Published in final edited form as:

Psychoneuroendocrinology. 2017 March ; 77: 284–294. doi:10.1016/j.psyneuen.2017.01.001.

Blunted HPA axis activity prior to suicide attempt and increased inflammation in attempters

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Abstract

Background—Hypothalamic-Pituitary-Adrenal (HPA) axis dysregulation is associated with increased risk for suicidal behavior. However, it is not clear whether such dysregulation exists prior to or is a consequence of attempt. Studies also show an activation of inflammatory responses in suicidal behavior but often combine attempters with those with ideation.

Methods—The sample consisted of psychiatric inpatients, aged 15-30 years, admitted for suicide attempt (SA, n=38), inpatients admitted for suicidal ideation with no prior history of attempts (SI, n=40), and healthy controls (n=37). We compared SA, SI, and controls on hair cortisol concentrations (HCC), which provides retrospective levels of cortisol and thus prior to the attempt in SA. We also compared them on the expression of genes in the HPA axis and inflammatory pathways previously implicated in suicidal behavior (GR or *NR3C1*, *SKA2*, *FKBP5*, *IL-1 β* , *TNF- α*); plasma C-Reactive Protein (CRP); and cellular measures of glucocorticoid receptor (GR) sensitivity and stimulated production of IL-6.

Results—We found lower HCC [β =−0.55, 95% CI (−0.96, −0.13), p =0.01, ES=−0.54] in first-time SA compared to SI and controls. In addition, SA showed lower GR or *NR3C1* (α isoform) mRNA [β =−5.11, 95% CI (−10.9, 0.73), p =0.09, ES=−0.46], higher CRP [β =0.94, 95% CI (−0.004, 1.9), p =0.05, ES=0.60], and higher *TNF- α* mRNA [β =26.4, 95% CI (7.7, 45.2), p =0.006, ES=0.73].

Conclusions—This is the first study to differentiate youth who attempt suicide from those with suicidal ideation on HCC and to show that low HCC precedes suicide attempt. Suicide attempters

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also showed a distinct biological profile on several markers in *both* the HPA axis and inflammatory pathways. Future longitudinal studies are needed to examine the ability of these biomarkers to predict suicidal behavior.

Keywords

Suicide attempt; Ideation; Youth; Hair Cortisol; Inflammation; Gene Expression

1. INTRODUCTION

One of the most challenging tasks for clinicians is to identify patients at high risk for suicidal behavior. Studies find no association between clinicians' prediction for a patient and patient's actual suicidal behavior (Dawes et al., 1989). Thus, it is critical to identify objective biological signatures for suicidal behavior.

The diathesis of suicidal behavior is hypothesized to include a vulnerability that is exacerbated by an acute stressor, which often precedes suicidal behavior. Indeed, dysregulation in the hypothalamic-pituitary-adrenal (HPA) axis, which regulates reactions to stress, is associated with 4.5-fold increased risk for suicide (Mann and Currier, 2007). Both HPA axis hypo- and hyper- activity are described in attempters and relatives of people who died by suicide (Dietz et al., 2013; Jokinen et al., 2010; Mann and Currier, 2007; McGirr et al., 2010; McGirr et al., 2011). We previously reported lower cortisol levels in response to an experimental stressor in attempters compared to high-risk suicidal non-attempters and non-suicidal subjects and compared to healthy controls; and low baseline cortisol prior to the stressor in attempters (Keilp et al., 2016; Melhem et al., 2016). Our results were also recently replicated by another study (O'Connor et al., 2017). One of the limitations of extant studies is that it is not clear whether HPA axis dysregulation exists prior to suicidal behavior or is a consequence of an attempt because extant studies often examine cortisol levels in subjects with history of suicidal behavior. Recent development of reliable methods to assess levels of cortisol in hair samples permits the retrospective assessment of HPA axis activity (Stalder and Kirschbaum, 2012). Hair grows at ~1 cm per month and thus, analysis of the 3 cm segment of hair closest to the scalp represents cortisol levels over the past three months (Stalder and Kirschbaum, 2012). In this study, we conducted the first examination of hair cortisol concentrations (HCC) among inpatients admitted for a suicide attempt (SA), which reflect cortisol levels *prior* to the attempt, and compared them to HCC in those admitted for suicidal ideation (SI) with no prior history of attempt and healthy controls.

In order to better understand the biological pathways implicated in HPA axis dysregulation and suicidal behavior, we also compared SA, SI, and controls on peripheral markers of HPA axis gene expression (*GR* or *NR3C1*, *SKA2*, *FKBP5*) and cellular measures of glucocorticoid receptor (GR) sensitivity. Reduced hippocampal *GR* mRNA is reported in brains of people who died by suicide and suicides with history of abuse (Labonte et al., 2012; McGowan et al., 2009; Sequeira et al., 2012). Studies in postmortem brains and living subjects have also implicated the expression of GR-related genes, specifically, *SKA2* and *FKBP5*, in suicidal ideation and behavior (Guintivano et al., 2014; Niculescu et al., 2015; Pandey et al., 2016; Perez-Ortiz et al., 2013; Yin et al., 2016).

There is also accumulating evidence for the role of inflammation in suicidal behavior. There is increased risk for suicidal behavior with the activation of inflammatory responses following treatment with interferon for patients with cancer, hepatitis C, and multiple sclerosis (Baron et al., 1993; Fragoso et al., 2010; Janssen et al., 1994); in autoimmune disorders (Fuller-Thomson et al., 2016; Ludvigsson et al., 2011); in infections (Brundin and Grit, 2016; Lund-Sorensen et al., 2016); and in diseases that involve exaggerated immune responses, such as allergies and asthma (Goodwin, 2012). Inflammation is also associated with impulsivity and aggression (Coccaro et al., 2014; Isung et al., 2014), which predict suicidal behavior (Melhem et al., 2007); and with sleep problems (Prather et al., 2009), which often precede suicidal behavior. In addition, meta-analyses show direct evidence of increased circulating markers of inflammation in suicidal patients (Black and Miller, 2015); and high C-Reactive Protein (CRP) levels are associated with a 4-fold increased risk for suicide (Batty et al., 2016). Finally, increased expression of inflammatory genes [e.g., interleukin (*IL*)- 1β , *IL*-6, and Tumor Necrosis Factor- α (*TNF*- α)] is reported in postmortem brains of suicides (Hoyo-Becerra et al., 2013; Pandey et al., 2012; Tonelli et al., 2008); and in peripheral blood among subjects at risk for suicidal behavior (Le-Niculescu et al., 2013; Mostafavi et al., 2014). Thus, we also compared SA, SI, and controls on mRNA of inflammatory genes (*TNF*- α and *IL*- 1β), plasma CRP, and a cellular measure of stimulated production of IL-6.

Indeed, there is a tight signaling between the nervous, endocrine, and immune systems to maintain homeostasis. In an adaptive response to stress, peripheral cortisol is released and binds to GR and inhibits pro-inflammatory transcription factors, which regulate the expression of genes for many cytokines (O'Connor et al., 2000). Cytokines can also act to down-regulate the HPA axis and affect GR function at multiple levels (Miller et al., 2009). Increases in inflammation that accompany stress could result from downregulation of GR sensitivity, possibly as a function of chronic exposure to increased cortisol (Pace and Miller, 2009; Rohleder, 2012). However, childhood adversity, a risk factor for suicidal behavior, is associated with low cortisol and increased inflammation (Baumeister et al., 2016; Raison and Miller, 2003). Studies suggest that hypocortisolism may occur after a prolonged period of HPA axis hyperactivity due to chronic stress; and the impaired immunosuppressive effects of low cortisol may also result in increased inflammation (Heim et al., 2000).

This study is the first to measure HCC in SA, which reflects cortisol levels prior to attempt; and to combine several biological markers of the HPA axis and inflammatory pathways to examine their relationship to suicidal behavior. Based on our previous results, we hypothesize that SA will show lower HCC, downregulation of *GR* mRNA and its co-chaperones (*SKA2* & *FKBP5* mRNA), enhanced GR sensitivity, and increased inflammation compared to SI and controls. HCC is the only biological marker with retrospective recording; all other biological markers reflect post-hospitalization state measures. We also hypothesize that childhood history of abuse will be associated with reduced HCC and that HCC will be associated with post-hospitalization biological measures, perceived stress, and clinical correlates of suicidal behavior (e.g., impulsivity, aggression, and sleep disturbances).

2. MATERIALS AND METHODS

2.1. Sample

The sample consisted of psychiatric inpatients, aged 15-30 years, admitted following a suicide attempt (SA, n=38), inpatients admitted for suicidal ideation with no prior history of attempts (SI, n=40), and healthy controls (n=37). Inpatients were recruited from Western Psychiatric Institute and Clinic (WPIC), the psychiatric hospital of the University of Pittsburgh Medical Center (UPMC). Inpatients whose attempt resulted in brain injury affecting their cognitive ability to participate were excluded. Healthy controls with no previous history of psychiatric disorders and suicidal and/or self-injurious behavior were recruited from the University of Pittsburgh Clinical and Translational Science Institute Research Participant registry, which engages participants at points of routine clinical care in the UPMC health system. Inpatients and controls with no hair; those with chronic conditions that could affect their HPA axis and/or inflammatory pathways (e.g., Cushing's and Addison's diseases); subjects receiving oral corticosteroids during the prior year or prescribed medications that influence immune or neuroendocrine function [such as chemotherapy (e.g., cyclosporine or methotrexate), TNF- α inhibitors (e.g., Infliximab)]; pregnancy; and those with cold, flu, or other acute infections or on antibiotics in the two weeks prior to blood draw were excluded. Inpatients and controls were recruited in accordance with the Institutional Review Board (IRB) guidelines for Human Subjects Protection at the University of Pittsburgh.

2.2. Assessment

Suicidal ideation and behavior were assessed during the clinical interview using the Columbia Suicide Severity Rating Scale (C-SSRS) (Posner et al., 2011), which assesses current and lifetime suicidal ideation and attempts, intensity of ideation, and medical lethality of suicide attempts. Lifetime history and current psychiatric disorders were obtained using the Family History-Research Diagnostic Criteria (FH-RDC) with criteria modified to DSM-IV (Andreasen et al., 1986). Self-reported instruments were also administered to assess the severity of depression symptoms using the Patient Health Questionnaire-9 (PHQ-9) (Kroenke et al., 2001); anxiety symptoms using the Generalized Anxiety Disorder-7 (GAD-7) (Spitzer et al., 2006); hopelessness using the Beck Hopelessness Scale (BHS) (Beck et al., 1974), PTSD symptoms using the PTSD Symptom Scale (Foa, 1993); and alcohol and substance use using the Drug Use Screening Inventory-Revised (DUSI-R) (Tarter and Kirisci, 1997). We also assessed impulsivity using the Barratt Impulsivity Scale (Barratt, 1965); aggression using the Aggression Questionnaire (Buss, 1992); and sleep disturbances using the Pittsburgh Sleep Quality Index (Buysse et al., 1989). Intercurrent stressors prior to admission were measured using the Life Events Checklist in subjects <18 years of age (Brand, 1982) and the shortened version of the Social Readjustment Rating Scale for older subjects (Holmes and Rahe, 1967). Childhood history of abuse was assessed using the Child Trauma Questionnaire (Bernstein et al., 1994). We also measured perceived stress using the 14-item Perceived Stress Scale (Cohen et al., 1983). Finally, we measured height and weight, and waist-hip circumference; collected information about medications used; and socio-economic status (SES) using the Hollingshead index of social status (Hollingshead, 1975).

2.3. Biological assays

2.3.1. Hair cortisol—We collected hair from the 3 cm segment closest to the scalp from a posterior vertex position. Hair samples were processed at the Behavioral Immunology Laboratory at the University of Pittsburgh following methods described by Laudenslager et al. (Laudenslager et al., 2011) from the Behavioral Immunology and Endocrinology Laboratory at the University of Colorado. Cortisol was quantified in pg/mg using an enzyme immunoassay run according to manufacturer's instructions (Salimetrics, LLC, PA). All samples were run in duplicate with an average coefficient of variation (CV) < 5% and intra-plate CV < 10%. In a cross-lab validation study, we found high correlation for HCC assayed from the same individuals between the Pittsburgh and Colorado laboratories ($r=0.96$, $p<0.001$). We found no association between HCC and hair dyeing ($p=0.42$) and chemical processing ($p=0.8$) in our sample.

2.3.2. High sensitivity C-Reactive Protein (CRP)—was determined in mg/dl using a CRPH reagent on a SYNCHRON LX System (Beckman Coulter, Inc., Brea, California).

2.3.3. GR sensitivity and Stimulated production of IL-6—Whole blood samples were incubated with lipopolysaccharide from *E.coli* (LPS, serotype 026:B6, Sigma) at a final concentration of 50 ng/ml with 0, 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , and 10^{-6} M of hydrocortisone (H-0888, 5g, Sigma) to assess corticosteroid suppression of cytokine (IL-6) production. After an 18-hour incubation at 37° C and 5% CO₂, supernatants were harvested and stored at -80° C until analysis. Analyses for GR sensitivity were conducted using the area under the curve and controlling for levels at 0 hydrocortisone concentration. Stimulated production of IL-6 is obtained at 0 hydrocortisone concentration and provides a measure of IL-6 derived solely from immune cells. All samples were run in duplicate with an average CV < 5%.

2.3.4. RNA analyses—PAXgene Blood RNA Tubes were used for blood collection, stabilization, and transport, and the PAXgene Blood RNA Kit for RNA isolation and purification. Samples were run using Illumina's HumanHT-12 v4 Expression BeadChip Kit. We excluded two samples with low RNA integrity number (RIN < 4). The average RIN for samples analyzed was 8.1 (SD=0.56). We conducted background adjustment to remove signal due to non-specific hybridization and normalized the data to correct for systematic processing biases using the average normalization method (Schmid et al., 2010). All the genes analyzed passed quality control (QC) analysis and showed a detection p-value that was significant in 50% or more of the samples. *IL-6* mRNA was excluded from our list of genes because it did not meet our QC criterion. When there were multiple probes per gene, we examined whether they correspond to different transcript variants and encode different isoforms using Ensembl Genome Browser. We analyzed probes for different isoforms separately, which was the case for the *NR3C1* or *GR* gene with α and β isoforms. For transcript variants encoding the same isoform, we examined their correlation and used the average intensities if highly correlated. This was conducted for *SKA2* ($r=0.58$, $p<0.001$).

2.4. Statistical analyses

We examined the normality assumption for the biological measures and applied appropriate transformations when this assumption was violated. A natural logarithmic transformation

was applied for HCC, CRP, and stimulated production of IL-6. We compared the three groups on demographic and clinical characteristics using chi-square tests and analysis of variance (ANOVA) with post-hoc comparisons if $p < 0.05$. We corrected for post-hoc multiple comparisons, where 3 two-group comparisons (SA vs. Controls; SI vs. Controls; SA vs. SI) were conducted, using a Bonferroni correction ($p < 0.017$ or $0.05/3$). We examined the relationship of HCC and other biological markers to potential covariates including sex, age, race, SES, Body Mass Index (BMI), adiposity as measured by waist-hip circumference (WHC), smoking, and time of blood draw (O'Connor et al., 2009). We used linear regression to examine the relationship between each biological measure and group controlling for significantly associated covariates. Biological assays were conducted in two batches and thus we controlled for batch effects. We also controlled for childhood abuse in these models given its long-lasting impact on the HPA axis and inflammatory pathways (Danese and McEwen, 2012). Group was used as a dummy-coded variable with healthy controls as the reference category. We assessed the impact of alcohol and substance use and regular use of medications and controlled for those that were significantly associated with the biological measure. In addition, we examined the impact of medications and substances used among SA, who attempted by overdose, on biological measures. When only few subjects report the use of a substance or medication (whether used regularly or in an overdose) that is significantly associated with the biological measure, we excluded these subjects from the analyses of that biological measure (e.g., 4 subjects used diet pills, 2 used gasoline & fumes). We also excluded one subject who attempted suicide by gunshot and had severe injuries that affected CRP levels in that subject. Given our sample size, we removed covariates from the final model that were not significant at $p < 0.05$. We also examined the relationships between biological measures and lethality of most recent attempt using Spearman's rho correlations. Finally, we examined the relationships between HCC and post-hospitalization biological measures; and between each of HCC and post-hospitalization biological measures with childhood history of abuse and clinical correlates of suicidal behavior using Pearson's correlations. We conducted all analyses with and without subjects with multiple attempts ($n=15$) to eliminate any potential effects of prior attempts on HCC; and subjects less than 18 years of age ($n=5$), a period of developmental changes that could impact the studied biological pathways. Thus, the reduced sample sizes were 21 SA, 38 SI, and 37 controls.

3. RESULTS

3.1. Demographic and clinical characteristics

SA, SI, and controls were similar on demographic characteristics with 43% females, mean age of 23 (Standard Deviation or $SD=3.4$) years, and 82% Caucasian (**Table 1**). They were also similar on BMI and time of blood draw, which was standardized to be around 2 pm (± 2 hours). However, SA and SI had significantly lower SES compared to controls. The average time between admission and recruitment was 3.1 days ($SD=1.6$) and was not significantly different between SA and SI. For SA, the average time between attempt and admission for psychiatric care was 1.9 days ($SD=2.4$) because some patients were first admitted to another hospital to be medically stabilized. The average time between attempt and blood draw was 5.3 days ($SD=2.7$). The method of attempt was overdose on medications and/or drugs in

76.3% (n=29) of SA, hanging in 10.5% (n=4), gunshot in 8% (n=3), cutting wrist with intent to die in 2.6% (n=1), and cutting wrist and walking into traffic in 2.6% (n=1). The average lethality of attempts on the C-SSRS was 2.2 (SD=1.2), which corresponds to moderate physical damage and medical attention needed.

SA and SI were also similar on primary psychiatric diagnoses (unipolar, bipolar, and psychotic disorders) and comorbid disorders (e.g., anxiety disorders, alcohol and substance use disorders) at the time of admission; family history of suicidal behavior; and regular use of antidepressants, antipsychotics, and mood stabilizers prior to admission (**Table 1**). SA and SI were also similar on most clinical and psychosocial correlates of suicidal behavior. However, SA showed significantly higher severity of current suicidal ideation as measured by the C-SSRS (4.8 ± 0.15 vs. 3.7 ± 0.16 , $t=6$, $df=58.6$, $p<0.001$) compared to SI. SA also reported higher rates of childhood history of abuse (73.7% vs. 52.5%, $\chi^2=3.7$, $df=1$, $p=0.05$), were more likely to be smokers compared to SI (78.9% vs. 57.5%, $\chi^2=4.1$, $df=1$, $p=0.04$), and had lower waist-hip circumference (0.82 ± 0.1 vs. 0.87 ± 0.08 , $t=-2.1$, $df=76$, $p=0.04$) although these post-hoc differences did not reach statistical significance at $p<0.017$. As expected, both SA and SI were significantly different from the controls on all clinical characteristics.

3.2. Comparing groups on Hair Cortisol Concentrations

SA showed significantly lower HCC compared to SI and controls even after controlling for significant covariates [$\beta=-0.42$, 95% CI (-0.78, -0.05), $p=0.02$, ES=-0.38] (**Table 2, Figure 1**). Similar results were obtained when excluding multiple attempters (n=15) and subjects <18 years of age (n=5) [$\beta=-0.55$, 95% CI (-0.96, -0.13), $p=0.01$, ES=-0.54] (**reduced sample, Table 2, Figure 2**). Among SA, lower HCC was associated with increased lethality of the attempt in the full sample but the result did not reach statistical significance ($r=-0.38$, $p=0.08$). A similar correlation was observed in the reduced sample and was not significant ($r=-0.26$, $p=0.25$).

3.3. Comparing groups on post-hospitalization biological markers

SA also showed significantly lower *GR-α* mRNA compared to SI and controls [$\beta=-4.7$, 95% CI (-9.45, 0.02), $p=0.05$, ES=-0.40]; Similar results were obtained in the reduced sample [$\beta=-5.11$, 95% CI (-10.9, 0.73), $p=0.09$, ES=-0.46] although it did not reach statistical significance (**Table 2, Figures 1 & 2**). *GR-β* mRNA and GR sensitivity were not significantly different in the full and reduced samples (**Table 2, Figures 1 & 2**); however, both SA and SI show a direction towards higher GR sensitivity compared to controls with effect sizes (ES) of -0.3 and -0.2, respectively (lower values reflect higher GR sensitivity) (**Table 2, Figure 1**). ES were reduced to -0.14 and -0.11, respectively, in the reduced sample (**Table 2, Figure 2**). *FKBP5* and *SKA2* mRNA were also not significantly different; however, *FKBP5* mRNA was consistently reduced (ES=-0.29) and *SKA2* mRNA consistently increased in SI in the full and reduced samples (ES=0.35 and 0.40, respectively) (**Table 2, Figures 1 & 2**).

Regarding inflammation, SA showed significantly higher CRP [$\beta=0.99$, 95% CI (0.15, 1.84), $p=0.02$, ES=0.64] and *TNF-α* mRNA [$\beta=16.8$, 95% CI (1.9, 31.7), $p=0.03$, ES=0.50]

compared to SI and controls (**Table 2, Figure 1**). Similar results for CRP [$\beta=0.94$, 95% CI (−0.004, 1.9), $p=0.05$, ES=0.60] and *TNF- α* mRNA [$\beta=26.4$, 95% CI (7.7, 45.2), $p=0.006$, ES=0.73] were obtained in the reduced sample (**Table 2, Figure 2**). SA, SI, and controls were not significantly different on *IL-1 β* mRNA in the full and reduced samples (**Table 2, Figures 1 & 2**). We also find SA to show a trend towards higher stimulated production of IL-6 compared to SI and controls in the reduced sample [$\beta=0.33$, 95% CI (−0.04, 0.7), $p=0.08$, ES=0.49] (**Table 2, Figure 2**).

3.4. Relationship between HCC and post-hospitalization biological markers

We examined the relationship of HCC with other biological markers and find HCC to be significantly and consistently associated with *GR- α* mRNA ($r=0.41$, $p<0.001$) in the full (**Table 3**) and reduced samples ($r=0.41$, $p<0.001$) (**Tables S1**). HCC was not significantly correlated with GR sensitivity and with inflammatory markers that significantly differentiated SA from SI and controls. However, *GR- α* mRNA and GR sensitivity were significantly correlated in the full ($r=0.21$, $p<0.05$) (**Table 3**) and reduced samples ($r=0.22$, $p<0.05$) (**Tables S1**). Lower *GR- α* mRNA, which was found in SA, was associated with enhanced GR sensitivity (lower scores on GR sensitivity reflect higher sensitivity). *GR- α* mRNA was also negatively correlated with CRP levels in the full ($r=-0.32$, $p<0.001$) (**Table 3**) and reduced samples ($r=-0.40$, $p<0.001$) (**Tables S1**).

3.5. Relationship of HCC with childhood abuse and clinical correlates of suicidal behavior

HCC was significantly and negatively correlated with childhood abuse in the full ($r=-0.21$, $p=0.01$) (**Table 4**) and reduced samples ($r=-0.26$, $p=0.01$) (**Table S2**). However, HCC was significantly different in SA even after controlling for childhood abuse. Among the post-hospitalization correlates of suicidal behavior, HCC was only associated with perceived stress in the full ($r=-0.28$, $p<0.01$) and reduced ($r=-0.25$, $p<0.05$) samples. There were no significant correlations between HCC and severity of depression, anxiety, and PTSD symptoms; hopelessness; impulsivity and aggression; and sleep disturbances (**Table 4**).

3.6. Relationship of post-hospitalization biological markers with childhood abuse, and clinical correlates of suicidal behavior

Among the post-hospitalization biological markers, only *GR- α* mRNA was significantly and negatively associated with childhood abuse in the full sample ($r=-0.24$, $p<0.01$) (**Table 4**). *TNF- α* mRNA was significantly and positively correlated with childhood abuse in the reduced sample ($r=0.22$, $p<0.05$) (**Table S2**). However, *GR- α* and *TNF- α* mRNA were significantly different in SA even after controlling for childhood abuse in the full and reduced samples. *TNF- α* mRNA was also significantly and positively correlated with ongoing stressful life events ($r=0.26$, $p<0.01$) and perceived stress ($r=0.25$, $p<0.01$); with clinical correlates of depression ($r=0.30$, $p<0.01$) and anxiety symptoms ($r=0.24$, $p<0.05$), impulsivity ($r=0.40$, $p<0.001$), aggression ($r=0.21$, $p<0.05$), hopelessness ($r=0.30$, $p<0.01$), and sleep disturbances ($r=0.31$, $p<0.01$); and with the number of cigarettes smoked per day ($r=0.31$, $p=0.001$). Similar correlations were obtained in the reduced sample (**Table S2**).

4. DISCUSSION

Suicide attempters and those with suicidal ideation were similar on all clinical characteristics at the time of a suicidal crisis, except for the severity of suicidal ideation. However, attempters showed a distinct biological profile on several markers in the HPA axis and inflammatory pathways. Most importantly, SA showed lower HCC, which reflect retrospective levels of cortisol prior to their attempt. In addition, SA consistently showed lower *GR* or *NR3C1* (α isoform) mRNA, higher CRP, and higher *TNF- α* mRNA compared to those with ideation and controls. Childhood history of abuse was associated with lower HCC, lower *GR- α* mRNA, and higher *TNF- α* mRNA. HCC was not associated with any of the clinical correlates of suicidal behavior except for perceived stress; however, *TNF- α* mRNA was associated with almost all of these clinical correlates.

This is the first study to examine HCC in SA reflecting cortisol levels prior to attempt; to examine several biological markers in *both* the HPA axis and inflammatory pathways in the context of suicidal behavior; and to compare SA to high-risk subjects, i.e., those with ideation and no prior history of attempt, on these biological markers. Our study is a pilot study and thus limited by small sample size and cross-sectional design. In addition, except for HCC, it is not clear whether post-hospitalization biological alterations are due to the attempt or the method of attempt itself (e.g., overdose, cutting wrist) or existed prior to the attempt. Longitudinal studies of psychiatric patients with no prior history of suicidal behavior are needed to examine whether these biological alterations precede and predict future suicidal behavior.

We found SA and SI to be similar on stressful life events and perceived stress but SA showed lower HCC, which reflects a diminished ability to mount an adaptive response to stress. Similar results were obtained in first-time attempters and all attempters including those with multiple attempts; and when excluding subjects less than 18 years of age. Previous studies have reported both HPA axis hypo- and hyper- activity in attempters and relatives of people who died by suicide (Dietz et al., 2013; Jokinen et al., 2010; Mann and Currier, 2007; McGirr et al., 2010; McGirr et al., 2011). These mixed findings could be due to methodological variations and difficulties inherent in the assessment of peripheral cortisol, which varies as a function of circadian rhythm and environmental factors. HCC overcomes these methodological difficulties and provides a retrospective measure of chronic HPA axis activity (Stalder and Kirschbaum, 2012). We did not assess plasma or salivary cortisol levels in this study to examine whether current cortisol levels were also reduced in SA compared to SI and controls. These HCC results are consistent with our previous findings of blunted cortisol response to an acute social stressor in SA compared to suicidal non-attempters, non-suicidal, and healthy controls; and low baseline salivary cortisol levels prior to the social stress task in SA (Keilp et al., 2016; Melhem et al., 2016). They are also consistent with recent reports replicating these findings (O'Connor et al., 2017). In this sample, SA and SI were also similar on primary psychiatric diagnosis, comorbidity profile, and their use of medications; however, SA showed lower HCC prior to the attempt compared to SI and controls. Hence, lower HCC in SA are unlikely due to major depressive or bipolar disorders, the two most common primary psychiatric diagnoses in our sample and known to be associated with HPA axis dysregulation (Belvederi Murri et al., 2016; Murri et al., 2014).

In addition, lower HCC in SA are unlikely due to medications, which affect HPA axis function (Pariante et al., 2004). Indeed, HCC was not associated with major depressive disorder, bipolar disorder, other primary or comorbid psychiatric diagnoses, or regular use of medications in our sample of psychiatric patients. HCC were also not associated with the severity of depression, anxiety, and PTSD symptoms. Finally, lower HCC in SA are also unlikely due to medications used in an overdose among attempters because we used the 3 cm segment closest to the scalp, which reflects chronic HPA axis activity over the past three months.

Our results are consistent with low HCC and hypocortisolism in subjects with PTSD and those exposed to trauma (Heim et al., 2000; Hinkelmann et al., 2013; Steudte et al., 2013). SA were more likely to report a history of childhood abuse, which was also associated with lower HCC. However, childhood abuse was not a significant covariate in the model differentiating attempters on HCC. We also find that SA have lower *GR-α* mRNA compared to SI and controls. Low hippocampal *GR* mRNA has been previously reported in brains of suicides and suicides with history of abuse (Labonte et al., 2012; McGowan et al., 2009; Pandey et al., 2013). *GR-α* mRNA was positively and moderately correlated with HCC in our sample. Similar to HCC, *GR-α* mRNA was also negatively correlated with childhood abuse. Future longitudinal studies in patients without prior history of suicidal behavior are needed to examine whether peripheral *GR-α* mRNA precede and predict suicide attempt; and to examine whether childhood abuse is a moderator of the relationship between each of HCC and *GR-α* mRNA with suicidal behavior.

Enhanced GR sensitivity has been one of the proposed mechanisms for hypocortisolism observed in stress-related disorders (Heim et al., 2000). SA and SI showed a direction towards enhanced GR sensitivity; however, SA, SI, and controls were not significantly different on GR sensitivity. Our results suggest additional mechanisms likely contribute to the reduced HCC and *GR-α* mRNA in SA only. *FKBP5* and *SKA2* mRNA were also not significantly different between groups. However, *FKBP5* mRNA was consistently reduced ($ES=-0.29$) and *SKA2* mRNA consistently increased in SI in the full and reduced samples ($ES=0.35$ and 0.40 , respectively). Lower *FKBP5* mRNA has been reported in brains of suicides and in subjects with PTSD (Perez-Ortiz et al., 2013; Sarapas et al., 2011; Yin et al., 2016); and *FKBP5* polymorphisms have been previously associated with suicidal events in adolescents (Brent et al., 2010). Reduced *SKA2* expression is also reported in postmortem brains of suicides and in patients with suicidal ideation and behavior (Guintivano et al., 2014; Niculescu et al., 2015; Pandey et al., 2016). However, a recent study did not replicate these results and showed no differences in *SKA2* expression between brains of suicides compared to brains of those with major depressive disorder (MDD) and without MDD who died from other causes (Yin et al., 2016). Niculescu et al.'s study (Niculescu et al., 2015) reporting reduced *SKA2* expression in living suicidal patients is limited by small sample size and included males only. Consistent with the direction of our *SKA2* mRNA results, a recent study showed decreases in *SKA2* methylation over time, which would result in increased expression of the gene, to be associated with increased PTSD symptoms in deployed military personnel exposed to trauma (Boks et al., 2016). Future studies are needed to clarify the role of *SKA2* expression in suicidal behavior; and to examine co-expression

networks of HPA axis genes to shed light on the mechanisms of HPA axis dysregulation implicated in suicidal behavior.

Our results of increased CRP and *TNF- α* mRNA in SA are consistent with studies showing an activation of inflammatory responses in suicidal behavior (Black and Miller, 2015). None of the post-hospitalization biological measures were significantly related to primary or comorbid psychiatric disorders among inpatients except for higher CRP in those with unipolar depression ($p=0.01$); however, SA showed higher CRP even after controlling for unipolar vs. bipolar disorder. High CRP levels in the general population have been recently reported to be associated with a 4-fold increased risk for suicide (Batty et al., 2016). However, Batty et al.'s study cannot establish a causal relationship between CRP levels and suicide given the long follow-up period (8.6 ± 3.3 years). Future longitudinal studies in high-risk samples are needed to examine whether CRP and other peripheral inflammatory markers predict imminent risk for suicidal behavior. While hypocortisolism and the impaired immunosuppressive effects of low cortisol may result in increased inflammation, which is observed in patients with stress-related disorders (Heim et al., 2000), we find no significant correlations between HCC and each of CRP and *TNF- α* mRNA. Similar to HCC, we also find childhood abuse to be associated with increased *TNF- α* mRNA in the reduced sample, consistent with extant studies of increased inflammation in subjects with history of childhood abuse (Danese and McEwen, 2012). *TNF- α* mRNA also continued to be increased in SA even after controlling for childhood abuse. Similar to HCC, *TNF- α* mRNA was also associated with perceived stress; however, *TNF- α* mRNA was associated with several clinical predictors of suicidal behavior of increased severity of depression and anxiety symptoms, impulsivity, aggression, hopelessness, and sleep disturbances. *TNF- α* is a potent pro-inflammatory cytokine and is one of the cytokines that can cross the blood brain barrier (BBB) without BBB disruption and affect brain function; in addition, large doses of *TNF- α* can disrupt the BBB (Banks, 2015). Future studies are needed to examine whether *TNF- α* mRNA translates into increased plasma levels of *TNF- α* , which we did not measure in our study. It is unlikely that a single biomarker will predict a complex phenotype like suicidal behavior. Our results suggest that it is possible that combining HCC with *TNF- α* mRNA and other biological markers could improve our ability to predict suicidal behavior. Indeed, combining biological (HCC, CRP, *TNF- α* mRNA, *GR* mRNA) and clinical measures (severity of suicidal ideation, childhood abuse, smoking) differentiating SA resulted in a significantly increased Area Under the Curve or AUC compared to the model including clinical measures only (0.96 vs. 0.79, respectively, $p<0.001$); as well as increased sensitivity (75% vs. 24%), specificity (96% vs. 89%), positive predictive value (82% vs. 38%), negative predictive value (95% vs. 80%), % correctly classified (93% vs. 74%), and pseudo R^2 (0.54 vs. 0.18). We recognize the limitations of our sample size to conduct these analyses, which results in model over-fitting; however, we present them here as preliminary evidence to emphasize the need for future longitudinal studies with large sample sizes to combine an *array* of biological markers in *both* the HPA axis and inflammatory pathways and to combine them with well-characterized clinical measures in order to improve our prediction of suicidal behavior.

In conclusion, this is the first study to differentiate youth who attempt suicide from those with suicidal ideation on HCC and to show that low hair cortisol levels precede suicide

attempt. Suicide attempters also showed a distinct biological profile on several biological markers in the HPA axis and inflammatory pathways. Future longitudinal studies are needed to examine the ability of these biomarkers to help clinicians in one of their most challenging tasks, i.e., predict those at highest-risk for suicidal behavior for appropriate interventions among patients who are often clinically indistinguishable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES

- Andreasen NC, Rice J, Endicott J, Reich T, Coryell W. The family history approach to diagnosis. How useful is it? *Arch Gen Psychiatry*. 1986; 43:421–429. [PubMed: 3964020]
- Banks WA. The blood-brain barrier in neuroimmunology: Tales of separation and assimilation. *Brain Behav Immun*. 2015; 44:1–8. [PubMed: 25172555]
- Baron DA, Hardie T, Baron SH. Possible association of interleukin-2 treatment with depression and suicide. *J Am Osteopath Assoc*. 1993; 93:799–800. [PubMed: 8365929]
- Barratt ES. Factor analysis of some psychometric measures of impulsiveness and anxiety. *Psychological Reports*. 1965; 16:547–554. [PubMed: 14285869]
- Batty GD, Bell S, Stamatakis E, Kivimaki M. Association of Systemic Inflammation With Risk of Completed Suicide in the General Population. *JAMA Psychiatry*. 2016; 73:993–995. [PubMed: 27532220]
- Baumeister D, Akhtar R, Ciufolini S, Pariante CM, Mondelli V. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-alpha. *Mol Psychiatry*. 2016; 21:642–649. [PubMed: 26033244]
- Beck AT, Weissman A, Lester D, Trexler L. The measurement of pessimism: the hopelessness scale. *J Consult Clin Psychol*. 1974; 42:861–865. [PubMed: 4436473]
- Belvederi Murri M, Prestia D, Mondelli V, Pariante C, Patti S, Olivieri B, Arzani C, Masotti M, Respino M, Antonioli M, Vassallo L, Serafini G, Perna G, Pompili M, Amore M. The HPA axis in bipolar disorder: Systematic review and meta-analysis. *Psychoneuroendocrinology*. 2016; 63:327–342. [PubMed: 26547798]
- Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K, Sapareto E, Ruggiero J. Initial reliability and validity of a new retrospective measure of child abuse and neglect. *Am J Psychiatry*. 1994; 151:1132–1136. [PubMed: 8037246]
- Black C, Miller BJ. Meta-Analysis of cytokines and chemokines in suicidality: Distinguishing suicidal versus nonsuicidal patients. *Biol Psychiatry*. 2015; 78:28–37. [PubMed: 25541493]
- Boks MP, Rutten BP, Geuze E, Houtepen LC, Vermetten E, Kaminsky Z, Vinkers CH. SKA2 Methylation is involved in cortisol stress reactivity and predicts the development of post-traumatic stress disorder (PTSD) after military deployment. *Neuropsychopharmacology*. 2016; 41:1350–1356. [PubMed: 26361058]
- Brand AH, Johnson JH. Note on reliability of the Life Events Checklist. *Psychological Reports*. 1982; 50:1274.
- Brent D, Melhem N, Ferrell R, Emslie G, Wagner KD, Ryan N, Vitiello B, Birmaher B, Mayes T, Zelazny J, Onorato M, Devlin B, Clarke G, DeBar L, Keller M. Association of FKBP5 polymorphisms with suicidal events in the Treatment of Resistant Depression in Adolescents (TORDIA) study. *Am J Psychiatry*. 2010; 167:190–197. [PubMed: 20008943]
- Brundin LC, Grit J. Ascertaining whether suicides are caused by infections. *JAMA Psychiatry*. 2016; 73:895–896. [PubMed: 27533721]
- Buss AH, Perry M. The aggression questionnaire. *J Pers Soc Psychol*. 1992; 63:452–459. [PubMed: 1403624]

- Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 1989; 28:193–213. [PubMed: 2748771]
- Coccato EF, Lee R, Coussons-Read M. Elevated plasma inflammatory markers in individuals with intermittent explosive disorder and correlation with aggression in humans. *JAMA Psychiatry.* 2014; 71:158–165. [PubMed: 24352431]
- Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav.* 1983; 24:385–396. [PubMed: 6668417]
- Danese A, McEwen BS. Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiol Behav.* 2012; 106:29–39. [PubMed: 21888923]
- Dawes RM, Faust D, Meehl PE. Clinical versus actuarial judgment. *Science.* 1989; 243:1668–1674. [PubMed: 2648573]
- Dietz LJ, Stoyak S, Melhem N, Porta G, Matthews KA, Walker Payne M, Brent DA. Cortisol response to social stress in parentally bereaved youth. *Biol Psychiatry.* 2013; 73:379–387. [PubMed: 23021533]
- Foa EB, Johnson JM, Feeny NC. Reliability and validity of a brief instrument for assessing post traumatic stress disorder. *J Trauma Stress.* 1993; 6:459–473.
- Fragozo YD, Frota ER, Lopes JS, Noal JS, Giacomo MC, Gomes S, Goncalves MV, da Gama PD, Finkelsztejn A. Severe depression, suicide attempts, and ideation during the use of interferon beta by patients with multiple sclerosis. *Clin Neuropharmacol.* 2010; 33:312–316. [PubMed: 21079457]
- Fuller-Thomson E, Ramzan N, Baird SL. Arthritis and suicide attempts: findings from a large nationally representative Canadian survey. *Rheumatol Int.* 2016; 36:1237–1248. [PubMed: 27306384]
- Goodwin RD. Asthma and suicide: current knowledge and future directions. *Curr Psychiatry Rep.* 2012; 14:30–35. [PubMed: 22094981]
- Guintivano J, Brown T, Newcomer A, Jones M, Cox O, Maher BS, Eaton WW, Payne JL, Wilcox HC, Kaminsky ZA. Identification and replication of a combined epigenetic and genetic biomarker predicting suicide and suicidal behaviors. *Am J Psychiatry.* 2014; 171:1287–1296. [PubMed: 25073599]
- Heim C, Ehler U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology.* 2000; 25:1–35. [PubMed: 10633533]
- Hinkelmann K, Muhtz C, Dettenborn L, Agorastos A, Wingenfeld K, Spitzer C, Gao W, Kirschbaum C, Wiedemann K, Otte C. Association between childhood trauma and low hair cortisol in depressed patients and healthy control subjects. *Biol Psychiatry.* 2013; 74:e15–17. [PubMed: 23726317]
- Hollingshead, A. Four-factor Index of Social Status. Yale University; New Haven: 1975.
- Holmes TH, Rahe RH. The Social Readjustment Rating Scale. *J Psychosom Res.* 1967; 11:213–218. [PubMed: 6059863]
- Hoyo-Becerra C, Huebener A, Trippler M, Lutterbeck M, Liu ZJ, Truebner K, Bajanowski T, Gerken G, Hermann DM, Schlaak JF. Concomitant interferon alpha stimulation and TLR3 activation induces neuronal expression of depression-related genes that are elevated in the brain of suicidal persons. *PLoS One.* 2013; 8:e83149. [PubMed: 24391741]
- Isung J, Aeinband S, Mobarrez F, Nordstrom P, Runeson B, Asberg M, Piehl F, Jokinen J. High interleukin-6 and impulsivity: determining the role of endophenotypes in attempted suicide. *Transl Psychiatry.* 2014; 4:e470. [PubMed: 25335166]
- Janssen HL, Brouwer JT, van der Mast RC, Schalm SW. Suicide associated with alfa-interferon therapy for chronic viral hepatitis. *J Hepatol.* 1994; 21:241–243. [PubMed: 7989716]
- Jokinen J, Ouda J, Nordstrom P. Noradrenergic function and HPA axis dysregulation in suicidal behaviour. *Psychoneuroendocrinology.* 2010; 35:1536–1542. [PubMed: 20576361]
- Keilp JG, Stanley BH, Beers SR, Melhem NM, Burke AK, Cooper TB, Oquendo MA, Brent DA, John Mann J. Further evidence of low baseline cortisol levels in suicide attempters. *J Affect Disord.* 2016; 190:187–192. [PubMed: 26519639]

- Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. 2001; 16:606–613. [PubMed: 11556941]
- Labonte B, Yerko V, Gross J, Mechawar N, Meaney MJ, Szyf M, Turecki G. Differential glucocorticoid receptor exon 1(B), 1(C), and 1(H) expression and methylation in suicide completers with a history of childhood abuse. *Biol Psychiatry*. 2012; 72:41–48. [PubMed: 22444201]
- Laudenslager ML, Jorgensen MJ, Grzywa R, Fairbanks LA. A novelty seeking phenotype is related to chronic hypothalamic-pituitary-adrenal activity reflected by hair cortisol. *Physiol Behav*. 2011; 104:291–295. [PubMed: 21396388]
- Le-Niculescu H, Levey DF, Ayalew M, Palmer L, Gavrin LM, Jain N, Winiger E, Bhosrekar S, Shankar G, Radel M, Bellanger E, Duckworth H, Olesek K, Vergo J, Schweitzer R, Yard M, Ballew A, Shekhar A, Sandusky GE, Schork NJ, Kurian SM, Salomon DR, Niculescu AB 3rd. Discovery and validation of blood biomarkers for suicidality. *Mol Psychiatry*. 2013; 18:1249–1264. [PubMed: 23958961]
- Ludvigsson JF, Sellgren C, Runeson B, Langstrom N, Lichtenstein P. Increased suicide risk in coeliac disease-A Swedish nationwide cohort study. *Digest Liver Dis*. 2011; 43:616–622.
- Lund-Sorensen H, Benros ME, Madsen T, Sorensen HJ, Eaton WW, Postolache TT, Nordentoft M, Erlangsen A. A nationwide cohort study of the association between hospitalization with infection and risk of death by suicide. *JAMA Psychiatry*. 2016; 73:912–919. [PubMed: 27532502]
- Mann JJ, Currier D. A review of prospective studies of biologic predictors of suicidal behavior in mood disorders. *Archives of suicide research*. 2007; 11:3–16. [PubMed: 17178639]
- McGirr A, Diaconu G, Berlim MT, Pruessner JC, Sable R, Cabot S, Turecki G. Dysregulation of the sympathetic nervous system, hypothalamic-pituitary-adrenal axis and executive function in individuals at risk for suicide. *Journal of psychiatry & neuroscience*. 2010; 35:399–408. [PubMed: 20731961]
- McGirr A, Diaconu G, Berlim MT, Turecki G. Personal and family history of suicidal behaviour is associated with lower peripheral cortisol in depressed outpatients. *J Affect Disord*. 2011; 131:368–373. [PubMed: 21093058]
- McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci*. 2009; 12:342–348. [PubMed: 19234457]
- Melhem NM, Brent DA, Ziegler M, Iyengar S, Kolko D, Oquendo M, Birmaher B, Burke A, Zelazny J, Stanley B, Mann JJ. Familial pathways to early-onset suicidal behavior: familial and individual antecedents of suicidal behavior. *Am J Psychiatry*. 2007; 164:1364–1370. [PubMed: 17728421]
- Melhem NM, Keilp JG, Porta G, Oquendo MA, Burke A, Stanley B, Cooper TB, Mann JJ, Brent DA. Blunted HPA axis activity in suicide attempters compared to those at high risk for suicidal behavior. *Neuropsychopharmacology*. 2016; 41:1447–1456. [PubMed: 26450815]
- Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry*. 2009; 65:732–741. [PubMed: 19150053]
- Mostafavi S, Battle A, Zhu X, Potash JB, Weissman MM, Shi J, Beckman K, Haudenschild C, McCormick C, Mei R, Geleroff MJ, Gindes H, Adams P, Goes FS, Mondimore FM, MacKinnon DF, Notes L, Schweizer B, Furman D, Montgomery SB, Urban AE, Koller D, Levinson DF. Type I interferon signaling genes in recurrent major depression: increased expression detected by whole-blood RNA sequencing. *Mol Psychiatry*. 2014; 19:1267–1274. [PubMed: 24296977]
- Murri MB, Pariante C, Mondelli V, Masotti M, Atti AR, Mellacqua Z, Antonioli M, Ghio L, Menchetti M, Zanetidou S, Innamorati M, Amore M. HPA axis and aging in depression: Systematic review and meta-analysis. *Psychoneuroendocrinology*. 2014; 41:46–62. [PubMed: 24495607]
- Niculescu AB, Levey DF, Phalen PL, Le-Niculescu H, Dainton HD, Jain N, Belanger E, James A, George S, Weber H, Graham DL, Schweitzer R, Ladd TB, Learman R, Niculescu EM, Vanipenta NP, Khan FN, Mullen J, Shankar G, Cook S, Humbert C, Ballew A, Yard M, Gelbart T, Shekhar A, Schork NJ, Kurian SM, Sandusky GE, Salomon DR. Understanding and predicting suicidality using a combined genomic and clinical risk assessment approach. *Mol Psychiatry*. 2015; 20:1266–1285. [PubMed: 26283638]

- O'Connor DB, Green JA, Ferguson E, O'Carroll R, O'Carroll RC. Cortisol reactivity and suicidal behavior: investigating the role of hypothalamic-pituitary-adrenal axis responses to stress in suicide attempters and ideators. *Psychoneuroendocrinology*. 2017; 75:183–191. [PubMed: 27837698]
- O'Connor MF, Bower JE, Cho HJ, Creswell JD, Dimitrov S, Hamby ME, Hoyt MA, Martin JL, Robles TF, Sloan EK, Thomas KS, Irwin MR. To assess, to control, to exclude: effects of biobehavioral factors on circulating inflammatory markers. *Brain Behav Immun*. 2009; 23:887–897. [PubMed: 19389469]
- O'Connor TM, O'Halloran DJ, Shanahan F. The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. *QJM*. 2000; 93:323–333. [PubMed: 10873181]
- Pace TW, Miller AH. Cytokines and glucocorticoid receptor signaling. Relevance to major depression. *Ann N Y Acad Sci*. 2009; 1179:86–105. [PubMed: 19906234]
- Pandey GN, Rizavi HS, Ren X, Dwivedi Y, Palkovits M. Region-specific alterations in glucocorticoid receptor expression in the postmortem brain of teenage suicide victims. *Psychoneuroendocrinology*. 2013; 38:2628–2639. [PubMed: 23845513]
- Pandey GN, Rizavi HS, Ren X, Fareed J, Hoppensteadt DA, Roberts RC, Conley RR, Dwivedi Y. Proinflammatory cytokines in the prefrontal cortex of teenage suicide victims. *J Psychiatr Res*. 2012; 46:57–63. [PubMed: 21906753]
- Pandey GN, Rizavi HS, Zhang H, Bhaumik R, Ren X. The expression of the suicide-associated Gene SKA2 is decreased in the prefrontal cortex of suicide victims but not of nonsuicidal patients. *Int J Neuropsychopharmacol*. 2016; 19:pyw015. [PubMed: 26902949]
- Pariante CM, Thomas SA, Lovestone S, Makoff A, Kerwin RW. Do antidepressants regulate how cortisol affects the brain? *Psychoneuroendocrinology*. 2004; 29:423–447. [PubMed: 14749091]
- Perez-Ortiz JM, Garcia-Gutierrez MS, Navarrete F, Giner S, Manzanares J. Gene and protein alterations of FKBP5 and glucocorticoid receptor in the amygdala of suicide victims. *Psychoneuroendocrinology*. 2013; 38:1251–1258. [PubMed: 23219223]
- Posner K, Brown GK, Stanley B, Brent DA, Yershova KV, Oquendo MA, Currier GW, Melvin GA, Greenhill L, Shen S, Mann JJ. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. *Am J Psychiatry*. 2011; 168:1266–1277. [PubMed: 22193671]
- Prather AA, Marsland AL, Hall M, Neumann SA, Muldoon MF, Manuck SB. Normative variation in self-reported sleep quality and sleep debt is associated with stimulated pro-inflammatory cytokine production. *Biol Psychol*. 2009; 82:12–17. [PubMed: 19427354]
- Raison CL, Miller AH. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *Am J Psychiatry*. 2003; 160:1554–1565. [PubMed: 12944327]
- Rohleder N. Acute and chronic stress induced changes in sensitivity of peripheral inflammatory pathways to the signals of multiple stress systems --2011 Curt Richter Award Winner. *Psychoneuroendocrinology*. 2012; 37:307–316. [PubMed: 22226321]
- Sarapas C, Cai G, Bierer LM, Golier JA, Galea S, Ising M, Rein T, Schmeidler J, Muller-Myhsok B, Uhr M, Holsboer F, Buxbaum JD, Yehuda R. Genetic markers for PTSD risk and resilience among survivors of the World Trade Center attacks. *Dis Markers*. 2011; 30:101–110. [PubMed: 21508514]
- Schmid R, Baum P, Itrich C, Fundel-Clemens K, Huber W, Brors B, Eils R, Weith A, Mennerich D, Quast K. Comparison of normalization methods for Illumina BeadChip HumanHT-12 v3. *BMC Genomics*. 2010; 11:349. [PubMed: 20525181]
- Sequeira A, Morgan L, Walsh DM, Cartagena PM, Choudary P, Li J, Schatzberg AF, Watson SJ, Akil H, Myers RM, Jones EG, Bunney WE, Vawter MP. Gene expression changes in the prefrontal cortex, anterior cingulate cortex and nucleus accumbens of mood disorders subjects that committed suicide. *PLoS One*. 2012; 7:e35367. [PubMed: 22558144]
- Spitzer RL, Kroenke K, Williams JB, Lowe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med*. 2006; 166:1092–1097. [PubMed: 16717171]
- Stalder T, Kirschbaum C. Analysis of cortisol in hair--state of the art and future directions. *Brain Behav Immun*. 2012; 26:1019–1029. [PubMed: 22366690]

- Steudte S, Kirschbaum C, Gao W, Alexander N, Schonfeld S, Hoyer J, Stalder T. Hair cortisol as a biomarker of traumatization in healthy individuals and posttraumatic stress disorder patients. *Biol Psychiatry*. 2013; 74:639–646. [PubMed: 23623187]
- Tarter RE, Kirisci L. The Drug Use Screening Inventory for adults: psychometric structure and discriminative sensitivity. *Am J Drug Alcohol Abuse*. 1997; 23:207–219. [PubMed: 9143634]
- Tonelli LH, Stiller J, Rujescu D, Giegling I, Schneider B, Maurer K, Schnabel A, Moller HJ, Chen HH, Postolache TT. Elevated cytokine expression in the orbitofrontal cortex of victims of suicide. *Acta Psychiatr Scand*. 2008; 117:198–206. [PubMed: 18081924]
- Yin H, Galfalvy H, Pantazatos SP, Huang YY, Rosoklija GB, Dwork AJ, Burke A, Arango V, Oquendo MA, Mann JJ. Glucocorticoid receptor-related genes: genotype and brain gene expression relationships to suicide and major depressive disorder. *Depress Anxiety*. 2016; 33:531–540. [PubMed: 27030168]

Highlights

- This is the first study to show low hair cortisol concentrations in attempters
- In our study, hair cortisol concentrations reflect levels prior to suicide attempt
- Attempters also show low *GR-α* mRNA and increased C-reactive Protein and *TNF-α* mRNA
- This is a distinct biological profile compared to ideators and healthy controls
- Hair cortisol was associated with *GR-α* mRNA, childhood abuse, and perceived stress

Figure 1.

Effect sizes (ES), Cohen's *d*, for group differences on biological markers between suicide attempters, those with ideation and no prior history of attempts, and healthy controls controlling for covariates. Group was used as a dummy-coded variable with healthy controls as the reference category. *d*=0.2, *d*=0.5, and *d*=0.8 represent small, medium, and large ES, respectively. *0.05<*p*<0.1; ***p*<0.05; ****p*<0.01. §Lower values reflect higher GR sensitivity.

Figure 2.

Effect sizes (ES), Cohen's *d*, for group differences on biological markers between suicide attempters, those with ideation and no prior history of attempts, and healthy controls controlling for covariates in the reduced sample, i.e., excluding multiple attempters and subjects < 18 years of age. Group was used as a dummy-coded variable with healthy controls as the reference category. *d*=0.2, *d*=0.5, and *d*=0.8 represent small, medium, and large ES, respectively. *0.05<*p*<0.1; ***p*<0.05; ****p*<0.01. [§]Lower values reflect higher GR sensitivity.

Table 1

Demographic, clinical, and psychosocial characteristics by group

	Suicide Attempt (N=38)	Suicidal Ideation (N=40)	Controls (N=37)	Stat	P
Demographics					
Sex, N (% Female)	17 (44.7)	11 (27.5)	20 (54.1)	$\chi^2=5.8$	0.06
Age, M (SD)	22.8 (3.8)	23.6 (3.9)	22.1 (2.2)	F=1.8	0.18
Race, N (% Caucasian)	34 (89.5)	30 (75.0)	31 (83.8)	$\chi^2_2=2.9$	0.24
Socio-economic status (SES), M (SD)	4.8 (2.6) ^a	5.8 (3.1) ^a	8.1 (1.8) ^b	F=15.8	<0.001
Body mass index (BMI), M (SD)	24.6 (5.1)	24.7 (4.1)	22.9 (2.9)	F=2.2	0.12
Waist-Hip Circumference (WHC)	0.82 (0.15) ^a	0.87 (0.08) ^a	0.82 (0.07) ^a	F=3.3	0.04
Time of blood draw	14:28 (1:40)	14:57 (1:16)	14:13 (1:18)	F=2.6	0.08
Number of days between Admission & Interview, M (SD)	3.3 (1.7)	3.1 (1.6)	—	t=0.71	0.48
Psychiatric diagnoses					
Major Depressive Disorder, N (%)	19 (50.0)	25 (62.5)	—	FET	0.58
Bipolar disorder, N (%)	16 (42.1)	13 (32.5)	—		
Psychotic disorders, N (%)	0 (0.0)	3 (7.5)	—	FET	0.24
Anxiety disorders, N (%)	27 (71.1)	24 (60.0)	—	$\chi^2_1=1.05$	0.31
Posttraumatic Stress Disorder (PTSD), N (%)	14 (36.8)	16 (40.0)	—	$\chi^2_1=0.08$	0.77
Attention Deficit Hyperactivity Disorder (ADHD), N (%)	8 (21.1)	3 (7.5)	—	$\chi^2_1=2.95$	0.09
Eating disorders, N (%)	2 (5.26)	2 (5.0)	—	FET	>0.99
Alcohol dependence, N (%)	9 (23.7)	6 (15.0)	—	$\chi^2_1=0.95$	0.33
Substance dependence, N (%)	15 (39.5)	17 (42.5)	—	$\chi^2_1=0.07$	0.79
Personality disorders, N (%)	6 (15.8)	2 (5.0)	—	FET	0.15
Family history of suicide attempt, N (%)	6 (15.8)	4 (10.0)	—	FET	0.51
Medications use prior to admission					
Antidepressants, N (%)	47.4 (18)	35.0 (14)	—	$\chi^2_1=1.2$	0.27
Antipsychotics, N (%)	10.5 (4)	15 (6)	—	FET	0.74
Mood stabilizers, N (%)	7.9 (3)	7.5 (3)	—	FET	1.00
Severity of current symptomatology					
CSSRS- ideation, M (SD)	4.8 (0.09)	3.7 (0.16)	—	t= 5.9	<0.001
Depression (PHQ-9) score, M (SD)	18.1 (7.0) ^a	18.3 (6.6) ^a	1.1 (1.6) ^b	F=112.7	<0.001
Anxiety (GAD-7) score, M (SD)	14.3 (5.3) ^a	14.3 (5.8) ^a	1.2 (1.6) ^b	F=98.7	<0.001
PTSD (PSSI) score ^I , M (SD)	15.5 (13.4) ^a	12.8 (13.8) ^a	0 (0.0) ^b	t=0.81	0.001
Clinical correlates of suicidal behavior					
Impulsivity, M (SD)	79.7 (11.0) ^a	76.9 (13.6) ^a	55.4 (8.1) ^b	F= 53.2	<0.001
Aggression, M (SD)	82.7 (22.8) ^a	81.8 (24.6) ^a	46.7 (12.1) ^b	F=36.8	<0.001

	Suicide Attempt (N=38)	Suicidal Ideation (N=40)	Controls (N=37)	Stat	P
Sleep disturbances ² , M (SD)	10.5 (3.4) ^a	11.6 (3.7) ^a	3.2 (1.9) ^b	F=79.6	<0.001
Hopelessness, M (SD)	11.6 (5.0) ^a	11.1 (5.1) ^a	1.8 (1.9) ^b	F=61.9	<0.001
Psychosocial history					
Childhood abuse, N (%)	28 (73.7) ^a	21 (52.5) ^a	3 (8.1) ^b	$\chi^2_2=33.9$	<0.001
Stressful life events, M (SD)	6.5 (3.9) ^a	5.8 (4.3) ^a	2.0 (1.7) ^b	F=17.2	<0.001
Perceived stress, M (SD)	36.7 (8.1) ^a	37.3 (5.5) ^a	17.7 (5.5) ^b	F=99.2	<0.001
Cigarette smoking, N (%)	30 (78.9) ^a	23 (57.5) ^a	4 (10.8) ^b	$\chi^2_2=36.4$	<0.001

Dark grey shading represents differences at $p<0.05$; Similar letters represent no differences on post-hoc comparisons at $p<0.017$; Light grey shading represents post-hoc differences with $0.05<p<0.1$

¹ There were 16 controls who reported exposure to trauma but none reported PTSD symptoms

² Higher scores on the Pittsburgh Sleep Quality Index reflect worse sleep.

Table 2

Relationship between biological markers and suicidal behavior controlling for covariates

Biological measure	Suicide Attempt (SA) ¹			Suicidal Ideation (SI) ¹		
	β (95% CI)	p	Effect Size (d)	β (95% CI)	p	Effect Size (d)
Full sample						
Hair Cortisol Concentrations (HCC), $\ln^2$	-0.42 (-0.78, -0.05)	0.02	-0.38	-0.13 (-0.46, 0.2)	0.43	-0.12
<i>NR3C1</i> (α isoform) mRNA	-4.7 (-9.45, 0.02)	0.05	-0.40	1.3 (-3.45, 6.03)	0.59	0.10
<i>NR3C1</i> (β isoform) mRNA	-1.8 (-18.6, 15.0)	0.84	-0.05	9.0 (-6.83, 24.8)	0.26	0.27
<i>SKA2</i> mRNA	8.1 (-0.75, 16.9)	0.07	0.42	6.7 (-2.0, 15.5)	0.13	0.35
<i>FKBP5</i> mRNA ³	-18.8 (-97.2, 59.6)	0.64	-0.11	-43.2 (-119.2, 32.8)	0.26	-0.29
GR sensitivity ⁴	-23.1 (-50.6, 4.35)	0.09	-0.30	-14.2 (-41.1, 12.8)	0.30	-0.20
C-Reactive Protein (CRP), $\ln^{1,5}$	0.99 (0.15, 1.84)	0.02	0.64	0.42 (-0.33, 1.17)	0.27	0.26
Tumor Necrosis Factor (<i>TNF</i>)- α mRNA	16.8 (1.9, 31.7)	0.03	0.50	11.6 (-4.1, 27.2)	0.15	0.37
<i>IL-1\beta</i> mRNA ⁶	-9.3 (-70.8, 52.3)	0.77	-0.08	5.6 (-50.5, 61.7)	0.84	0.05
Stimulated production of IL-6, $\ln^2$	0.14 (-0.19, 0.48)	0.41	0.20	0.07 (-0.25, 0.39)	0.65	0.12
Reduced sample (excluding multiple attempters and those <18 years of age)⁷						
Hair Cortisol Concentrations (HCC), $\ln^2$	-0.55 (-0.96, -0.13)	0.01	-0.54	-0.23 (-0.57, 0.12)	0.19	-0.21
<i>NR3C1</i> (α isoform) mRNA ⁸	-5.11 (-10.9, 0.73)	0.09	-0.46	-0.76 (-5.97, 4.45)	0.77	-0.06
<i>NR3C1</i> (β isoform) mRNA ⁹	-8.59 (-27.5, 10.4)	0.37	-0.27	6.1 (-10.1, 22.3)	0.46	0.18
<i>SKA2</i> mRNA	3.4 (-6.7, 13.5)	0.51	0.18	7.5 (-1.2, 16.2)	0.09	0.40
<i>FKBP5</i> mRNA ³	8.5 (-90.3, 107.2)	0.87	0.05	-45.4 (-127, 36.2)	0.27	-0.29
GR sensitivity	-10.9 (-43.7, 22.1)	0.51	-0.14	-8.1 (-38.1, 22)	0.60	-0.11
C-Reactive Protein (CRP), $\ln^{2,10}$	0.94 (-0.004, 1.9)	0.05	0.60	0.44 (-0.31, 1.19)	0.25	0.28
Tumor Necrosis Factor (<i>TNF</i>)- α mRNA	26.4 (7.7, 45.2)	0.006	0.73	6.5 (-9.8, 22.7)	0.43	0.20
<i>IL-1\beta</i> mRNA ⁶	-1.57 (-70.3, 67.2)	0.96	-0.01	3.44 (-54.1, 60.9)	0.91	0.03
Stimulated production of IL-6, $\ln^2$	0.33 (-0.04, 0.70)	0.08	0.49	0.08 (-0.22, 0.39)	0.59	0.14

Grey shading represents $p < 0.05$ ¹ Dummy coded variable with healthy controls as the reference category² Natural logarithmic transformation³ Time of blood draw and use of ecstasy were significant covariate⁴ Lower values reflect higher GR sensitivity⁵ Sex, BMI and abuse were significant covariates⁶ Smoking was a significant covariate

⁷Reduced sample sizes are 21 SA, 38 SI, and 37 controls

⁸Use of antidepressants was a significant covariate

⁹SES was a significant covariate

¹⁰Sex and BMI were significant covariates.

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Table 3

Correlations between biological markers in the full sample

	HCC	NR3C1 mRNA (α isoform)	NR3C1 mRNA (β isoform)	SKA2 mRNA	FKBP5 mRNA	GR Sensitivity ^I	CRP	TNF- α mRNA	IL-1 β mRNA	Stimulated production of IL-6
HCC	1.00									
NR3C1 mRNA (α isoform)	0.41***	1.00								
NR3C1 mRNA (β isoform)	0.19*	-0.11	1.00							
SKA2 mRNA	0.18	-0.15	0.73***	1.00						
FKBP5 mRNA	0.15	-0.01	0.32***	0.12	1.00					
GR Sensitivity ^I	0.12	0.21*	0.15	0.13	0.18	1.00				
CRP	-0.07	-0.32***	-0.02	0.15	0.01	-0.05	1.00			
TNF- α mRNA	0.15	0.05	0.39***	0.41***	0.14	0.02	-0.07	1.00		
IL-1 β mRNA	-0.04	0.03	0.20*	-0.01	0.39***	0.10	-0.17	0.34***	1.00	
Stimulated production of IL-6	0.18	0.10	0.08	0.10	0.01	0.48***	-0.07	0.18	0.03	1.00

Grey shading represents correlations with $p < 0.05$

*** $p < 0.01$

* $p < 0.05$

*** $p < 0.001$

^I Lower values reflect higher GR sensitivity.

Correlations of biological markers with clinical and psychosocial correlates of suicidal behavior in the full sample

Table 4

	HCC	NR3C1 mRNA (α isoform)	NR3C1 mRNA (β isoform)	SKA2 mRNA	FKBP5 mRNA	GR-Sensitivity ¹	CRP	TNF- α mRNA	IL-1 β mRNA	Stimulated production of IL-6
Depression symptoms (PHQ-9)	-0.05	-0.11	0.16	0.21 [*]	0.07	0.09	0.12	0.30 ^{**}	0.18	0.18
Anxiety symptoms (GAD-7)	-0.10	-0.16	0.12	0.16	0.02	0.07	0.07	0.24 [*]	0.12	0.12
PTSD symptoms (PSS)	0.05	-0.10	0.02	0.14	0.05	-0.01	0.11	-0.11	-0.005	0.10
Impulsivity	0.01	-0.06	0.16	0.19 [*]	0.07	0.15	0.08	0.40 ^{***}	0.21 [*]	0.15
Aggression	0.02	-0.09	0.06	0.06	0.003	0.18	-0.03	0.21 [*]	0.13	0.06
Sleep disturbances ²	-0.10	-0.14	0.16	0.17	-0.03	0.06	0.08	0.31 ^{**}	0.14	0.21 [*]
Hopelessness	0.09	0.03	0.14	0.18	-0.01	0.09	0.06	0.30 ^{**}	0.10	0.17
Childhood abuse	-0.21 [*]	-0.24 ^{**}	0.04	0.02	-0.08	-0.04	0.02	0.16	0.03	-0.01
Stressful life events	0.08	-0.03	0.12	0.12	-0.04	-0.04	0.07	0.26 ^{**}	0.14	0.11
Perceived stress	-0.28 ^{**}	-0.11	0.03	0.03	-0.10	0.01	0.02	0.25 ^{**}	0.07	0.11
Cigarettes per day	0.14	-0.002	0.09	0.11	0.06	0.07	0.002	0.31 ^{***}	0.28 ^{**}	0.07

Dark grey shading represents correlations with $p < 0.05$ ^{*} $p < 0.05$ ^{**} $p < 0.01$ ^{***} $p < 0.001$ ¹ Lower values reflect higher GR sensitivity² Higher scores on the PSQI reflect worse sleep.