**Neuropsychopharmacology (2016) 41,** 1447–1456

**www.neuropsychopharmacology.org**

# Blunted HPA Axis Activity in Suicide Attempters Compared to those at High Risk for Suicidal Behavior

**Nadine M Melhem*[,1], John G Keilp[2], Giovanna Porta[3], Maria A Oquendo[2], Ainsley Burke[2], Barbara Stanley[2], Thomas B Cooper[4], J John Mann[2] and David A Brent[1]**

*[1]Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; [2]Department of Psychiatry, Columbia University, New York State Psychiatric Institute, New York, NY, USA; [3]University of Pittsburgh Medical Center, Pittsburgh, PA, USA; [4]Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA*

Studies looking at the relationship of the hypothalamic–pituitary–adrenal (HPA) axis to suicidal behavior and its risk factors, such as depression, childhood abuse, and impulsive aggression, report inconsistent results. These studies also do not always differentiate between subjects who go on to attempt suicide, suicidal subjects who never attempted suicide, and non-suicidal subjects with psychiatric disorders. In this study, we examined cortisol responses to an experimental stressor, the Trier Social Stress Test (TSST), in 208 offspring of parents with mood disorder. Offspring suicide attempters showed lower total cortisol output ($\beta = -0.47$, 95% CI (−0.83, −0.11), $p = 0.01$) compared with offspring with suicide-related behavior (SRB) but never attempted, non-suicidal offspring, and a healthy control group. The result remained significant even after controlling for sex, age, race, ethnicity, site, socio-economic status, and hour of the day when the TSST was conducted. Suicide attempters also showed lower baseline cortisol before the TSST ($\beta = -0.45$, 95% CI (−0.74, −0.17), $p = 0.002$). However, there were no significant differences between the groups on cortisol reactivity to stress ($\beta = 4.5$, 95% CI (−12.9, 22), $p = 0.61$). Although subjects with suicide attempt and SRB have similar clinical and psychosocial characteristics, this is the first study to differentiate them biologically on HPA axis indices. Blunted HPA axis activity may increase risk for suicide attempt among individuals with psychopathology by reducing their ability to respond adaptively to ongoing stressors. These results may help better identify subjects at high risk for suicidal behavior for targeted prevention and intervention efforts.
*Neuropsychopharmacology* (2016) **41,** 1447–1456; doi:10.1038/npp.2015.309; published online 4 November 2015

## INTRODUCTION

The diathesis for suicidal behavior is believed to include a vulnerability that is exacerbated by an acute stressor (Mann, 2003). One of the most consistent finding is that resistance of cortisol to dexamethasone suppression test is associated with increased risk of future suicide (Coryell *et al*, 2006; Jokinen and Nordstrom, 2009; Yerevanian *et al*, 2004). However, other studies found no association (Fountoulakis *et al*, 2004; Pitchot *et al*, 2008). Lower plasma cortisol are also found in relatives of suicide completers and predict suicide (Jokinen *et al*, 2010; McGirr *et al*, 2011); and one study reported elevated bedtime salivary cortisol in suicide attempters (Kamali *et al*, 2012). High basal cortisol and reduced glucocorticoid receptor (GR) responsiveness are reported in depressed individuals and offspring of parents with mood disorders, groups at elevated risk for suicidal behavior (Ellenbogen *et al*, 2006, 2011; Kamali *et al*, 2012; Mannie *et al*, 2007; Owens *et al*, 2014). We previously reported impulsive aggression to be a significant predictor of suicide attempts (SAs; Melhem *et al*, 2007). However, low basal cortisol is reported with increased aggression (Poustka *et al*, 2010). The risk for suicidal behavior is also increased in individuals who have experienced childhood adversity (Teicher and Samson, 2013). However, studies of adults with such a history show an attenuated cortisol response (Danese and McEwen, 2012). Methodological variations in the assessment of hypothalamic–pituitary–adrenal (HPA) axis could explain these discrepant findings. The heterogeneity of populations studied with respect to psychiatric morbidity, suicidal behavior, and the risk factors profile associated with them could also contribute to these discrepancies.

Because early-onset suicidal behavior may involve dysfunctional response to a stressor, we examine HPA axis response to an experimental stressor, the Trier Social Stress Test (TSST), in a high-risk sample of offspring of probands with mood disorders from the Familial Pathways for Suicidal Behavior Study, and examine its relationship with suicidal behavior in offspring. The sample is unique with a prospective phenotypic characterization of suicidal behavior, personal and family history of psychopathology, and associated risk factors such as childhood abuse and impulsive aggression. We previously reported that subjects with a

*Correspondence: Dr NM Melhem, Department of Psychiatry, University of Pittsburgh School of Medicine, 3811 O'Hara Street, BFT 318, Pittsburgh, PA 15213, USA, Tel: +412 246 6166, Fax: +412 246 5344, E-mail: melhemnm@upmc.edu
Received 2 February 2015; revised 26 August 2015; accepted 20 September 2015; accepted article preview online 9 October 2015

history of an actual SA are similar on clinical and psychosocial characteristics to those who engaged in suicide-related behavior (SRB) but never attempted suicide; however, this is the first study to compare them biologically in terms of HPA axis responses to stress to reduce the heterogeneity of the phenotype. The standards for the classification of suicidal behavior include completed suicide, actual attempt, interrupted, aborted, and preparatory actions indicating imminent suicidal behavior; and these are distinguished from non-suicidal self-injurious behavior (US Department of Health and Human Services, 2012; Crosby *et al*, 2011; Meyer *et al*, 2010). In this study, SRB includes aborted and interrupted attempts, and preparatory behavior that did not result in an actual attempt. We also include suicidal ideation that prompted emergency referral during the study because the referral may have prevented suicidal behavior, which we have previously included with SRBs and is commonly included with suicidal events in treatment studies (Brent *et al*, 2009; Melhem *et al*, 2007). There were no deaths by suicide during the study. Because of the mixed findings related to the direction of HPA axis dysregulation in suicidal behavior and its correlates, we hypothesize that SA will show a distinct cortisol response to stress compared with SRB, non-suicidal (NS) offspring, and healthy controls (HC) but we do not make specific hypothesis about the direction of cortisol response in SA.

## MATERIALS AND METHODS

### Sample

There are 208 offspring who completed the TSST, from the larger sample of 701 offspring of 334 parents or probands with mood disorders. The TSST was added to the study protocol at follow-up and thus only a subset of subjects completed the test. Slightly over half (52.7%) of the probands had a history of SA. Most probands were recruited from inpatient units at Western Psychiatric Institute and Clinic (Pittsburgh) and New York State Psychiatric Institute (New York). The sample of 208 offspring is almost equally distributed by sex ($n=92$ female, 44.2%), has a mean age of 23.3 years (SD = 5.3, range 16–38; Table 1), and 55.3% is Caucasian and non-Hispanic (89.9%). There were 20 offspring with SA and 20 with SRB (six aborted, three interrupted, nine ideation prompting emergency referral, and two preparatory behavior). Offspring with non-suicidal self-injurious behavior only were not included. Of the 208 offspring, 126 (60.6%) were offspring of proband with lifetime history of suicide attempt and 82 (39.4%) were offspring of a non-attempter proband. Almost all offspring (95%) had lifetime history of axis I psychiatric disorder and 38% reported history of childhood abuse or neglect. As for the probands ($n=134$), the majority is female (87.8%) and Caucasian (67.7%) with mean age of 51.9 years (SD = 7.3 years, range 37–77), and average reported annual household income of $21 151 (SD = $20 784). All probands had a history of mood disorder and 34.3% (46/134) had bipolar disorder.

We compared offspring who completed the TSST to the remaining sample using logistic regression and taking into account the clustering effect of multiple offspring per family. Offspring completing the TSST had longer person-years of follow-up ($\beta=4.3$, 95% CI (3.8, 4.8), $p<0.001$), were younger ($\beta=-4.82$, 95% CI (−6.38, −3.25), $p<0.001$), less likely to be Caucasian (OR = 0.34, 95% CI (0.22, 0.54), $p<0.001$), less likely to have lifetime history of alcohol/substance use disorders (OR = 0.62, 95% CI (0.39, 1.01), $p=0.05$), and more likely to have history of anxiety disorders (OR = 1.78, 95% CI (1.22, 2.59), $p=0.003$).

A group of 35 healthy control subjects who participated in the same TSST protocol were available to the investigators through the Conte Center for the Neuroscience of Mental Disorders at New York State Psychiatric Institute. HC were selected to have no personal or family history of psychiatric disorders and no history of childhood abuse or neglect; consisted of 37.1% ($n=13$) females, 32.4% ($n=11$) Caucasian, and their mean age was 27.2 (SD = 5.5, range 19–39; Table 1).

### Trier Social Stress Task

The TSST is a well-established protocol to induce psychological and endocrine indices of stress response (Kirschbaum *et al*, 1993). It involves a 5-min personal introduction speech followed by 9 min of a speeded mental arithmetic task, in the presence of an observer ('confederate'), a staff person unknown to the subject. Because the sample is at high risk for suicidal behavior, we did not apply the social evaluation part of the TSST where the confederate is supposed to provide negative feedback and replaced criticism with indifference. We performed the test in the afternoon when cortisol levels have dropped to lower levels to improve the signal to baseline ratio (Kudielka *et al*, 2004). Saliva samples were obtained upon subject's arrival to our laboratory ($\text{Cort}_{-30}$) and 10 min before the procedure ($\text{Cort}_{-10}$) after participants watched a 20-min relaxing video, and then at four intervals of time after completion of the task: 15, 20, 30, and 40 min. The same protocol was conducted in the HC group except that pre-task cortisol was measured once at −10. However, the −30 and −10 measurements in the high-risk sample were highly correlated ($r=0.87$, $p<0.001$). Subjective mood was evaluated using the Profile of Mood States (POMS; McNair *et al*, 1992) at −10, 15, and 40 min. We found a significant increase in cortisol over time using mixed effects linear regression ($\beta=0.24$, 95% CI (0.17–0.32), $p<0.001$), with the peak observed 15–20 min after the stressor. Thus, the TSST induced the expected HPA axis response to stress. We computed total cortisol output using 'area under the curve with respect to ground, $\text{AUC}_G$', and cortisol reactivity using 'AUC with respect to increase, $\text{AUC}_I$' following the trapezoid method using raw values (Figure 1; Pruessner *et al*, 2003). We also analyzed baseline or pre-task cortisol ($\text{Cort}_{-10}$). We examined the distribution of $\text{Cort}_{-10}$, $\text{AUC}_G$ and $\text{AUC}_I$; and found $\text{Cort}_{-10}$ and $\text{AUC}_G$ were not normally distributed and thus were transformed using a natural logarithmic transformation. $\text{Cort}_{-10}$ was highly correlated with $\text{AUC}_G$ ($r=0.78$, $p<0.001$) and negatively correlated with $\text{AUC}_I$ ($r=-0.24$, $p=0.006$); $\text{AUC}_G$ and $\text{AUC}_I$ were also correlated ($r=0.28$, $p=0.0001$).

### Salivary Samples Collection and Assay

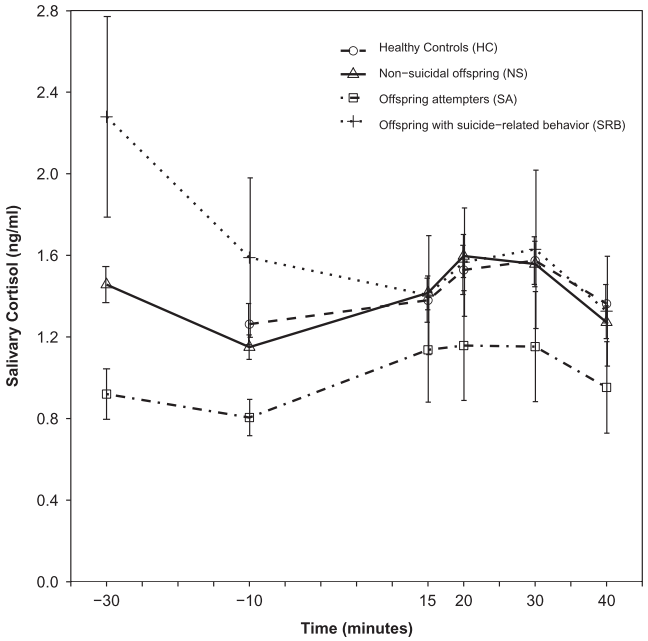
Saliva was collected via the Sarstedt Salivette Synthetic Swab saliva collection system (Catalogue # 51.1534.500 Sarstedt,

Newton, NC 28658, USA). Samples were stored at −30 °C until assayed for cortisol by radioimmunoassay. Primary antibodies raised against cortisol-3-O-carboxymethyloxime-BSA and iodine labeled cortisol were purchased from MP Biomedicals. Cortisol standards were purchased from Sigma Chemical, anti rabbit globulin serum in con-

**Table 1** Demographic and Clinical Characteristics of the Study Sample

| | Total offspring sample (*N* = 208) | Offspring with SA (*n* = 20) | Offspring with SRB (*n* = 20) | NS offspring (*n* = 168) | Healthy controls *n* = 35 | Test statistic | *p*-values |
|---|---|---|---|---|---|---|---|
| *Offspring demographics* | | | | | | | |
| Age, M (SD)[a] | 23.3 (5.3) | 25.4 (4.0)[a,b] | 22.8 (5.8)[a] | 23.2 (5.4)[a] | 27.2 (5.5)[b] | 6.44 | <0.001 |
| Sex, *N* (% female) | 92 (44.2) | 13 (65.0) | 9 (45.0) | 70 (41.7) | 13 (37.1) | 4.58 | 0.21 |
| Caucasian, *N* (%) | 114 (55.3) | 7 (35.0) | 12 (60.0) | 95 (57.2) | 11 (32.4) | 9.91 | 0.02 |
| Hispanic, *N* (%) | 21 (10.1) | 2 (10.0) | 0 (0.0) | 19 (11.4) | 4 (11.4) | FET[b] | 0.52 |
| Site, *N* (% Pittsburgh) | 151 (72.6) | 15 (75.0)[a] | 17 (85.0)[a] | 119 (70.8)[a] | 0 (0.0)[b] | 68.69 | <0.001 |
| Socio-economic status, M (SD) | 19.5 (18.8) | 13.6 (11.1) | 16.7 (13.5) | 20.5 (19.8) | 22.6 (19.0) | 1.03 | 0.38 |
| *Offspring lifetime psychiatric diagnoses* | | | | | | | |
| Depression, *N* (%) | 110 (53.1) | 20 (100.0)[b] | 20 (100.0)[b] | 90 (53.6)[a] | 0 (0.0)[c] | 75.04 | <0.001 |
| Bipolar disorder, *N* (%) | 20 (9.7) | 4 (20.0)[a,b] | 8 (40.0)[a] | 8 (4.8)[b] | 0 (0.0)[b] | FET | <0.001 |
| Anxiety, *N* (%) | 112 (54.6) | 16 (80.0)[b] | 16 (80.0)[b] | 80 (48.5)[a] | 0 (0.0)[c] | 48.70 | <0.001 |
| Posttraumatic stress disorder, *N* (%) | 41 (19.8) | 9 (45.0)[b] | 7 (35.0)[a,b] | 25 (15.0)[a,c] | 0 (0.0)[c] | FET | <0.001 |
| Behavioral disorders, *N* (%) | 43 (62.3) | 9 (100.0)[a] | 2 (50.0)[a] | 32 (57.1)[a] | 0 (0.0)[b] | FET | <0.001 |
| Attention-deficit hyperactivity disorder, *N* (%) | 43 (20.7) | 8 (40.0)[a] | 3 (15.0)[a,b] | 32 (19.0)[a] | 0 (0.0)[b] | FET | 0.001 |
| Alcohol/substance abuse disorders, *N* (%) | 74 (35.8) | 15 (75.0)[b] | 7 (35.0)[a,b] | 52 (31.1)[a] | 0 (0.0)[c] | 34.22 | <0.001 |
| Any axis I disorder, *N* (%) | 169 (94.9) | 20 (100.0)[a] | 20 (100.0)[a] | 129 (93.5)[a] | 0 (0.0)[b] | FET | <0.001 |
| Any axis II disorder, *N* (%) | 14 (9.2) | 2 (11.8) | 2 (14.3) | 10 (8.3) | 0 (0.0) | FET | 0.11 |
| Functional impairment[c], M (SD) | 77.9 (11.3) | 71 (11.1)[a] | 70.4 (13.9)[a] | 79.5 (10.6)[b] | 91.2 (5.7)[b] | 23.6 | <0.001 |
| NSSI behavior, *N* (%) | 11 (5.3) | 6 (30.0)[a] | 5 (25.0)[a] | 0 (0.0)[b] | 0 (0.0)[b] | FET | <0.001 |
| *Offspring self-reported measures at the time of TSST* | | | | | | | |
| Depression symptomatology, M (SD) | 5.1 (6.4) | 7.4 (5.5)[b] | 9.1 (8.2)[b] | 4.2 (5.7)[a] | 1.2 (2.0)[c] | 10.27 | <0.001 |
| Impulsive aggression, M (SD) | 0.3 (0.9) | 0.9 (1.0)[b] | 0.6 (0.9)[a,b] | 0.1 (0.9)[a] | −0.9 (0.9)[c] | 21.84 | <0.001 |
| Childhood abuse/neglect, *N* (%) | 76 (38.0) | 13 (65.0)[b] | 11 (55.0)[a,b] | 52 (32.5)[a] | 0 (0.0)[c] | 31.17 | <0.001 |
| *Offspring TSST measures* | | | | | | | |
| Time of day, M (SD) range | 14:04 (1:28) 10:09–18:04 | 13:54 (1:27)[a] 12:04–18:04 | 13:53 (1:30)[a] 12:00–16:19 | 14:07 (1:28)[a] 10:09–17:39 | 14:58 (0:57)[b] 13:09–17:19 | 4.38 | 0.01 |
| Habitual smoking | 48 (23.3) | 9 (45.0)[a] | 4 (20.0)[a,b] | 35 (21.1)[a,b] | 2 (5.7)[b] | FET | 0.01 |
| Smoking on day of TSST | 28 (20.0) | 4 (28.6) | 2 (13.3) | 22 (19.8) | 2 (5.7) | FET | 0.12 |
| Current alcohol/substance abuse | 46 (22.1) | 9 (45.0)[a] | 3 (15.0)[a,b] | 34 (20.2)[a] | 0 (0.0)[b] | FET | <0.001 |
| Pre-task POMS (−10), M (SD) | 8.6 (20.9) | 13.2 (15.3)[a,b] | 27.0 (32.1)[a] | 5.7 (18.5)[b] | 3.5 (21.6)[b] | 7.59 | <0.001 |
| Change in POMS scores, post (15)—pre-task (−10), M (SD) | 9.3 (16.3) | 7.7 (24.2) | 8.3 (13.3) | 9.6 (15.5) | 3.4 (19.8) | 1.18 | 0.32 |
| Change in POMS scores, rest (40)—pre-task (−10), M (SD) | −2.5 (10.3) | −4.3 (12.1) | −3.5 (12.0) | −2.1 (9.9) | −5.4 (13.8) | 0.97 | 0.41 |
| *Offspring treatment at the time of the TSST* | | | | | | | |
| Use of any medications, *N* (%) | 98 (47.1) | 9 (45.0)[a] | 14 (70.0)[a] | 75 (44.6)[a] | 0 (0.0)[b] | 32.45 | <0.001 |
| Psychotropic medication, *N* (%) | 44 (21.4) | 5 (25.0)[a] | 8 (40.0)[a] | 31 (18.7)[a] | 0 (0.0)[b] | FET | <0.001 |
| Non-psychotropic medication, *N* (%) | 70 (34.0) | 6 (30.0)[a] | 12 (60.0)[a] | 52 (31.3)[a] | 0 (0.0)[b] | 24.05 | <0.001 |
| Psychotherapy, *N* (%) | 61 (29.5) | 7 (36.8)[a,b] | 12 (60.0)[b] | 42 (25.0)[a] | 0 (0.0)[c] | FET | <0.001 |
| *Proband lifetime psychiatric diagnoses* | | | | | | | |
| Bipolar disorder[d], *N* (%) | 76 (36.9) | 11 (55.0)[a] | 9 (45.0)[a] | 56 (33.7)[a] | 0 (0.0)[b] | 23.27 | <0.001 |
| Suicide attempt, *N* (%) | 126 (60.6) | 15 (75.0)[a] | 16 (80.0)[a] | 95 (56.5)[a] | 0 (0.0)[b] | 49.82 | <0.001 |

Abbreviations: NS, non-suicidal offspring; NSSI, non-suicidal self-injurious behavior; POMS, Profile of Mood States; SA, suicide attempt; SRB, suicide-related behavior.
[a]M (SD), mean and SD.
[b]Fisher's exact test; letters indicate *post hoc* comparisons where different letters represent significant *post hoc* differences and similar letters represent no *post hoc* differences.
[c]Lower scores on the GAS reflect worse functional impairment.
[d]Compared with unipolar. Similar to healthy controls, NS offspring had no previous history of NSSI; they also had lower rates of bipolar disorder compared with the SRB. Both NS and SA offspring were similar to healthy controls on their subjective ratings of mood before the task, which were lower than the scores in the SRB group.




| Measure | SA | SRB | NS | HC |
|---|---|---|---|---|
| Cort(−10), ng/ml | 0.81±0.09 | 1.59±0.39 | 1.15±0.06 | 1.26±0.10 |
| AUCg | 52.09±43.18 | 72.62±68.83 | 67.81±45.71 | 70.51±28.33 |
| AUCi | 11.83±34.99 | −8.05±31.66 | 9.40±29.01 | 7.36±19.15 |

**Figure 1** Cortisol levels (raw values) before, during, and after the Trier Social Stress Test (TSST) by offspring suicide attempt status. Cortisol levels were not measured at −30 for HC. Mean and standard deviation for $Cort_{-10}$, $AUC_G$, and $AUC_I$ computed based on raw values are reported in table; $Cort_{-10}$, Baseline or pre-task cortisol 10 min prior to the task; $AUC_G$, Area under the curve with respect to ground; $AUC_I$, Area under the curve with respect to increase.

junction with polyethylene glycol was used for separation of the bound and free fractions. All samples and standards were analyzed in duplicate. The intra and inter-assay coefficient of variation was 4.7% at 1 ng/ml and 7.4% at 0.25 ng/ml, respectively.

## Assessment

Recruitment and informed consent were conducted in accordance with protocols approved by the Institutional Review Boards of the University of Pittsburgh and New York State Psychiatric Institute. Probands and offspring were interviewed at intake and yearly follow-ups. Lifetime and current psychiatric disorders were ascertained using the Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime Version (Kaufman *et al*, 1997) and the Structured Clinical Interview (Spitzer *et al*, 1992) for DSM-IV for those 18 years and older. Suicidal behavior was assessed using the Columbia-Suicide History Form and the Medical Damage Lethality Rating Scale (Mann *et al*, 1992). Functional impairment was assessed using the Global Assessment Scale (Endicott *et al*, 1976). A battery of self-reported questionnaires was also administered including the Beck Depression Inventory to assess depression symptomatology (Beck *et al*, 1961), the Buss-Durkee Hostility Inventory for impulsive aggression (Buss and Durkee, 1957), and the Child Trauma Questionnaire for childhood abuse and neglect (Bernstein *et al*, 1994).

## Group Comparisons

We compared SA, SRB, NS, and HC on demographic and clinical characteristics using standard statistics ($\chi^2$, Fisher's exact test, analysis of variance (ANOVA); Table 1). As expected, HC were significantly different from all other groups on psychiatric diagnoses and other clinical characteristics. They were also administered the TSST almost 1 h later, on average, than the other groups. SA, SRB, and NS offspring were similar in their overall rate of axis I disorders, parental history of bipolar disorder and suicide attempt, and use of medications. However, SA and SRB showed higher rates of depression and anxiety disorders, higher scores on depression symptomatology, and worse functional impairment than the NS and HC. The most frequently reported psychotropic medications were selective serotonin reuptake inhibitors (52.3%) and stimulants (31.8%); and for non-psychotropic medications, birth control pills (22.9%), asthma inhalers (17%), and non-steroidal anti-inflammatory drugs and pain medications (14.3%).

## Statistical Analyses

We examined the relationship of offspring suicidal behavior to total cortisol output, pre-task cortisol, and cortisol reactivity using linear regression where cortisol reactivity ($AUC_I$), eg, is the dependent variable. Suicidal behavior was coded as a dummy variable with HC as the reference category. We controlled for sex, age, race, socio-economic status as measured by household income of adult working offspring or that of the proband for young offspring, and hour of the day when the TSST was conducted due to their well-established effects on HPA axis function (Golden *et al*, 2011; Kalsbeek *et al*, 2012). We also controlled for site and ethnicity because subjects at the NY site were more likely to be of Hispanic origin (23.1 *vs* 2.6%, $p<0.001$). We use regression instead of analysis of covariance, which are equivalent approaches under the generalized linear models, for ease of interpretation of regression coefficients for the relationships between dependent and independent variables. Given the above-mentioned group differences, we examined offspring lifetime history of specific psychiatric diagnoses, functional impairment, self-reported measures at the time of the TSST, TSST measures, use of medications and psychotherapy at the time when the TSST was conducted, and proband lifetime history of bipolar disorder (*vs* unipolar depression) and suicide attempt as potential confounders of the relationship between suicidal behavior and cortisol outcomes (Table 2). We also examined offspring smoking and current alcohol and substance abuse at the time of the TSST in relation to cortisol outcomes. Only variables significantly associated with cortisol outcomes were included in the multivariate model. Since our study subjects are clustered within families, we used the vce (cluster) command that can be applied to regression models in Stata (version 11; StataCorp, 2009) to estimate the variance–covariance matrix with correlated or clustered observations and compute clustered robust standard errors. Our sample size has 80% power to detect effect sizes (ES) in the order of $d>0.67$ at

**Table 2** Regression Models for Clinical Covariates in Relation to Total Cortisol Output, Baseline or Pre-Task Cortisol Levels, and Cortisol Reactivity

| | Total cortisol output[a] | | | Baseline or pre-task cortisol[b] | | | Cortisol reactivity[c] | | |
|---|---|---|---|---|---|---|---|---|---|
| | β (95% CI) | *t* | *p* | β (95% CI) | *t* | *p* | β 95% CI | *t* | *p* |
| *Offspring lifetime psychiatric diagnoses* | | | | | | | | | |
| Depression | −0.24 (−0.40, −0.08) | −2.92 | **0.004** | −0.16 (−0.32, 0.01) | −1.92 | 0.06 | −3.91 (−10.4, 2.6) | −1.20 | 0.23 |
| Bipolar disorder | −0.08 (−0.45, 0.29) | −0.42 | 0.68 | −0.28 (−0.60, 0.04) | −1.71 | 0.09 | −3.84 (−18.8, 11.1) | −0.51 | 0.61 |
| Anxiety | −0.14 (−0.32, 0.03) | −1.63 | 0.10 | −0.17 (−0.33, −0.01) | −2.08 | **0.04** | −2.11 (−8.6, 4.4) | −0.64 | 0.52 |
| Posttraumatic stress disorder | −0.15 (−0.40, 0.09) | −1.22 | 0.22 | −0.10 (−0.33, 0.14) | −0.80 | 0.42 | −6.63 (−14.3, 1.1) | −1.70 | 0.09 |
| Behavioral disorders | −0.20 (−0.49, 0.09) | −1.36 | 0.18 | −0.07 (−0.32, 0.19) | −0.51 | 0.61 | −1.71 (−11.3, 7.8) | −0.36 | 0.72 |
| Attention-deficit hyperactivity disorder | −0.09 (−0.32, 0.12) | −0.83 | 0.41 | −0.07 (−0.27, 0.13) | −0.68 | 0.49 | 1.66 (−7.8, 11.1) | 0.35 | 0.73 |
| Alcohol/substance abuse disorders | −0.01 (−0.21, 0.19) | −0.11 | 0.91 | 0.03 (−0.16, 0.21) | 0.30 | 0.76 | −2.60 (−10.4, 5.2) | −0.66 | 0.51 |
| Personality disorders | −0.37 (−0.82, 0.09) | −1.59 | 0.11 | −0.32 (−0.65, 0.01) | −1.95 | **0.05** | −4.57 (−15.4, 6.3) | −0.83 | 0.41 |
| Functional impairment | 0.004 (−0.003, 0.01) | 1.10 | 0.27 | −0.002 (−0.01, 0.01) | −0.41 | 0.68 | 0.26 (0.04, 0.5) | 2.30 | **0.02** |
| NSSI behavior | −0.30 (−0.76, 0.17) | −1.26 | 0.21 | −0.18 (−0.54, 0.19) | −0.95 | 0.35 | −1.97 (−13.3, 9.4) | −0.34 | 0.73 |
| *Offspring self-reported measures at the time of TSST* | | | | | | | | | |
| Depression symptomatology | −0.004 (−0.02, 0.01) | −0.54 | 0.59 | 0.01 (−0.01, 0.03) | 1.18 | 0.24 | −0.61 (−1.4, 0.1) | −1.62 | 0.11 |
| Impulsive aggression | −0.04 (−0.13, 0.04) | −1.03 | 0.31 | −0.02 (−0.09, 0.06) | −0.48 | 0.63 | −1.72 (−4.9, 1.4) | −1.08 | 0.28 |
| Childhood abuse/neglect | −0.17 (−0.38, 0.04) | −1.60 | 0.11 | −0.07 (−0.23, 0.09) | −0.87 | 0.39 | −2.52 (−10.2, 5.1) | −0.65 | 0.52 |
| *Offspring TSST measures* | | | | | | | | | |
| Time of day | −1.35 (−2.78, 0.09) | −1.86 | 0.07 | −1.45 (−2.83, −0.07) | −2.07 | **0.04** | 43.43 (−19.8, 106.7) | 1.36 | 0.18 |
| Habitual smoking | −0.21 (−0.43, 0.005) | −1.93 | 0.06 | −0.07 (−0.29, 0.14) | −0.67 | 0.50 | −8.17 (−15.3, −1.02) | −2.26 | **0.03** |
| Smoking on day of TSST | −0.08 (−0.37, 0.22) | −0.51 | 0.61 | 0.06 (−0.21, 0.34) | 0.47 | 0.64 | −11.90 (−21.8, −2.1) | −2.40 | **0.02** |
| Current alcohol/substance use | 0.01 (−0.22, 0.24) | 0.09 | 0.93 | 0.10 (−0.11, 0.31) | 0.94 | 0.35 | −4.06 (−13.43, 5.3) | −0.86 | 0.39 |
| Pre-task POMS (−10) | −0.001 (−0.01, 0.002) | −0.74 | 0.46 | 0.001 (−0.003, 0.01) | 0.53 | 0.60 | −0.19 (−0.33, −0.06) | −2.77 | **0.006** |
| Change in POMS score, post (15)—pre-task (−10) | −0.0001 (−0.01, 0.01) | −0.04 | 0.97 | −0.0001 (−0.004, 0.004) | −0.04 | 0.97 | −0.01 (−0.18, 0.18) | −0.06 | 0.95 |
| Change in POMS score, rest (40)—pre-task (−10) | −0.003 (−0.01, 0.004) | −0.93 | 0.35 | −0.004 (−0.01, 0.003) | −1.14 | 0.26 | 0.05 (−0.27, 0.37) | 0.30 | 0.76 |
| *Offspring treatment at the time of the TSST* | | | | | | | | | |
| Use of psychotropic medications[d] | −0.29 (−0.53, −0.06) | −2.44 | **0.02** | −0.24 (−0.46, −0.03) | −2.24 | **0.03** | 3.21 (−6.3, 12.7) | 0.67 | 0.51 |
| Use of non-psychotropic medication[d] | −0.09 (−0.28, 0.10) | −0.96 | 0.34 | −0.10 (−0.29, 0.10) | −0.98 | 0.33 | −4.03 (−11.7, 3.7) | −1.04 | 0.30 |
| Psychotherapy | −0.19 (−0.39, 0.01) | −1.90 | 0.06 | −0.07 (−0.26, 0.13) | −0.66 | 0.51 | −3.45 (−11.4, 4.5) | −0.86 | 0.39 |
| *Proband lifetime psychiatric diagnoses* | | | | | | | | | |
| Bipolar disorder[e] | −0.09 (−0.29, 0.11) | −0.90 | 0.37 | −0.12 (−0.29, 0.06) | −1.33 | 0.19 | 3.12 (−4.7, 10.9) | 0.79 | 0.43 |
| Suicide attempt | −0.18 (−0.35, −0.01) | −2.13 | **0.04** | −0.11 (−0.27, 0.05) | −1.30 | 0.20 | −4.98 (−11.6, 1.6) | −1.49 | 0.14 |

Abbreviations: NSSI, non-suicidal self-injurious behavior; POMS, Profile of Mood States.
[a]Total cortisol output = $\ln(AUC_G)$.
[b]Baseline or pre-task cortisol = $\ln(Cort_{-10})$.
[c]Cortisol reactivity = $AUC_I$.
[d]Dummy variables for medication use with no medication as the reference category.
[e]Compared with unipolar, the reference category.
Bold values indicate $p < 0.05$.

$p < 0.05$. We present ES as Cohen's *d* (Cohen, 1988) for differences in means of cortisol outcomes (unadjusted and adjusted means) between suicidal behavior groups.

## RESULTS

### Total Cortisol Output and Suicidal Behavior

Offspring SA showed lower total cortisol output compared with SRB, NS, and HC ($\beta = -0.47$, 95% CI (−0.83, −0.11), ES = −0.75, $p = 0.01$; Table 3, Figure 1). The result remained significant even when controlling for demographic variables, site, socio-economic status, and hour of the day ($\beta = -0.47$, 95% CI (−0.89, −0.05), ES = −0.85, $p = 0.03$; Table 3). In addition, sex, race, and hour of the day were significant covariates. We also find lower cortisol output in offspring with major depression, offspring whose parent had history of suicide attempt, and offspring using psychotropic medications (Table 2). We repeated our model in Table 3 controlling for these variables and for functional impairment as an overall measure of severity of psychopathology. SA continued to show lower cortisol output ($\beta = -0.15$, 95% CI

**Table 3** Regression Models for the Relationships Between Suicidal Behavior and Total Cortisol Output With and Without Controlling for Demographics and Hour of the Day When the TSST was Conducted

| | Total Cortisol Output[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Unstand[b] β** | **95% CI** | ***t*** | ***p*** | **Unstand[b] β** | **95% CI** | ***t*** | ***p*** |
| SA | −0.47 | −0.83, −0.11 | −2.58 | **0.01** | −0.47 | −0.89, −0.05 | −2.2 | **0.03** |
| SRB | −0.13 | −0.49, 0.23 | −0.70 | 0.49 | −0.14 | −0.58, 0.29 | −0.65 | 0.52 |
| NS | −0.15 | −0.32, 0.03 | −1.61 | 0.11 | −0.11 | −0.42, 0.20 | −0.69 | 0.49 |
| Age | | | | | 0.002 | −0.02, 0.02 | 0.27 | 0.79 |
| Sex, female[c] | | | | | −0.22 | −0.40, −0.04 | −2.43 | **0.02** |
| Race, Caucasian[c] | | | | | 0.21 | 0.04, 0.38 | 2.45 | **0.02** |
| Ethnicity, Hispanic[c] | | | | | 0.18 | −0.23, 0.59 | 0.88 | 0.38 |
| Site, Pittsburgh[c] | | | | | −0.21 | −0.49, 0.08 | −1.4 | 0.16 |
| SES[d] | | | | | 0.001 | −0.003, 0.005 | 0.51 | 0.61 |
| Time of day | | | | | −2.53 | −4.03, −1.03 | −3.34 | **0.001** |

Abbreviations: NS, non-suicidal; SA, suicide attempt; SRB, suicide-related behavior.
[a]Total cortisol output = ln($AUC_G$).
[b]Unstandardized coefficients.
[c]Compared with reference categories males, non-Caucasians, non-Hispanic, and NY.
[d]SES, socio-economic status. There were 208 high-risk offspring from 134 families with an average number of 1.6 (SD = 0.7, range 1–5) offspring per family participating in the study. Regression models take into account the correlation between subjects.
Bold values indicate $p < 0.05$.

(−0.76, 0.45), ES = −0.26, $p = 0.62$) than SRB ($\beta = 0.19$, 95% CI (−0.4, 0.79), ES = 0.32, $p = 0.52$) and NS ($\beta = 0.20$, 95% CI (−0.23, 0.63), ES = 0.33, $p = 0.37$) but the ES is smaller than this sample is powered to detect as statistically significant. In addition, none of the clinical variables were significantly associated with total cortisol in the multivariate model. Thus, the model in Table 3 is the most parsimonious model. SA also continued to show lower cortisol output when excluding subjects reporting the use of anti-inflammatory and pain medications ($n = 10$; $\beta = -0.47$, 95% CI (−0.9, −0.04), ES = −0.82, $p = 0.03$) and asthma inhalers ($n = 12$; $\beta = -0.38$, 95% CI (−0.77, 0.01), ES = −0.70, $p = 0.05$). Similar results were obtained when excluding subjects on psychotropic ($n = 44$; $\beta = -0.25$, 95% CI (−0.69, 0.19), ES = −0.42, $p = 0.27$); and non-psychotropic medications ($n = 70$; $\beta = -0.22$, 95% CI (−0.67, 0.21), ES = −0.47, $p = 0.31$); however, our sample sizes were reduced for these analyses and thus our power to detect these differences as statistically significant was limited.

Within suicide attempters, there was no relationship between total cortisol output and age of onset of attempt ($r = 0.16$, $p = 0.51$), number of attempts ($r = -0.12$, $p = 0.61$), duration between the most recent attempt and the TSST ($r = 0.19$, $p = 0.43$), and lethality of the most recent attempt ($r = 0.01$, $p = 0.96$).

## Baseline or Pre-Task Cortisol and Suicidal Behavior

Offspring SA showed lower pre-task cortisol compared with SRB, NS, and HC ($\beta = -0.45$, 95% CI (−0.74, −0.17), ES = −0.87, $p = 0.002$; Table 4, Figure 1). The result remained significant even after controlling for demographics, site, socio-economic status, and hour of the day ($\beta = -0.42$, 95% CI (−0.82, −0.01), ES = −0.82, $p = 0.046$; Table 4). In addition, sex and hour of the day were significant covariates. We also find lower pre-task cortisol in offspring with anxiety disorders, personality disorders, and those using psychotropic medications (Table 2). Similar results were obtained for those with major depression and bipolar disorders although the result did not reach statistical significance. We repeated our model in Table 4 controlling for these variables and for functional impairment; however, they were not significant in the multivariate model. Thus, the model in Table 4 is the most parsimonious model. SA also continued to show lower baseline cortisol when excluding subjects reporting the use of anti-inflammatory and pain medications ($\beta = -0.45$, 95% CI (−0.9, −0.03), ES = −0.87, $p = 0.04$); asthma inhalers ($\beta = -0.35$, 95% CI (−0.73, 0.03), ES = −0.71, $p = 0.07$); psychotropic ($\beta = -0.13$, 95% CI (−0.51, 0.24), ES = −0.3, $p = 0.48$); and non-psychotropic medications ($\beta = -0.43$, 95% CI (−0.91, 0.05), ES = −0.86, $p = 0.08$); however, our sample sizes, and accordingly our power to detect these differences as statistically significant were reduced for these analyses. Similar to total cortisol output, there were no relationships between pre-task cortisol and characteristics of attempt within SA.

## Cortisol Reactivity and Suicidal Behavior

There were no significant group differences on cortisol reactivity or $AUC_I$ with and without controlling for demographic variables, site, socio-economic status, and hour of the day (Table 4). Functional impairment was associated with cortisol reactivity where subjects with higher Global Assessment Scale scores and thus higher functioning showing higher reactivity (Table 2). Higher scores on subjective ratings of mood before task, habitual smoking, and smoking on the day of the TSST were associated with lower cortisol reactivity in response to stress. There were no significant group differences on cortisol reactivity when controlling for these variables and none of these variables were significant when added to the model in Table 4. Similar

**Table 4** Regression Models for the Relationships Between Suicidal Behavior and Each of Baseline or Pre-Task Cortisol Levels and Cortisol Reactivity With and Without Controlling for Demographics and Hour of the Day When the TSST was Conducted

| | Unstand[a] β | 95% CI | t | p | Unstand[a] β | 95% CI | t | p |
|---|---|---|---|---|---|---|---|---|
| *Baseline or pre-task cortisol*[b] | | | | | | | | |
| SA | −0.45 | −0.74, −0.17 | −3.11 | **0.002** | −0.42 | −0.82, −0.01 | −2.02 | **0.046** |
| SRB | −0.07 | −0.39, 0.26 | −0.40 | 0.69 | −0.27 | −0.66, 0.13 | −1.33 | 0.19 |
| NS | −0.16 | −0.36, 0.04 | −1.60 | 0.11 | −0.22 | −0.51, 0.08 | −1.46 | 0.15 |
| Age | | | | | 0.004 | −0.01, 0.02 | 0.55 | 0.58 |
| Sex, female[c] | | | | | −0.17 | −0.33, −0.01 | −2.04 | **0.04** |
| Race, Caucasian[c] | | | | | 0.14 | −0.04, 0.31 | 1.53 | 0.13 |
| Ethnicity, Hispanic[c] | | | | | 0.21 | −0.12, 0.54 | 1.27 | 0.21 |
| Site, Pittsburgh[c] | | | | | −0.06 | −0.30, 0.18 | −0.46 | 0.65 |
| SES[d] | | | | | 0.003 | −0.0003, 0.01 | 1.79 | 0.08 |
| Time of day | | | | | −2.24 | −3.72, −0.76 | −2.99 | **0.003** |
| *Cortisol reactivity*[e] | | | | | | | | |
| SA | −1.00 | −14.89, 12.88 | −0.15 | 0.89 | −1.74 | −13.78, 10.30 | −0.29 | 0.78 |
| SRB | −15.41 | −32.42, 1.59 | −1.79 | 0.08 | −7.95 | −24.65, 8.74 | −0.94 | 0.35 |
| NS | −0.29 | −7.83, 7.25 | −0.08 | 0.94 | 0.27 | −9.07, 9.60 | 0.06 | 0.96 |
| Age | | | | | −0.44 | −1.19, 0.31 | −1.16 | 0.25 |
| Sex, female[c] | | | | | −5.57 | −12.13, 0.98 | −1.68 | 0.10 |
| Race, Caucasian[c] | | | | | 1.06 | −6.32, 8.44 | 0.28 | 0.78 |
| Ethnicity, Hispanic[c] | | | | | 3.63 | −9.11, 16.38 | 0.56 | 0.57 |
| Site, Pittsburgh[c] | | | | | 0.03 | −9.47, 9.53 | 0.01 | >0.99 |
| SES[d] | | | | | −0.08 | −0.24, 0.09 | −0.92 | 0.36 |
| Time of day | | | | | 50.66 | −15.53, 116.84 | 1.51 | 0.13 |

[a]Unstandardized coefficients. [b]Baseline or pre-task cortisol = ln(Cort$_{-10}$). [c]Compared with reference categories males, non-Caucasians, non-Hispanic and NY. [d]SES, socio-economic status. [e]Cortisol reactivity = AUC$_I$.
Bold values indicate $p < 0.05$.

results were also obtained when excluding subjects using different types of medications.

## DISCUSSION

This study shows lower total cortisol output in response to an experimental stressor and lower baseline cortisol in suicide attempters compared with subjects with SRB who never attempted suicide and NS subjects in a high-risk sample, namely offspring of probands with mood disorders, and HC. However, there were no group differences in cortisol reactivity between the groups.

Subjects who participated in this study come from a longitudinal study with up to 13 years of follow-up and are well characterized on suicidal behavior, psychiatric diagnoses and adversity, a major strength of the study. Although these subjects were different from offspring who did not participate in the TSST in terms of history of alcohol and substance use and anxiety disorders, those variables were not associated with cortisol outcomes except for anxiety disorders being associated with lower pre-task cortisol; however, we controlled for anxiety in those analyses. In addition, our sample is similar to other high-risk samples in rates of psychopathology and specifically, bipolar disorder (Mesman *et al*, 2013). Our control group was recruited at one of the sites; however, we used exactly the same TSST procedures as those performed in the high-risk sample except for collecting cortisol samples at −30. However, pre-task cortisol at −30 and −10 were highly correlated in the high-risk sample. We did not collect data about menstrual cycle phase (luteal *vs* follicular) in females, which affects HPA axis responses to stress (Kirschbaum *et al*, 1999), and hence were not able to examine its effects in our sample. We examine HPA axis responses to an experimental stressor and thus our results may not be generalizable to HPA axis functioning in response to real-life stressors (Wolfram *et al*, 2013).

Our results are consistent with studies finding lower resting cortisol in subjects with personal or family history of suicidal behavior (Jokinen *et al*, 2010; McGirr *et al*, 2011; Pfennig *et al*, 2005). However, they are not consistent with a pilot study reporting blunted cortisol reactivity to the TSST in first-degree relatives of suicide completers compared with HC (McGirr *et al*, 2010), and our report of blunted cortisol reactivity in offspring bereaved by parental suicide compared with those bereaved by accidental and sudden natural death (Dietz *et al*, 2013). In this study, we find lower total cortisol output in SA compared with other high-risk groups, which could be mainly attributed to their lower baseline cortisol. As SA's ratings of subjective mood before the task and changes in these ratings by the end of the task were similar to those of the other groups, current mood state did not contribute

to the findings. One should be cautious in the interpretation of pre-task levels as they may not be representative of naturalistic levels of cortisol and may reflect some reactivity in anticipation of the acute stressor. Future studies looking at diurnal variation in cortisol secretions are needed to examine whether SA have lower cortisol in general or a shifted diurnal variation. Although subjects with SA and SRB had similar clinical and psychosocial characteristics, consistent with our previous report (Melhem *et al*, 2007), this is the first study to differentiate them biologically on total cortisol output throughout exposure to stress and on baseline cortisol. Although suicidal behavior occurs in the context of mood and other psychiatric disorders, only 10–15% of subjects with psychiatric disorder attempt or die by suicide (Angst *et al*, 2005; Arsenault-Lapierre *et al*, 2004). Our results differentiate subjects who go on to attempt suicide among a high-risk sample where 95% had a lifetime history of psychopathology, and thus further delineating the suicidal behavior phenotype. We previously reported impulsive aggression to be a significant predictor of suicide attempts (Melhem *et al*, 2007). However, we find no associations between impulsive aggression and cortisol outcomes across all groups (Table 2, Supplementary Figure S1). Within groups, we find a moderate correlation with each of total output ($r = -0.39$, $p = 0.1$), pre-task cortisol ($r \sim = -0.38$, $p = 0.1$), and reactivity ($r = -0.3$, $p = 0.22$) among SA and a strong correlation with cortisol reactivity among those with SRB ($r = -0.46$, $p = 0.09$; Supplementary Figure S1). Further studies with larger sample sizes are needed to have the power to detect these correlations to a significant level. These results suggest that blunted cortisol and cortisol responses to stress may underlie increased impulsive aggression in subjects at high risk for suicidal behavior; however, SA and SRB groups were similar on impulsive aggression. Blunted total cortisol output in SA may be linked to the familial transmission of suicidal behavior. Offspring whose parent had a lifetime history of suicide attempt are at five times greater risk for suicide attempt (Brent *et al*, 2015) and in this study, they showed lower total cortisol output. Thus, total cortisol output in response to stress may be an underlying vulnerability that puts individuals with family history of suicidal behavior at risk for suicidal behavior. We also find subjects with lifetime history of major depression to show lower total cortisol. Subjects with moderate to severe depression have been previously shown to exhibit a blunted cortisol response to the TSST (Harkness *et al*, 2011). Thus, it is also possible that major depression dampens the HPA axis response to stress, which puts individuals at risk for suicidal behavior when faced with repeated stressors. Offspring using psychotropic medications also showed lower total output and baseline cortisol. Antidepressants have been shown to increase GR and reduce resting and stimulated HPA axis activity (Pariante *et al*, 2004). However, use of psychotropic medications does not completely explain the differences between SA and the other groups as evident by the substantial ES for total output and baseline levels (−0.4 and −0.3, respectively) remaining when we excluded subjects using psychotropic medications. Finally, it is possible that lower total output and baseline cortisol in SA are the consequence of suicide attempt rather than a pre-existing vulnerability or the consequence of their exposure to the adversity of parental suicide attempt. However, we cannot address the temporal sequence because most offspring had their attempt before the TSST assessment and had their parental suicide attempt occur in their lifetime. Future studies are needed to establish whether alterations in HPA axis response to stress predict future risk for suicidal behavior.

We find no direct relationship between cortisol outcomes and offspring childhood exposure to abuse/neglect, a finding that is not consistent with extant literature (Danese and McEwen, 2012). However, our subjects are a high-risk sample of offspring of parents with mood disorders, which itself is reported to affect cortisol responses to stress (Waugh *et al*, 2012). In addition, 95% of the offspring had a lifetime history of psychiatric disorder and thus it is difficult to disentangle the relationship between childhood adversity and cortisol outcomes in such a high-risk sample. A postmortem study of subjects who completed suicide and had a history of childhood abuse report less *GR* mRNA and more *GR* promotor DNA methylation compared with controls and suicides without such history (McGowan *et al*, 2009). A blunted stress response has also been reported in PTSD (Yehuda *et al*, 2010). Future studies are needed to explain the link between adversity, genetic and epigenetic effects, and cortisol dynamics that may increase risk for suicidal behavior.

In conclusion, blunted HPA axis activity may increase risk for suicide attempt among individuals with psychopathology by reducing their ability to respond adaptively to ongoing stressors. These results may help better identify subjects at high risk for suicidal behavior for targeted preventions and interventions.

## FUNDING AND DISCLOSURE

Drs Melhem, Keilp, Cooper, and Ms Porta have no conflicts of interest to disclose. Dr Oquendo receives royalties for use of the Columbia-Suicide Severity Rating Scale (C-SSRS) and received financial compensation from Pfizer for the safety evaluation of a clinical facility, unrelated to this study. She has received unrestricted educational grants and/or lecture fees from Astra-Zeneca, Bristol Myers Squibb, Eli Lilly, Janssen, Otsuko, Pfizer, Sanofi-Aventis, and Shire. Her family owns stock in Bristol Myers Squibb. Drs Burke and Stanley receive royalties from the use of the Columbia-Suicide Severity Rating Scale. Dr Mann receives royalties for commercial use of the C-SSRS from the Research Foundation for Mental Health and has stock options in Qualitas Health, a startup company developing EPA as a nutroceutical. Dr Brent receives royalties from Guilford Press, have or will receive royalties from the electronic self-rated version of the C-SSRS from ERT, is on the Editorial board of UpToDate, and receives honoraria for presenting at Continuing Medical Education events. This study work was supported by an R01 grant (MH56612, Brent DA; MH056390, Mann JJ) and a K01 grant (MH077930, Melhem NM) from the National Institute of Mental Health.

## REFERENCES

Angst J, Angst F, Gerber-Werder R, Gamma A (2005). Suicide in 406 mood-disorder patients with and without long-term medication: a 40 to 44 years' follow-up. *Arch Suicide Res* **9**: 279–300.

Arsenault-Lapierre G, Kim C, Turecki G (2004). Psychiatric diagnoses in 3275 suicides: a meta-analysis. *BMC Psychiatry* **4**: 37.

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961). An inventory for measuring depression. *Arch Gen Psychiatry* **4**: 561–571.

Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K *et al* (1994). Initial reliability and validity of a new retrospective measure of child abuse and neglect. *Am J Psychiatry* **151**: 1132–1136.

Brent DA, Emslie GJ, Clarke GN, Asarnow J, Spirito A, Ritz L *et al* (2009). Predictors of spontaneous and systematically assessed suicidal adverse events in the treatment of SSRI-resistant depression in adolescents (TORDIA) study. *Am J Psychiatry* **166**: 418–426.

Brent DA, Melhem NM, Oquendo M, Burke A, Birmaher B, Stanley B *et al* (2015). Familial pathways to early-onset suicide attempt: a 5.6-year prospective study. *JAMA Psychiatry* **72**: 160–168.

Buss AH, Durkee A (1957). An inventory for assessing different kinds of hostility. *J Consult Psychol* **21**: 343–349.

Cohen J (1988). *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. Lawrence Erlbaum Associates: New Jersey.

Coryell W, Young E, Carroll B (2006). Hyperactivity of the hypothalamic-pituitary-adrenal axis and mortality in major depressive disorder. *Psychiatry Res* **142**: 99–104.

Crosby A, Ortega L, Melanson C (2011). *Self-Directed Violence Surveillance: Uniform Definitions and Recommended Data Elements*. Centers for Disease Control and Prevention: Atlanta.

Danese A, McEwen BS (2012). Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiol Behav* **106**: 29–39.

Dietz LJ, Stoyak S, Melhem N, Porta G, Matthews KA, Walker Payne M *et al* (2013). Cortisol response to social stress in parentally bereaved youth. *Biol Psychiatry* **73**: 379–387.

Ellenbogen MA, Hodgins S, Linnen AM, Ostiguy CS (2011). Elevated daytime cortisol levels: a biomarker of subsequent major affective disorder? *J Affect Disord* **132**: 265–269.

Ellenbogen MA, Hodgins S, Walker CD, Couture S, Adam S (2006). Daytime cortisol and stress reactivity in the offspring of parents with bipolar disorder. *Psychoneuroendocrinology* **31**: 1164–1180.

Endicott J, Spitzer RL, Fleiss JL, Cohen J (1976). The global assessment scale. a procedure for measuring overall severity of psychiatric disturbance. *Arch Gen Psychiatry* **33**: 766–771.

Fountoulakis KN, Iacovides A, Fotiou F, Nimatoudis J, Bascialla F, Ioannidou C *et al* (2004). Neurobiological and psychological correlates of suicidal attempts and thoughts of death in patients with major depression. *Neuropsychobiology* **49**: 42–52.

Golden SH, Wand GS, Malhotra S, Kamel I, Horton K (2011). Reliability of hypothalamic-pituitary-adrenal axis assessment methods for use in population-based studies. *Eur J Epidemiol* **26**: 511–525.

Harkness KL, Stewart JG, Wynne-Edwards KE (2011). Cortisol reactivity to social stress in adolescents: role of depression severity and child maltreatment. *Psychoneuroendocrinology* **36**: 173–181.

Jokinen J, Nordstrom P (2009). HPA axis hyperactivity and attempted suicide in young adult mood disorder inpatients. *J Affect Disord* **116**: 117–120.

Jokinen J, Ouda J, Nordstrom P (2010). Noradrenergic function and HPA axis dysregulation in suicidal behaviour. *Psychoneuroendocrinology* **35**: 1536–1542.

Kalsbeek A, van der Spek R, Lei J, Endert E, Buijs RM, Fliers E (2012). Circadian rhythms in the hypothalamo-pituitary-adrenal (HPA) axis. *Mol Cell Endocrinol* **349**: 20–29.

Kamali M, Saunders EF, Prossin AR, Brucksch CB, Harrington GJ, Langenecker SA *et al* (2012). Associations between suicide attempts and elevated bedtime salivary cortisol levels in bipolar disorder. *J Affect Disord* **136**: 350–358.

Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P *et al* (1997). Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry* **36**: 980–988.

Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom Med* **61**: 154–162.

Kirschbaum C, Pirke KM, Hellhammer DH (1993). The 'Trier Social Stress Test—a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* **28**: 76–81.

Kudielka BM, Schommer NC, Hellhammer DH, Kirschbaum C (2004). Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day. *Psychoneuroendocrinology* **29**: 983–992.

Mann JJ (2003). Neurobiology of suicidal behaviour. *Nat Rev Neurosci* **4**: 819–828.

Mann JJ, McBride PA, Brown RP, Linnoila M, Leon AC, DeMeo M *et al* (1992). Relationship between central and peripheral serotonin indexes in depressed and suicidal psychiatric inpatients. *Arch Gen Psychiatry* **49**: 442–446.

Mannie ZN, Harmer CJ, Cowen PJ (2007). Increased waking salivary cortisol levels in young people at familial risk of depression. *Am J Psychiatry* **164**: 617–621.

McGirr A, Diaconu G, Berlim MT, Pruessner JC, Sable R, Cabot S *et al* (2010). Dysregulation of the sympathetic nervous system, hypothalamic-pituitary-adrenal axis and executive function in individuals at risk for suicide. *J Psychiatry Neurosci* **35**: 399–408.

McGirr A, Diaconu G, Berlim MT, Turecki G (2011). Personal and family history of suicidal behaviour is associated with lower peripheral cortisol in depressed outpatients. *J Affect Disord* **131**: 368–373.

McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M *et al* (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* **12**: 342–348.

McNair DM, Lorr M, Droppleman LF (1992). EdITS manual for the Profile of Mood States. EdITS Educational & Industrial Testing Service: San Diego: CA, 1992.

Melhem NM, Brent DA, Ziegler M, Iyengar S, Kolko D, Oquendo M *et al* (2007). Familial pathways to early-onset suicidal behavior: familial and individual antecedents of suicidal behavior. *Am J Psychiatry* **164**: 1364–1370.

Mesman E, Nolen WA, Reichart CG, Wals M, Hillegers MH (2013). The Dutch bipolar offspring study: 12-year follow-up. *Am J Psychiatry* **170**: 542–549.

Meyer RE, Salzman C, Youngstrom EA, Clayton PJ, Goodwin FK, Mann JJ *et al* (2010). Suicidality and risk of suicidedefinition, drug safety concerns, and a necessary target for drug development: a consensus statement. *J Clin Psychiatry* **71**: e1–e21.

Owens M, Herbert J, Jones PB, Sahakian BJ, Wilkinson PO, Dunn VJ *et al* (2014). Elevated morning cortisol is a stratified population-level biomarker for major depression in boys only with high depressive symptoms. *Proc Natl Acad Sci* **111**: 3638–3643.

Pariante CM, Thomas SA, Lovestone S, Makoff A, Kerwin RW (2004). Do antidepressants regulate how cortisol affects the brain? *Psychoneuroendocrinology* **29**: 423–447.

Pfennig A, Kunzel HE, Kern N, Ising M, Majer M, Fuchs B *et al* (2005). Hypothalamus-pituitary-adrenal system regulation and suicidal behavior in depression. *Biol Psychiatry* **57**: 336–342.

Pitchot W, Scantamburlo G, Pinto E, Hansenne M, Reggers J, Ansseau M *et al* (2008). Vasopressin-neurophysin and DST in major depression: relationship with suicidal behavior. *J Psychiatr Res* **42**: 684–688.

Poustka L, Maras A, Hohm E, Fellinger J, Holtmann M, Banaschewski T *et al* (2010). Negative association between plasma cortisol levels and aggression in a high-risk community sample of adolescents. *J Neural Transm* **117**: 621–627.

Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* **28**: 916–931.

Spitzer RL, Williams JB, Gibbon M, First MB (1992). The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. *Arch Gen Psychiatry* **49**: 624–629.

StataCorp (2009). *Stata Statistical Software: Release* 11. StataCorp LP: College Station, TX, USA.

Teicher MH, Samson JA (2013). Childhood maltreatment and psychopathology: a case for ecophenotypic variants as clinically and neurobiologically distinct subtypes. *Am J Psychiatry* **170**: 1114–1133.

US Department of Health and Human Services (2012). Guidance for Industry. *Suicidal Ideation and Behavior: Prospective Assessment of Occurrence in Clinical Trials*, Silver Spring, MD.

Waugh CE, Muhtadie L, Thompson RJ, Joormann J, Gotlib IH (2012). Affective and physiological responses to stress in girls at elevated risk for depression. *Dev Psychopathol* **24**: 661–675.

Wolfram M, Bellingrath S, Feuerhahn N, Kudielka BM (2013). Cortisol responses to naturalistic and laboratory stress in student teachers: comparison with a non-stress control day. *Stress Health* **29**: 143–149.

Yehuda R, Flory JD, Pratchett LC, Buxbaum J, Ising M, Holsboer F (2010). Putative biological mechanisms for the association between early life adversity and the subsequent development of PTSD. *Psychopharmacology (Berl)* **212**: 405–417.

Yerevanian BI, Feusner JD, Koek RJ, Mintz J (2004). The dexamethasone suppression test as a predictor of suicidal behavior in unipolar depression. *J Affect Disord* **83**: 103–108.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)