24.1 (4.9)	24.1 (5.5)	0.05
Socioeconomic status (SES)	53.3 (11.6)	54.7 (12.7)	0.49
	N (%)	*N* (%)	X^2/t
Sex			3.60~
Male	8 (25.0)	17 (47.2)	
Female	24 (75.0)	19 (52.8)	
Race			0.32
Caucasian	23 (71.9)	28 (77.8)	
Non-Caucasian	9 (28.1)	8 (22.2)	
	M (*SD*)	*M* (*SD*)	*t*
Depressive symptoms (BDI-II)	8.72 (6.6)	3.42 (3.4)	4.11**
Cortisol			
Low-stress condition			
Baseline (ng/ml)	0.96 (.16)	1.04 (.20)	1.32
Anticipatory	0.93 (.15)	1.02 (.19)	1.52
AUCg	39.36 (8.41)	43.90 (9.11)	1.49
High-stress condition			
Baseline (ng/ml)	1.03 (.12)	1.10 (.20)	1.27
Anticipatory	1.00 (.14)	1.04 (.18)	0.85
AUCg	43.98 (7.06)	50.28 (7.12)	2.59*

RD=remitted depressed; ND=never depressed; Baseline=T0 cortisol (log-transformed); Anticipatory=T1 cortisol (log-transformed); AUCg=area under the curve with respect to ground (log-transformed).

~ $p<.06$.
* $p<.05$.
** $p<.001$.

significant depressive symptoms for at least two months. Participants received 6 course credits or $30 for participation in the baseline assessment and $10 for participation in the follow-up assessment. Informed written consent was obtained from all participants at baseline and follow-up assessments. All procedures were approved by the Vanderbilt University Institutional Review Board.

2.2. Baseline measures

Depression. The Mood Disorders and PTSD modules of the SCID-I (First et al., 1997) were used to assess inclusion and exclusion DSM-IV diagnostic criteria. Detailed information about all previous depressive episodes was obtained to determine the number of prior MDEs. All interviews were conducted by a trained graduate student (MCM) who was supervised by an expert clinical interviewer (JG). Interviews were audio-taped and a random 20% were re-rated for reliability by an independent evaluator. Inter-rater reliability for history of depression yielded a kappa of 1.00.

The *Beck Depression Inventory-II* (BDI-II; Beck et al., 1996a) was used to assess depressive symptoms at baseline. The BDI-II is a 21-item, widely used, self-report inventory with good reliability and validity (Beck et al., 1996b). In this sample, coefficient alpha for the BDI-II was.85.

Psychosocial Stressor. A version of the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), modified to include two experimental conditions manipulating the degree of social evaluation, was used to elicit cortisol response to the stressor. The task consisted of a 5-min free-speech task and a 5-min mental arithmetic task administered sequentially either in a social evaluation ('high-stress') condition or in a no evaluation ('low-stress') condition.

Cortisol. Salivary cortisol samples were collected using a saliva collection device (Salivette; Sarstedt Inc., Newton, NC). Cortisol levels were determined in duplicate using a commercially available enzyme immunoassay kit (Enzyme-Linked ImmunoSorbent Assay, ALPCO diagnostics, Salem, NH). The lower detection limit, or sensitivity, of this assay is 1.0 ng/ml.

Socio-demographic Characteristics. Body Mass Index (BMI), an indicator of body fat, was calculated by dividing each participant's body weight by the square of his/her height. SES was calculated using the Hollingshead four-factor index (Hollingshead, 1975, Unpublished).

2.3. Follow-up measures

Depression. The Longitudinal Interval Follow-Up Evaluation (LIFE; Keller et al., 1987), which parallels the SCID-I, was administered at the follow-up assessment and used to assess depressive symptoms and disorders for each week of the follow-up interval period. Participants were provided with chronological 'anchors' (e.g., salient events, holidays) to increase the likelihood of symptom recall. The LIFE yields a depressive symptom rating (DSR) score from 1 to 6, reflecting the extent of depressive symptoms and impairment for each week of the follow-up period. A score of 3 indicates fewer symptoms (e.g., two to three symptoms) than full *DSM–IV–TR* criteria with mild or moderate impairment; 4 indicates four symptoms with moderate to marked impairment; and ≥ 5 indicates an MDE according to *DSM–IV–TR* criteria, and significant impairment.

Recent life events. The Perceived Events Scale (PES; Compas et al., 1987) was used to measure the number and severity of life events experienced by participants during the follow-up interval. Participants were asked to indicate whether each event occurred during the follow-up period, and to rate the valence of those events on a 9-point scale (-4=Extremely Bad; 0=Neither Good or Bad; +4=Extremely Good). Participants were interviewed by study staff following completion of the PES online to determine the timing and duration of all reported life events. Total weekly stress level scores were calculated by summing ratings for all negative events (rated −1 to −4 on desirability) occurring each week. Total stress level scores were multiplied by −1 so that higher scores indicated higher stress levels. Stress level was included as a covariate in all data analytic models to control for the effects of individual differences in stress exposure on depressive symptoms.

2.4. Procedures

Individuals were screened by telephone regarding inclusion and exclusion criteria; eligible persons were invited to participate. Participants were instructed to not drink alcohol, smoke, use illegal drugs, engage in strenuous exercise, or visit the dentist within 24 h prior to their appointment, and to refrain from drinking (except water), eating, or brushing their teeth 1 h before the session. Participants were screened for these behaviors at the beginning of the laboratory assessment. All laboratory sessions were conducted in the afternoon/early evening (i.e., between 2:00 and 7:00 PM) to minimize the effects of diurnal variation in cortisol.

After participants provided informed consent, they were administered the SCID-I modules. They completed the BDI-II and sat quietly for 10 min. Following this rest period, participants provided the first cortisol sample (T0: baseline). Then they were informed about the laboratory tasks and they provided the second cortisol sample at the end of the 10-min preparation period (T1: anticipatory stress). Participants then were escorted to another room where they were given their task assignment. Half of the participants in each group (i.e., RD and ND) were randomly assigned to the high-stress condition and the other half to the low-stress condition. Participants in the high-stress condition were informed that their performance would be judged by a panel of evaluators, whereas those in the low-stress condition were informed that they would perform the tasks while alone and unobserved. For all participants, the speech task preceded the mental arithmetic task. Participants provided the third (T2: mid-task) cortisol sample between the speech and arithmetic tasks and the fourth (T3: post-task) cortisol sample immediately following the arithmetic task. They then completed a demographics questionnaire, rested for 10 min, provided the fifth (T4: recovery 1) cortisol sample, rested another 10 min, and then provided the sixth (T5: recovery 2), and final, cortisol sample. At the end of the baseline assessment, participants were fully debriefed regarding the nature of the experimental manipulation. Participants were re-contacted 6 months after the baseline assessment and asked to complete an online questionnaire (PES) about life events that had occurred since the baseline assessment, and a phone interview to assess the timing of the life events reported on the PES and the frequency and duration of depressive symptoms occurring since baseline, as reported on the LIFE.

2.5. Data analytic plan

All variables were examined for distributional properties and cases were screened for univariate and multivariate outliers. Cortisol data were log-transformed to reduce skewness. Cortisol measures included anticipatory (T1) cortisol levels and area under the curve with respect to ground (AUCg; Pruessner et al., 2003). The AUCg characterizes total cortisol output during the TSST and represents both anticipatory stress cortisol (T1) output and stressor-induced change in cortisol levels. The anticipatory stress cortisol and AUCg cortisol capture independent aspects of stress

reactivity and should be examined simultaneously (e.g., Balodis et al., 2010). Multilevel models (MLM) were used to examine associations between independent variables (depression, history, stressor condition, and cortisol indices) and the dependent variable (weekly DSR from the LIFE). The interactions of stressor condition (i.e., low vs. high) by AUCg cortisol, and between the number of prior MDEs and the cortisol measures also were tested. Covariates included age, sex, SES, BMI, antidepressant use, birth control pills, baseline depressive symptoms, number of MDEs at baseline, baseline (T0) cortisol levels, and weekly stress levels during follow-up. Covariates significantly associated with the dependent variable were included in the MLM. Variables included in interactions were centered. Simple slope analyses were conducted on all significant interactions (Aiken and West, 1991).

To address the hypotheses regarding within- and between-individual change simultaneously, we specified a series of MLM using hierarchical linear models (HLM 6) (Raudenbush et al., 2004) consisting of a within-person (i.e., level-1) sub-model describing how each individual changed over time and a between-person (i.e., level-2) sub-model describing how these changes varied across individuals (Bryk and Raudenbush, 1992; Singer and Willett, 2003). Preliminary analyses revealed that 25% of the total variation in DSR intercepts and slopes could be explained by differences between participants, suggesting that sufficient heterogeneity existed to examine substantive level-1 and level-2 predictors. For MLM, all Level 1 predictors were person-centered (i.e., the means of these variables equaled zero for each person) and person means for time-varying predictors were included in level-2 models to remove between-person variance from within-person variables and prevent predictors from correlating with individual intercepts or between-person factors (Hoffman and Stawski, 2009). To address hypotheses regarding time to MDE, Cox regression models were run with SPSS Version 17.0 for Windows; the primary outcome was MDE onset (DSR ≥ 5 for two or more consecutive weeks).

The mean number of weeks between baseline and follow-up was 35.16 ($SD=9.03$). By the end of the follow-up, eight participants had experienced one MDE and one participant had experienced two MDEs. MLM and Cox regression are both well-suited to the analysis of longitudinal data with varying follow-up intervals.

3. Results

3.1. Descriptive analyses

AUCg cortisol levels were significantly higher in the high-stress ($M=549.35$, $SD=226.00$) compared to the low-stress ($M=431.26$, $SD=218.64$) condition [$t(66)=-2.19$, $p=.03$]. Anticipatory cortisol levels were not significantly different in the high-stress ($M=11.21$, $SD=4.70$) as compared to the low-stress ($M=10.39$, $SD=4.87$) condition [$t(66)=-0.71$, $p=.48$]. Individuals taking antidepressants ($n=9$) did not differ significantly from non-medicated individuals on severity of depressive symptoms or cortisol measures. Sex, age, oral contraceptive use and SES were not significantly associated with mean DSRs over the follow-up. Significant associations were found between mean weekly DSRs and number of prior MDEs ($B=.01$), $t(66)=4.04$, $p<.001$, and baseline depressive symptoms ($B=.01$), $t(66)=4.13$, $p<.001$; therefore, these variables were included as covariates in subsequent analyses. All models controlled for baseline (T0) cortisol levels.

A series of lagged effects models assessing the predictive association of within-subject stress levels to DSRs revealed that the strongest association was at a lag of 7 weeks; subsequent analyses controlled for prior stress levels and depressive symptom ratings lagged at 7 weeks. Thus, these analyses provided a rigorous test of interactive models predicting DSRs by controlling for the effects of prior stress levels and prior DSRs. We first examined whether stressor condition moderated the relation between AUCg cortisol and depressive symptom trajectories. In addition, we examined whether this interaction was associated with the probability of depression during follow-up. Next, we examined whether number of previous MDEs moderated the relation between predictors and depressive symptom trajectories and whether these interactions were associated with the probability of depression during follow-up.

Do cortisol responses to the laboratory stressor condition (i.e., high vs. low stress) predict depression (i.e., depressive symptom trajectories, the probability of a depressive episode) during the follow-up? Controlling for within-individual covariates (lagged DSRs and stress levels, time) and between-individual covariates (baseline depressive symptoms, number of prior MDEs, baseline cortisol levels, person means for DSRs and stress levels), the interaction of AUCg cortisol, stressor condition, and time was significant [$B=-.010$), $t(1,885)=-2.74$, $p=.007$]. Simple slope analyses revealed that for individuals in the high-stress condition, neither higher ($B=.007$, $t=1.27$, $p=.205$) nor lower ($B=.009$, $t=1.86$, $p=.063$) AUCg cortisol responses significantly predicted depressive symptom trajectories. In the low-stress condition, higher AUCg cortisol responses predicted significant increases in DSRs ($B=.024$, $t=5.35$, $p<.001$), whereas lower AUCg cortisol responses did not predict change in DSRs ($B=.005$, $t=1.54$, $p=.124$). The interaction of stressor condition and AUCg cortisol did not predict the probability of a depressive episode during follow-up (Table 2, Model 1).

Does the number of prior MDEs interact with anticipatory stress cortisol levels or cortisol responses to the laboratory psychosocial stressor to predict depression (i.e., depressive symptom trajectories, the probability of depressive episodes) during the follow-up? Controlling for within-individual covariates (lagged DSRs and stress levels, time) and between-individual covariates (baseline depressive symptoms, stress condition, baseline cortisol levels, person means for DSRs and stress levels), the

Table 2
Cox regression models predicting MDEs over follow-up.

	B (*SE*)	Wald	OR (95% *CI*)	*p*
Model 1				
Stressor Condition	−0.59 (0.52)	1.31	0.55 (0.20–1.52)	.25
BDI-II	0.62 (0.31)	3.88	1.85 (1.00–3.41)	.05
Baseline cortisol	0.28 (0.16)	2.93	1.32 (0.96–1.80)	.09
Depression History	0.51 (0.23)	5.16	1.67 (1.07–2.61)	.02
AUCg	−1.71 (1.11)	2.37	0.18 (0.02–1.60)	.12
AUCg × Stressor	−0.71 (0.47)	2.29	0.49 (0.20–1.23)	.13
Model 2				
Stressor condition	−0.59 (0.48)	1.52	0.56 (0.22–1.42)	.22
BDI-II	0.16 (0.43)	0.14	1.17 (0.51–2.69)	.71
Baseline cortisol	0.19 (0.17)	1.23	1.20 (0.87–1.67)	.27
Depression history	1.78 (0.81)	4.78	5.91 (1.20–29.04)	.03
Anticipatory	−0.17 (1.25)	0.02	0.84 (0.07–9.76)	.89
Anticipatory × Dep Hx	2.25 (1.09)	4.23	9.50 (1.11–81.14)	.04
Model 3				
Stressor condition	−0.54 (0.57)	0.89	0.59 (0.19–1.79)	.35
BDI-II	0.54 (0.32)	2.76	1.71 (0.91–3.23)	.10
Baseline cortisol	0.26 (0.15)	3.08	1.30 (0.97–1.74)	.08
Depression history	1.39 (0.59)	5.59	4.03 (1.27–12.80)	.02
AUCg	−0.91 (1.04)	0.76	0.40 (0.05–3.11)	.38
AUCg × Dep Hx	2.34 (1.53)	2.35	10.34 (0.52–205.55)	.13

Note: BDI-II=Beck Depression Inventory – II (baseline); Dep Hx=Depression History=number of prior MDEs; AUCg=cortisol area under the curve with respect to ground; Stressor=stressor condition; Anticipatory=T1 cortisol (ng/ml); *SE*=standard error; OR=odds ratio; *CI*=confidence interval. Model 1: $\chi^2=16.60$, df=6, $p=.011$. Model 2: $\chi^2=52.80$, df=6, $p<.001$. Model 3: $\chi^2=18.03$, df=6, $p=.006$.

Table 3
Multilevel models predicting depressive symptom ratings.

Predictors	Anticipatory stress cortisol *B* (*SE*)	AUCg cortisol *B* (*SE*)
Intercept	−.363 (.10)***	−.351 (.08)***
Week	.013 (.00)***	.013 (.00)***
Lagged weekly stress levels	.003 (.01)	−.001 (.01)
Lagged weekly DSR scores	−.171 (.05)***	−.168 (.05)***
Stress person mean	.006 (.00)	.006 (.00)
DSRs person mean	1.051 (.04)***	1.031 (.04)***
Baseline cortisol	.002 (.01)	.001 (.00)
Baseline depressive symptoms	−.024 (.02)	−.029 (.02)
Prior major depressive episodes	−.533 (.05)***	−.581 (.09)***
Stressor condition	−.036 (.03)	−.034 (.04)
Cortisol measure	−.251 (.06)***	−.221 (.08)**
Cortisol × week	.011 (.00)***	.010 (.00)**
Cortisol × prior MDEs	−.684 (.12)***	−.583 (.23)*
Prior MDEs × week	.025 (.00)***	.027 (.00)***
Cortisol × prior MDEs × week	.032 (.01)***	.025 (.01)**

Note: DSRs=depressive symptom ratings; MDEs=major depressive episodes; AUCg=area under the curve with respect to ground.

* $p<.05$.
** $p<.01$.
*** $p<.001$.

interaction of anticipatory stress cortisol levels, number of previous MDEs, and time significantly predicted depressive symptoms [($B=.032$), $t(1,885)=5.84$, $p<.001$; see Table 3 and Fig. 1]. Simple slope analyses revealed that higher anticipatory stress cortisol levels predicted increases in depressive symptom ratings for individuals with no prior MDEs ($B=.024$, $t=8.34$, $p<.001$), one prior MDE ($B=.081$, $t=13.66$, $p<.001$), two prior MDEs ($B=.137$, $t=12.93$, $p<.001$), and three or more prior MDEs ($B=.194$, $t=12.47$, $p<.001$).

Cox regression models revealed that the interaction of anticipatory stress cortisol levels and number of prior MDEs significantly predicted the probability of a depressive episode during the follow-up period (see Table 2, Model 2). To graph this interaction (see Fig. 2), we stratified the sample into the following four groups based on median splits of anticipatory stress cortisol and number of prior MDEs: (1) low-anticipatory stress cortisol and fewer prior MDEs ($n=13$); (2) high-anticipatory stress cortisol and fewer prior MDEs ($n=23$); (3) low-anticipatory stress cortisol and more prior MDEs ($n=20$); (4) high-anticipatory stress cortisol and more prior MDEs ($n=12$). Depressive episodes occurred in 0% of individuals in Group 1, 8.7% of those in Group 2, 10.0% of those in Group 3, and 33.3% of those in Group 4. The overall analysis comparing these four groups regarding the probability of a depressive episode during the follow-up period was not significant ($\chi^2=4.34$, df=3, $p=.23$). Exploratory post-hoc pairwise comparisons, however, revealed that individuals with high-anticipatory stress cortisol and more MDEs (Group 4) were at significantly greater risk of recurrence compared to individuals in Group 1with low-anticipatory stress cortisol and fewer MDEs ($\chi^2=4.15$, $p=.042$). The other comparisons were not statistically significant.

Controlling for within-individual covariates (lagged DSRs and stress levels, time) and between-individual covariates (baseline depressive symptoms, stressor condition, baseline (T0) cortisol levels, person means for DSRs and stress levels), the interaction of AUCg cortisol, number of previous MDEs, and time also significantly predicted DSR scores during follow-up [$B=.025$, $t(1,885)=2.68$, $p=.008$; Table 3]. Simple slope analyses revealed that higher AUCg cortisol significantly predicted increases in DSR scores for individuals with no prior MDEs ($B=.023$, $t=5.71$, $p<.001$), one prior MDE ($B=.075$, $t=6.64$, $p<.001$), two prior MDEs ($B=.127$, $t=6.18$, $p<.001$), and three or more prior MDEs ($B=.179$, $t=5.96$, $p<.001$). The interaction of AUCg cortisol and number of prior MDEs did not significantly predict the probability of a depressive episode during follow-up (Table 2, Model 3).

4. Discussion

The present study examined the effects of social-evaluative threat and number of previous MDEs as moderators of the relation between cortisol reactivity and depressive symptom trajectories in individuals with or without a history of a prior depressive episode. Consistent with our hypotheses, increases in depressive symptoms over the 35 week follow-up were found for individuals who exhibited higher cortisol reactivity to the low-stress (no social evaluation) condition. That is, individuals who showed higher cortisol reactivity to a relatively low-level stressor were at greater risk for experiencing subsequent depressive symptoms. In contrast, cortisol reactivity to the high-stress condition did not significantly predict depressive symptoms over follow-up.

These results expand upon previous studies testing stress sensitization hypotheses regarding prospective changes in the association of stress to depression (e.g., Hammen et al., 2000; Monroe et al., 2006; Morris et al., 2010) by demonstrating a significant relation between cortisol reactivity to a laboratory stressor and the trajectory of depressive symptoms over time. Whereas transient cortisol increases in response to acute stressors involving social evaluation are typical for healthy controls (Dickerson and Kemeny, 2004) and may be adaptive (Cicchetti and Rogosch, 2001; de Kloet et al., 1999; Oitzl et al., 2010), the results of the current study suggest that enhanced cortisol reactivity to a relatively minor stressor (i.e., the 'placebo TSST') may represent a risk for depression, and could reflect an endophenotype for sensitivity to the depressogenic effects of stress (Oldehinkel and Bouma, 2011). The interaction of the laboratory stressor condition and cortisol reactivity, however, did not predict the probability of an MDE during follow-up. This negative finding likely reflects the low incidence of MDEs during the relatively short follow-up period and the modest sample size.

Consistent with our hypotheses, both anticipatory stress cortisol levels and cortisol reactivity interacted with number of previous MDEs to predict depressive symptom trajectories. The rate of increase in DSR scores over time for individuals with higher anticipatory stress cortisol was stronger for those with more prior MDEs. Moreover, this interactive model also significantly predicted a shorter time to onset of an MDE during follow-up (Table 2, Model 2). These findings are partially consistent with a study by Chopra et al. (2008) who reported an increased risk of relapse among remitted depressed patients who had high pre-stress cortisol levels regardless of number of prior MDEs and for those with low pre-stress cortisol who had experienced three or more prior MDEs (Chopra et al., 2008). Pre-stress cortisol levels likely reflect anticipatory stress linked to the novelty of the laboratory environment and the uncontrollability of the experimental procedure. Both novelty and uncontrollability have been associated with cortisol responses to acute stressors (Dickerson and Kemeny, 2004). The current findings extend prior work examining the link between anticipatory stress and cortisol levels (Balodis et al., 2010) and suggest that anticipatory stress cortisol may be a predictor of depression recurrence.

In the present study, the rate of increase in DSR scores for individuals with greater cortisol reactivity was stronger for those with more prior MDEs; in contrast, Chopra et al. (2008) reported that remitted depressed patients did not show a cortisol response to a psychosocial stress task. This discrepancy may be due to their use of a mood induction procedure, which is not generally

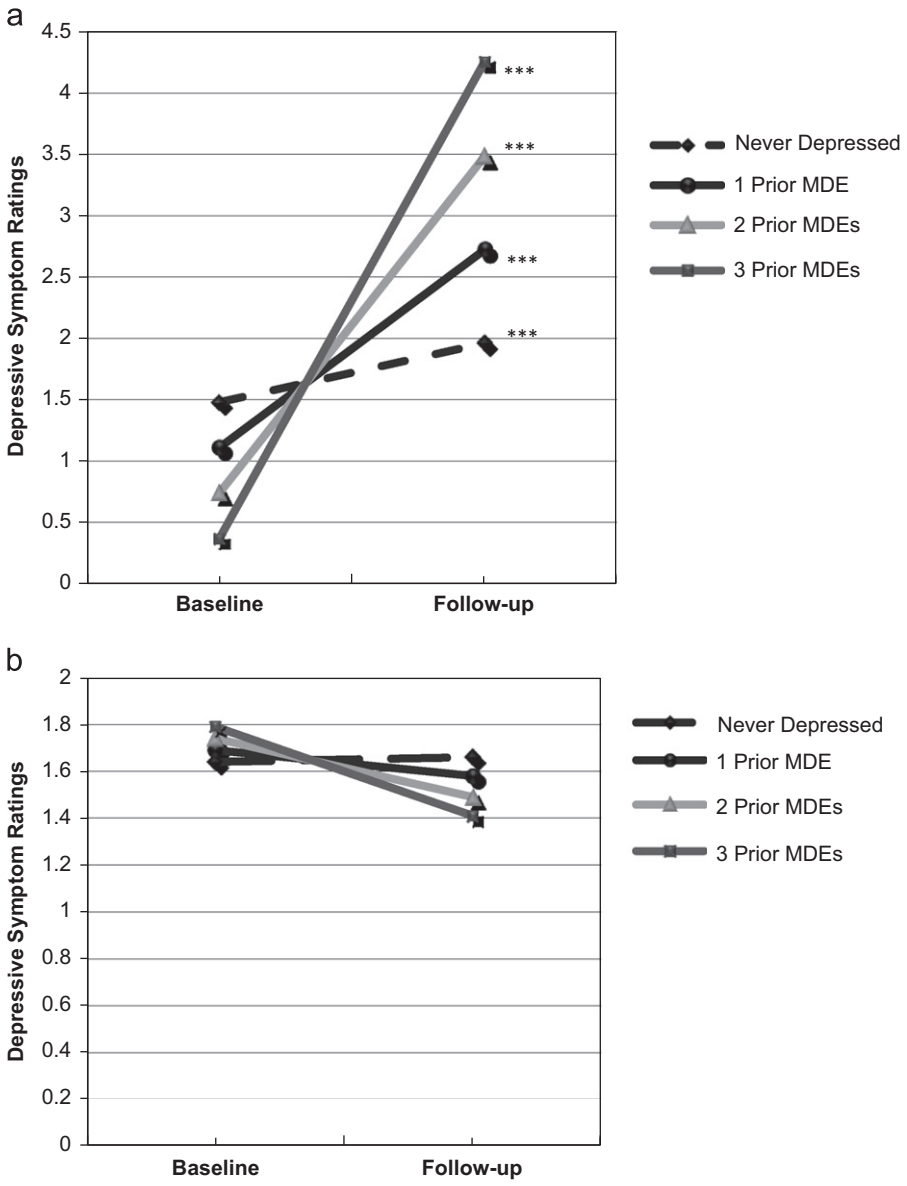

Fig. 1. Interaction of anticipatory stress cortisol, number of previous MDEs, and time predicting depressive symptom ratings (DSRs). Relations among number of previous MDEs and DSR trajectories are shown separately for individuals with higher anticipatory stress cortisol levels (Panel A) and lower anticipatory stress cortisol levels (Panel B). $^{***}p < .001$.

associated with significant cortisol responses (Dickerson and Kemeny, 2004); additionally, Chopra et al. did not adequately control for diurnal variation of cortisol levels. Thus, overall the results of the current study indicate that risk for depression recurrence can be predicted by interactions among HPA function, prior MDEs, and sensitivity to minor stressors.

Interpretation of these results should be tempered by limitations of the present study. First, the baseline cortisol sample was obtained within 1 h of participants' arrival to the laboratory and may reflect HPA activation due to novelty of the environment and anticipatory anxiety. Future studies should adopt more stringent assessment procedures to obtain "true" baseline values (e.g., Rao et al., 2008). Nevertheless, this sampling schedule was applied to all participants; support for our hypotheses was found despite the possibility of less than ideal baseline cortisol levels.

Second, the sample primarily consisted of undergraduate and graduate students from a private university and may not generalize to clinical populations. Nevertheless, our ability to detect significant interactions that predicted risk for recurrence in this non-clinical sample increases confidence in the robustness of these effects. Third, because HPA function was determined only at the laboratory assessment and not also at the follow-up, we cannot determine whether increased sensitivity to minor stressors represents a 'scar marker' of previous MDEs or a relatively stable 'trait marker' that predisposes individuals to depression. Prospective studies need to examine changes in cortisol reactivity to different stressor intensities by exposing participants to multiple psychosocial stress tasks over time.

Findings from the present study have several clinical implications. First, individual differences in HPA function during remission may serve as an indicator of vulnerability to recurrent depression (Adam et al., 2008), complementing previous studies of prognostic indicators in currently depressed individuals (Rao et al., 1996, 2010). Second, these findings highlight the importance of early intervention and prevention programs that promote the development of strategies for coping with stressors during early depressive episodes (e.g., Garber et al., 2009). Future studies should examine the extent to which cortisol elevations

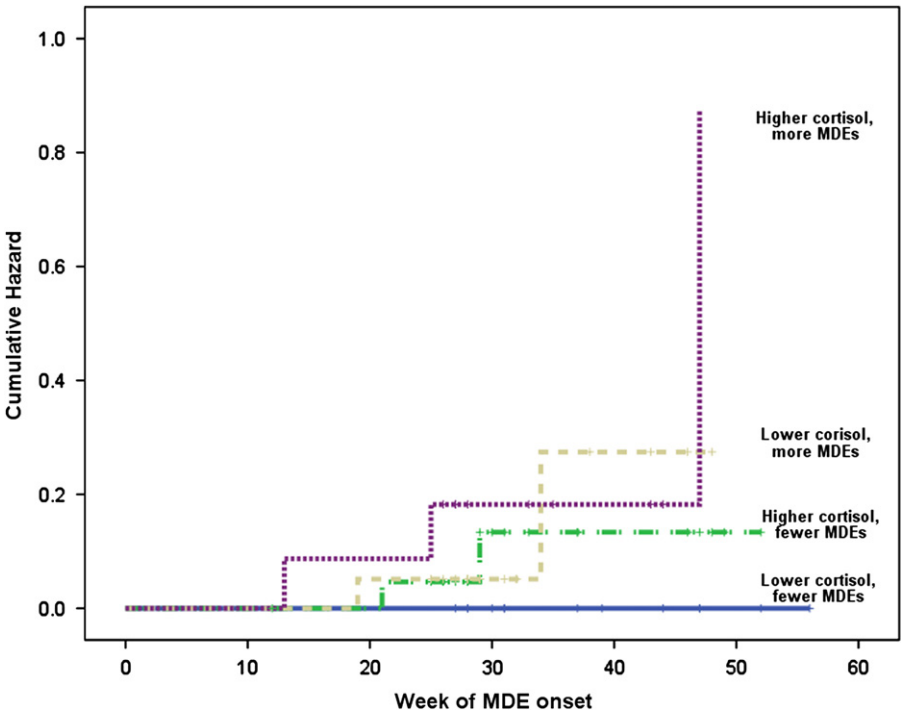

Fig. 2. Risk of incident depression during follow-up stratified on anticipatory stress cortisol levels during the initial evaluation and number of previous MDEs. *Note*: blue=lower cortisol and fewer MDEs; green=higher cortisol and fewer MDEs; gold=lower cortisol and more MDEs; purple=higher cortisol and more MDEs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

facilitate or constrain specific coping strategies. Finally, furthering knowledge of the interplay between stress reactivity and mood regulation may allow for more "personalized" interventions that match treatment approaches to an individual's profile of HPA function (Adam et al., 2008).

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Conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

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