Psychoneuroendocrinology 102 (2019) 216–224

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Hypothalamic-pituitary-adrenal axis dysregulation in depressed adolescents with non-suicidal self-injury

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ARTICLE INFO

Keywords:
Non-suicidal self-injury
Self-harm
Adolescents
Cortisol
HPA
Stress reactivity

ABSTRACT

Non-suicidal self-injury (NSSI) is characterized by causing harm to one's own body without the intent of suicide. While major depressive disorder (MDD) has been associated with elevated cortisol (at least in some subgroups), prior studies in NSSI have suggested that NSSI is associated with blunted reactivity to stress of the hypothalamic-pituitary-adrenal (HPA) axis, possibly consistent with an allostatic load model. The present study used a multi-level approach to examine salivary cortisol in the context of a social stressor in 162 adolescents (ages 12 to 19 years old) with MDD with a history of repeated engagement in NSSI (MDD/NSSI) versus MDD without repeated NSSI (MDD), and healthy controls (HC). Observed (expressed) and self-reported (experienced) ratings of stress were also obtained during the social stress paradigm. The results showed that MDD/NSSI exhibited lower salivary cortisol levels and differed in cortisol trajectories in the context of a social stressor compared to HC and MDD. Observed stress, but not self-reported stress, during the social stress paradigm was greater for the MDD/NSSI than HC. Follow-up analyses suggested the possibility that this pattern of lower cortisol for those who engage in NSSI was present in females and males, and was more pronounced in those with repeated NSSI (but not subthreshold NSSI) and those with a history of NSSI and suicide attempts. Overall, these findings add to the prior literature and begin to show a consistent pattern for how stress is processed in atypical ways for those who engage in repeated NSSI. Importantly, these results suggest that some of the heterogeneity across adolescent depression may be better represented by these underlying biological processes, perhaps even representing subgroups that will benefit from different types of intervention. Hypothalamic-Pituitary-Adrenal Axis Dysregulation in Depressed Adolescents with Non-Suicidal Self-Injury.

1. Introduction

Non-suicidal self-injury (NSSI) involves the self-directed act of causing harm to one's own body without the intent of suicide (Muehlenkamp, 2005). The estimated prevalence of adolescent NSSI is 17% (Swannell et al., 2014) and 40% or higher in clinical samples (Glenn and Klonsky, 2013). Those who self-injure are more prone to self-criticism and negative emotionality (Glassman et al., 2007). Most adolescents engage in NSSI as a method of stress-regulation, with the expectation that it will serve to relieve interpersonal difficulties or reduce negative affect (e.g., Zetterqvist et al., 2013). Complex psychological and biological systems are implicated in the activation and regulation of stress responses, including the hypothalamic-pituitary-adrenal (HPA) axis. However, these systems are just beginning to be examined in those who repeatedly engage in NSSI.

Under acute stress conditions, threat signals are relayed through neural and other physiological systems. This includes the hormonal cascade of the HPA axis, resulting in increased circulation of cortisol throughout the body. Excessive and prolonged stress impacts brain regions that have dense concentrations of glucocorticoid and mineralocorticoid receptors, including the hippocampus and the prefrontal cortex (PFC), which in turn may contribute to dysregulation of the HPA axis (Lupien et al., 2009). The impact is widespread, with emerging evidence showing that prolonged excessive stress also alters peripheral

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https://doi.org/10.1016/j.psyneuen.2018.11.004
Received 27 March 2018; Received in revised form 30 October 2018; Accepted 1 November 2018

immune system functioning and gene-specific expression through DNA methylation (Weaver et al., 2004). There is a wealth of evidence to suggest that chronic heightened activation of the HPA axis is associated with depression and other stress regulation disorders (Pariante and Lightman, 2008). In some cases, the body responds with diminished HPA axis activation (Juster et al., 2010) and potentially leaves an individual more susceptible to stress-related psychological disorders and other illnesses associated with immune suppression (McEwen, 2013).

Childhood and adolescence may be a particularly crucial time for alterations of these physiological stress systems, with evidence for significant HPA development during adolescence (Lupien et al., 2009; Klimes-Dougan et al., 2001). Further, adolescence is a time period during which an individual is highly susceptible to the development of psychiatric disorders and maladaptive behaviors such as NSSI (Costello et al., 2011). It is possible that circumstances associated with risk for NSSI, or even NSSI itself, may derail normative development in these systems. Two studies to date have used a well-validated stress paradigm, the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), to examine the HPA system in adolescents with NSSI. Kaess and colleagues (2012) found adolescent females with NSSI (n = 14) not only indicated significantly higher symptoms of depression and general distress, but also had blunted reactivity of salivary cortisol response following the TSST compared to healthy controls. A similar blunted pattern was noted when assessing blood cortisol levels in the context of the TSST in adolescent females who endorsed engaging in suicidal and/or non-suicidal self-harm (n = 21) than for those who did not report engaging in self-harm (Plener et al., 2017). Although this study included a wider range of self-harm behaviors, results remained significant after excluding participants who reported a history of suicide attempts (n = 4). Together these studies provide preliminary evidence that stress responding may be altered in those engaging in NSSI.

Considering multiple levels of analysis of stress system functioning is also critical for promoting an understanding of how biological, behavioral and psychological features of stress system functioning interact. From an organizational perspective of developmental psychopathology, a fundamental incoherence across different levels (e.g., what is experienced and what is expressed; physiological responses and what is experienced) would be predicted under risk conditions (Cicchetti et al., 2002). Preliminary evidence fails to support an association between self-reported mood and cortisol during the TSST for either the NSSI or control groups (Kaess et al., 2012). Regardless, it is necessary to continue using a multi-level approach, as we will further be able to conceptualize how one system works at multiple levels and also begin to explore how these levels interconnect.

2. Purpose of study

The primary goal of the present study was to use a multi-level approach to examine stress system functioning in adolescents with a history of repeated NSSI. Given that the rates of NSSI are higher and more persistent in clinical populations, especially in those with depressed mood (Brier and Gil, 1998), we address differences between healthy controls (HC) and adolescents who evidence significant levels of psychopathology. We focus on adolescents diagnosed with Major Depressive Disorder (MDD) who have (MDD/NSSI) or have not (MDD) had a history of repeated engagement in NSSI. We predicted that cortisol levels would be lower for the MDD/NSSI group than HC in the context of a social stressor, the TSST. We also examined if experiences of stress and expressions of stress differed across the groups, as well as if there was evidence of correspondence across levels of analysis (biological, psychological, and behavioral).

The secondary goal of this study was to conduct a series of secondary, exploratory analyses to consider salient factors associated with alterations in stress system functioning in the context of NSSI. First, we examine whether these findings extended to males. Despite evidence that both males and females engage in NSSI (e.g., Bresin and Schoenleber, 2015), and that elevations in cortisol during the TSST are more likely to be seen in males (Klimes-Dougan et al., 2001), past studies on HPA axis functioning in the context of NSSI have almost exclusively focused on females. Second, we explored if stress system functioning differed based on the frequency of NSSI, considering both subthreshold and repeated NSSI. Third, we examined differences in physiological responding for those who engage in NSSI with or without a history of suicide attempts, based on preliminary evidence that a blunted pattern of stress responding may be present in suicidal behavior as well as NSSI (Melhem et al., 2016). In addition to the standard control variables included in all the analyses (e.g., age, sex, depressive symptoms, time of cortisol collection), supplemental analyses were conducted in an attempt to rule out the impact of possible confounds (e.g., IQ, comorbid conditions, medication status).

3. Material and methods

3.1. Participants

Participants were 162 adolescents aged 12–19 years old (M = 16.46, SD = 1.94). The study sample was composed of three different groups: 39 patients with MDD who have engaged in repeated NSSI (MDD/NSSI), 59 patients with MDD who have not engaged in repeated NSSI (MDD), and 64 healthy controls (HC). Participants were recruited through community postings and letters to professionals serving inpatient and outpatient clinical services at the University of Minnesota and surrounding area. At the first visit, participants were consented and diagnostic interviews were conducted. The TSST was conducted on a separate, subsequent visit within about a month (M = 35.15 days, range = 0–260). Exclusionary criteria for all individuals included current or past pervasive developmental disorder, bipolar disorder, schizophrenia, a neurological disorder, or a chronic or serious medical condition; a current diagnosis of substance abuse disorder; and an IQ below 80 as assessed by the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999).

The study was approved by the Institutional Review Board at the University of Minnesota. All participants completed signed informed consent and/or assent (if under 18). All participants received monetary compensation for their participation after completing each of the visits.

3.2. Diagnostic and symptom assessment

Participants completed the Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (KSADS-PL; Kaufman et al., 1997), to determine the presence of an Axis I disorder as defined by the DSM-IV-TR. Participants and their parents were interviewed separately and a consensus on diagnosis was formed based on the two reports. A licensed doctoral-level clinician, or a trained graduate student under the supervision of a licensed psychiatrist or psychologist administered these interviews. Interview training included listening to and rating four audiotaped KSADS-PL interviews, observing a licensed clinician perform a minimum of two in-person interviews, and administering a minimum of two in-person interviews with a licensed clinician in the room. Inter-rater reliability was calculated to be 100% for MDD diagnosis, 84.63% for MDD symptom ratings, and 96% for the screen items. Participants were eligible for the patient group if they had a primary diagnosis of Major Depressive Disorder. HC were defined by not having an Axis I disorder as evaluated on the KSADS. Having completed the KSADS, clinicians rated depressed participants on the Global Assessment of Functioning (GAF) scale (APA, 1994) and documented their age of onset of MDD.

Clinicians assessed self-harm and other aspects of risk status in the context of the KSADS-PL interview and symptoms were based on either the parent or child report. Questions about NSSI included: “Did you ever try to hurt yourself?”, “Did you do this without intending to kill yourself?”, and “Did you cut, burn, hit, etc. yourself?” Clinicians then

rated if NSSI was *not present, subthreshold* - e.g., has engaged in NSSI on one or two episodes and has not caused serious injury to self, or *repeated* engagement in NSSI - e.g., has engaged NSSI on at least four episodes or has engaged in NSSI leading to significant injury to oneself (e.g., burn with scarring, cut requiring stitches). For the main analyses for this study, we relied on the definition of repeated engagement in NSSI (MDD/NSSI) or no repeated NSSI (MDD) for the patient groups. For some exploratory analyses, we considered both subthreshold (as defined by less than four lifetime episodes, the modal number was 1 reported episode for this group) and repeated engagement in NSSI. Notably, no participants in the control group reportedly engaged in NSSI at either a threshold or subthreshold level.

Additional information was obtained from the KSADS-PL for participants, including the presence or absence of suicide attempts. That is, after evaluating suicidal thoughts and plans, participants were asked, "Have you ever tried to kill yourself?" Suicide attempts were rated by the clinician on intent and perceived lethality resulting in a binary score of a history of no suicide attempt or a suicide attempt. This interview also assessed the presence of comorbid disorders (e.g., generalized anxiety disorder, social phobia, attention deficit hyperactivity disorder) and psychopharmacological intervention (based on the current use of antidepressant medications) (Table 1).

Evidence of the severity of current depressive symptoms over the past two weeks was based on the average Beck Depression Inventory-II (BDI-II) (Beck et al., 1996), a commonly-used, well-validated self-report measure of depressive symptoms. The analyses focused on the average BDI-II score across the visits to optimize the sample size (e.g., typically only the depressed participants completed the BDI-II after the initial visit and the BDI-II conducted at the initial and TSST visits were strongly correlated, $r(73) = .921$, $p < .001$.

Table 1
Participant and Procedure Characteristics.

	MDD/NSSI (n = 39)	MDD (n = 59)	HC (n = 64)
Age[c]	16.50 (1.62)	16.25 (2.08)	16.65 (2.01)
Gender[c], N (%)			
Male	8 (20.5)	21 (35.6)	24 (37.5)
Female	31 (79.5)	38 (64.4)	40 (62.5)
Race, N (%)			
Caucasian	27 (69.2)	47 (79.7)	43 (67.2)
African American	6 (15.4)	5 (8.5)	1 (1.6)
Asian	0 (0)	2 (3.4)	5 (7.8)
Native American	1 (2.6)	0 (0)	1 (1.6)
Other	5 (12.8)	5 (8.5)	14 (21.9)
BDI-II[c]	25.71 (13.50)	21.32 (10.83)	3.18 (3.35)
Onset	11.54 (3.03)	12.22 (2.67)	N/A
Duration	17.17 (31.74)	8.17 (10.17	N/A
GAF Score	51.08 (11.31)	55.04 (8.45)	N/A
Suicide Attempt, N (%)	17 (43.6)	12 (20.3)	0 (0)
IQ	104.95 (15.17)	109.69 (14.82)	111.61 (12.25)
Time of TSST CORT 0[c]	14:27:28 (1:28:03)	14:49:55 (1:13:45)	14:37:08 (1:23:01)
Medication, N (%)	22 (56.41)	21 (35.59)	0 (0)
Comorbidities, N (%)			
Anxiety	28 (71.8)	43 (72.9)	0 (0)
Externalizing	16 (41.0)	11 (18.6)	N/A

Notes: MDD/NSSI = major depressive disorder with repeated non-suicidal self-injury; MDD = major depressive disorder without repeated non-suicidal self-injury; HC = healthy control; BDI = Beck Depression Inventory 2; Onset = age of onset of depression: Duration = Duration of current illness; GAF = Global Assessment of Functioning Data are reported as mean (standard deviation) unless noted otherwise. Standard control variables used in group comparisons ([c]). With the exception of IQ, $F(2,156) = 2.746$, $p = .067$, there were no marginal or significant differences across group for demographic variables or cortisol collection times. As expected, there was a significant difference between the depressed (MDD/NSSI and MDD) and HC for lifetime Externalizing Disorders $X^2 = 6.13$, $p = 0.01$, lifetime Anxiety Disorders $X^2 = 82.56$, $p < 0.001$, BDI-II $F(2,157) = 85.19$, $p < .001$ (although BDI-II was not significantly higher for the MDD/NSSI than the MDD, $p = .09$), and medication use $X^2 = 168.8$, $p < 0.001$ (although medication use was significantly higher for the MDD/NSSI group than the MDD group, $X^2 = 4.13$, $p < 0.042$). For the variables only reported for the depressed participants, with the exception of the duration of illness $F(2,78) = 3.20$, $p < .073$, there were no marginal or significant differences for the MDD/NSSI and MDD groups (GAF, age of onset).

3.3. Procedures for collection of physiological measures

Participants provided salivary samples within the context of a slightly modified version of the TSST, a task that has been found to reliably elicit a stress response (Kirschbaum et al., 1993). In an attempt to control for diurnal variation and to maximize individual variation (Birkett, 2011), participants were typically scheduled for the TSST in the afternoon (M = 14:39:28; range 10:00:00 to 17:10:00). They were asked to spend 5 min preparing a speech to introduce themselves to a job committee. After the preparation period, participants were escorted to another room in front of two unfamiliar evaluators, who were wearing white lab coats and were trained to remain neutral and to avoid giving reassurance or feedback. Speaking into a microphone with a video-recorder running, participants were first asked to deliver their speech (5 min.) and then asked to do a serial subtraction task with corrective feedback provided by experimenters (5 min.). Participants were debriefed immediately following the completion of the TSST. A total of five salivary samples were collected throughout this visit: the first before speech preparation (TSST0: 0 min), the second immediately following the TSST (TSST1: approximately 20 min.), and the final three at approximately 35 (TSST2), 50 (TSST3), and 65 (TSST4) mins following the TSST. The measure of reactivity was based on TSST0, TSST1, and TSS2. Recovery was based on TSST2, TSST3, and TSST4.

Expressions (experimenter-ratings) and experiences (self-ratings) of stress were recorded during and after the TSST respectively. During the TSST, independent ratings by the two examiners were moderately correlated, $r(157) = .643$, $p < .001$, when asked to rate questions on a scale of 1 (not stressed at all) to 5 (considered discontinuing the procedure because they looked so stressed) that addressed "How stressed did the participant appear during the speech?" and "How stressful did the participant appear during the subtraction task?" Similarly, after completion of the TSST, participants were asked to rate a series of questions on a scale of 1 (calm) to 5 (high stress), including "How stressful was giving the speech (job interview)?" and "How stressful was the subtraction task?" A mean score across the speech and math tasks was used as the summary score for both experienced and expressed stress.

Specific directions were given to participants to refrain from eating, drinking caffeine, or smoking cigarettes for at least one hour before the afternoon visit in which the TSST was conducted. For each saliva sample, participants facilitated salivary excretion by chewing Trident Original gum for 20–30 seconds before spitting out the saliva and gum. Participants then pushed their saliva through a straw and into a 1.5 ml vial. Samples were labeled and stored in a -25-degree Celsius freezer until they were shipped to Universität Trier in Trier, Germany, for analysis. Researchers used assay methods consistent with Dressendörfer et al. (1992).

3.4. Data analysis

To address the primary study aims, we conducted a series of general linear model analyses (GLM) to evaluate MDD/NSSI, MDD, and HC groups on commonly reported *summary* indexes of cortisol within the context of the TSST. We considered two indexes of overall level of cortisol, (a) area under the curve ground (AUCg) and (b) the highest value to assess "Peak" cortisol as well as (c) area under the curve from the initial pretest sample (AUCi; commonly considered an index of reactivity) (Pruessner et al., 2003). Additionally, GLM analyses were conducted on summary indexes of experienced and expressed stress. Control variables included age, sex, BDI-II scores, and the time of day of the TSST. Regression analyses and correlations and Fisher's r to z

Table 2
Group Differences for Cortisol, Self-Report and Experimenter ratings in the Context of the Trier Social Stress Test.

	MDD/NSSI	MDD	HC	F	p
TSST					
CORT 0	0.22 (0.17)	0.28 (0.22)	0.23 (0.17)	2.52	0.084
CORT 1	0.21 (0.17)[a]	0.32 (0.20)[a]	0.28 (0.18)	3.33	0.038
CORT 2	0.21 (0.17)[a]	0.33 (0.23)[a]	0.32 (0.25)	3.55	0.031
CORT 3	0.15 (0.13)[a]	0.30 (0.22)[a]	0.22 (0.17)	7.26	0.001
CORT 4	0.17 (0.14)	0.26 (0.19)	0.22 (0.17)	2.26	0.108
TSST AUCg	774.54 (533.00)[a]	1219.07(770.77)[a]	1010.02(618.79)	4.25	0.016[I,A,E,M,B,D]
TSST Peak	0.30 (0.19)[a]	0.45 (0.25)[a]	0.41 (0.24)	3.67	0.028[I,A,E,D]
TSST AUCi	-87.00 (548.63)[a]	94.70 (752.94)	112.44 (581.05)[a]	3.60	0.030[I,A,E,D]
Experience	4.26 (0.77)	4.06 (0.73)	3.67 (0.88)	1.28	0.282
Expression	3.25 (0.88)[a]	2.99 (0.89)	2.68 (0.62)[a]	3.29	0.040[I,A,D]

Notes: MDD/NSSI = major depressive disorder with repeated non−suicidal self−injury; MDD = major depressive disorder without repeated non−suicidal self−injury; HC = healthy control. The five summary indexes that are the focus of this study are in bold. Data are reported as mean (standard deviation) unless noted otherwise. Post hoc contrasts with the same letter superscripts ([a]) note significant group differences between MDD/NSSI and MDD or between MDD/NSSI and HC. All analyses reported above control for age, sex, depressive symptoms, and time of day. Another series of supplemental analyses were conducted controlling for each of these possible confounds in turn, IQ ([I]), lifetime anxiety disorder comorbidities ([A]), lifetime externalizing comorbidities ([E]), medication use ([M]), days between diagnostic and TSST visit ([B]), and duration of illness ([D]) when including the standard control indexes. Some of these analyses focused on only the depressed group (e.g., duration of illness, medication use) and some of the variables did not include the whole sample (e.g., days between diagnostic and TSST visit). Corresponding superscripts on the far right column of the table indicate that significant group differences ($p \leq .05$) were found even when considering the corresponding control variable in addition to the standard control variables (e.g., age, sex, depressive symptoms BDI-II and time of day) in the analysis and in most of the other cases the group differences were marginally different ($p \leq .15$). The one exception was for AUCi when controlling for medication ($p = .223$). Given that TSST Peak cortisol values were higher for the 5 participants that did not complete the TSST we conducted follow up analyses and determined that group differences remained significant excluding these 5 cases in which the TSST was discontinued, $F(2, 142) = 5.49$, $p = .005$.

transformation were used to consider correspondence across levels of analysis (e.g., physiological, experience, expression).

A second approach to evaluate cortisol reactivity and recovery *trajectories* was used to address the primary study aims pertaining to HPA axis functioning by using mixed effects models (nlme package, R version 3.3.2; R Core Team, 2016) (Fitzmaurice et al., 2011). Cortisol values were square root transformed, as the measurements were right-skewed. These values were also multiplied by 100 to aide with interpretability (the pattern of significance does not change if the values are not multiplied this way). Several initial models were compared to accurately characterize changes in cortisol: a null model that did not include an effect of time (i.e., when sample was collected, centered at 35 min after speech preparation), a model with a linear effect of time across the measurements, a model with a quadratic effect, and a linear piecewise model. For the piecewise model, the measurements were separated into the estimates of reactivity and recovery. For each model (besides the null), a model with a random effect of intercept, and a model with a random effect of intercept and time were tested. Seven models were tested in all. The best fitting model was selected using the Bayesian Information Criterion (BIC) (Schwarz, 1978). Using the best-fitting model, the diagnostic group by time interaction terms were added to assess whether diagnostic group was related to differences in the cortisol response during the TSST. Control variables include age, sex, BDI-II scores, and the time of day of the TSST. Reference groups for categorical variables included the MDD/NSSI individuals and males.

Secondary study aims focused on *summary* indexes, conducting a series of GLM analyses to assess if patterns of stress functioning differed across group based on (a) the sex of the participant, and (b) the frequency of self-harm (subthreshold versus repeated) and (c) the type of self-harm (e.g., NSSI versus suicide attempts), using the standard control variables for all analyses (age, sex, BDI-II score, and the time of day of the TSST). Finally, follow-up, supplemental analyses explored other possible confounds (e.g., IQ, comorbid conditions, medication status, duration between visits, duration of illness). The possible confounds were assessed one at a time when accounting for the standard control variables to assess differences for the three groups because some of the variables were only available for the MDD/NSSI and MDD groups (e.g., duration of illness) and other variables did not have complete data (e.g., duration between visits) (e.g.,Funke et al., 2016). Group differences that were marginally significant at the $p < .15$ level were considered given the reduced power for these analysis.

4. Results

4.1. Preliminary results

Different summary indexes were significantly ($p < .05$) correlated: TSST AUCg with TSST AUCi ($r = 0.274$); TSST AUCg with TSST Peak ($r = 0.869$); TSST AUCi with TSST Peak ($r = 0.240$); Experience with Expression ($r = 0.476$).

4.2. Summary indexes: MDD/NSSI, MDD, and HC comparisons

The primary questions focused on comparisons for MDD/NSSI, MDD, and HC. As shown on Table 2 and Fig. 1, significant group differences were found for the TSST. Group differences were noted for the first through fourth cortisol sample (CORT0, CORT1, CORT2, CORT3), with MDD/NSSI having lower cortisol values than MDD. There were significant group differences for the main analyses that focused on the summary variables. For AUCg and Peak, cortisol was significantly lower for the MDD/NSSI than the MDD. For the cortisol level taking the initial value into account (AUCi), cortisol was lower for the MDD/NSSI and MDD than the HC.

Significant differences were found for observer ratings of expressed stress, but not self-report ratings of experienced stress, during the speech and math portions of the TSST (Table 2, Fig. 2), with MDD/NSSI having higher ratings of expressed (observed) stress than HC.

Correspondence across the biological (AUCg, AUCi, Peak), psychological (experience of stress) and behavioral (expression of stress) measures of stress responding were explored with a series of regression and correlation analyses. None of the regressions were significant. Overall, there were limited associations for biological and psychological/behavioral indexes. For the total sample, TSST experience of stress was correlated with TSST expression of stress, $r(156) = 0.476$, $p < .001$, and there was evidence of these associations for HC, $r(63) = 0.599$, $p < .001$, and MDD, $r(57) = 0.427$, $p = .001$, but not MDD/NSSI, $r(37) = 0.257$, $p = .124$. Fisher's r to z transformation, $z = 2.00$, $p = .022$, showed that correspondence was significantly higher for HC than MDD/NSSI. Additionally, there was evidence that Peak cortisol levels were differentially correlated with ratings of TSST expressions of stress. There were significant associations for MDD/NSSI, $r(37) = 0.367$, $p < .026$, but not MDD, $r(56) = 0.084$, $p = .539$, or

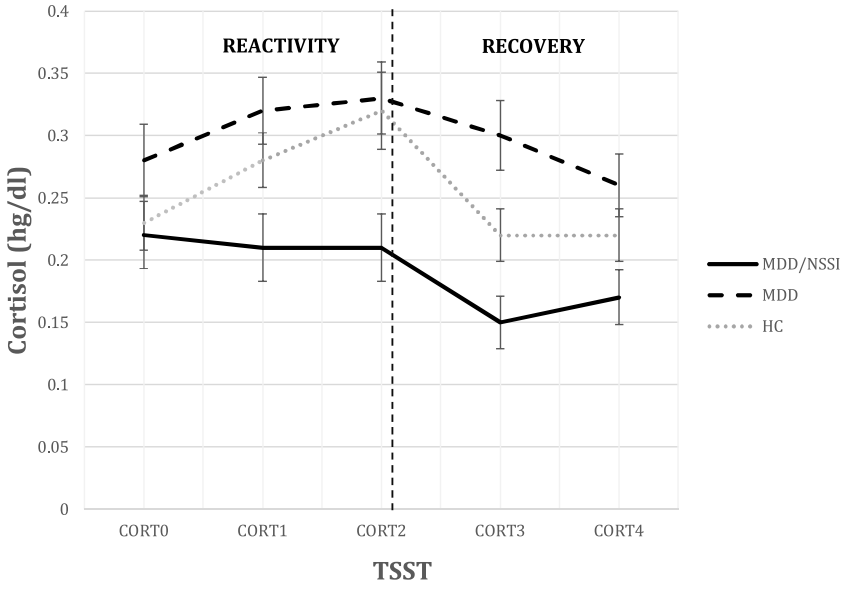

Fig. 1. Cortisol Levels for the Trier Social Stress Test by Group.
Note. HC = Healthy control group. MDD = Major depressive disorder without repeated non-suicidal self-injury group. MDD/NSSI = Major depressive disorder with repeated non-suicidal self-injury. Area under the curve (ground) and peak cortisol were significantly lower for MDD/NSSI than MDD. Area under the curve (initial) cortisol was significantly lower for MDD/NSSI than HC. There was a significantly less steep slope for the reactivity and recovery response for MDD/NSSI than HC.

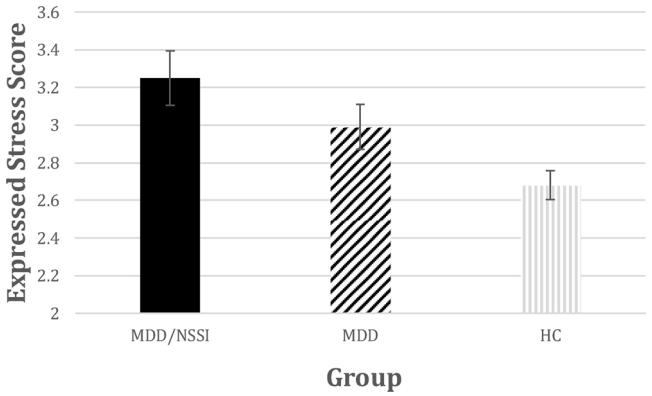

Fig. 2. Expressions of Stress during the Trier Social Stress Test by Group.
Note. HC = Healthy control group. MDD = Major depressive disorder without repeated non-suicidal self-injury group. MDD/NSSI = Major depressive disorder with repeated non-suicidal self-injury. Experimenters rated expressions of stress significantly higher for MDD/NSSI than HC.

HC, $r(63) = -0.071$, $p = .581$. Fisher's r to z transformation, $z = -2.12$, $p = .034$, showed that correspondence was significantly higher for the MDD/NSSI than HC.

4.3. Mixed effect models: MDD/NSSI, MDD, and HC comparisons

A linear piecewise model with random effects of intercept and measurement fit the cortisol data best, relative to the other models (e.g., null, linear, quadratic). Consistent with our prediction of group differences for the TSST, the interaction terms for diagnostic group and measurement were significant. Specifically, relative to MDD/NSSI, HC displayed a steeper reactivity response ($\beta = 0.29$, $p = .034$), with a more positive slope across the first three samples, and also displayed a stronger recovery response ($\beta = -0.20$, $p = .049$), in which there was a more negative slope across the last three samples. Thus, relative to HC, MDD/NSSI showed evidence of blunted reactivity and recovery to psychosocial stress. There was a marginally significant difference between individuals with MDD/NSSI and MDD for reactivity, where MDD demonstrated a steeper response than MDD/NSSI ($\beta = 0.25$, $p = 0.068$). There was also a main effect of diagnostic group with MDD/NSSI demonstrating lower cortisol responses overall during the TSST relative to both HC ($\beta = 9.65$, $p = 0.049$) and MDD ($\beta = 12.85$, $p = 0.002$). There were no significant differences between HC and MDD individuals regarding TSST cortisol trajectories. Please see Table 3 for model results.

Table 3
Results of Mixed Effects Models Comparing the Best-Fitting Model of Cortisol Change (Unadjusted Model) During the Trier Social Stress Test and the Final Model.

Fixed Effects	Unadjusted Model	Final Model
Intercept	49.80**	44.15**
(SE)	(1.58)	(3.88)
Reactivity	0.10*	−0.09
(SE)	(.05)	(0.11)
Recovery	−0.23**	−0.11
(SE)	(.04)	(0.08)
Age	–	.02
(SE)		(0.59)
BDI-II	–	−0.03
(SE)		(0.12)
Time of TSST	–	−0.64
(SE)		(0.85)
Gender	–	−4.00
(SE)		(2.46)
HC	–	9.65*
(SE)		(4.87)
HC by Reactivity	–	0.29*
(SE)		(0.13)
HC by Recovery	–	−0.20*
(SE)		(0.10)
MDD	–	12.85**
(SE)		(4.12)
MDD by Reactivity	–	0.25+
(SE)		(0.14)
MDD by Recovery	–	−0.12
(SE)		(0.10)
Variance Components		
Intercept	354.32	328.14
Reactivity	0.31	0.30
Recovery	0.09	0.08
Residual	80.24	82.90
Model Fit		
BIC	6650	6595

Notes. BDI-II = Beck Depression Inventory 2 score; TSST = Trier Social Stress Test; SE = Standard error; BIC = Bayesian Information Criterion; HC = Healthy control group; MDD = Major depressive disorder group; Individuals with MDD and non-suicidal self-injury are the reference. + $p = .07$, * $p \leq .05$, ** $p \leq .01$.

4.4. Summary indexes: exploratory follow-up analyses

A series of follow-up GLM analyses were conducted evaluating summary indexes (using standard control variables) based on sex, level of NSSI and type of self-harm. There was no evidence that the pattern of cortisol differed for males and females across groups (no group by sex interaction). When follow-up analyses were limited to females or limited to males the patterns of means paralleled the results for the total sample (Fig. 3 and 4). However, the only significant group differences were in AUCi, $F(2,106) = 3.19$, $p = .045$ for females and expressed stress, $F(2,51) = 3.32$, $p = .044$ for males (results provided upon request). Additionally, replicating past research with adolescents (e.g., Klimes-Dougan et al., 2001), overall males showed higher cortisol levels (Peak) and more pronounced reactions (AUCi) during the TSST than females, respectively, $F(1,158) = 12.22$, $p = .001$ and $F(1,158) = 5.27$, $p = .023$. By contrast, overall females were more likely than males to experience and express higher levels of stress, respectively, $F(1, 152) = 31.24$, $p < .001$ and $F(1,152) = 9.48$, $p = .002$.

Next, a series of GLM follow-up analyses were conducted with four groups (HC, MDD, MDD/NSSI-subthreshold, and MDD/NSSI-repeated), that also accounted for those who have a history of engaging in NSSI on a more limited basis (subthreshold, as defined by reports of between one and three lifetime episodes of NSSI). The results for the TSST differed for the AUCg, $F(3, 151) = 2.95$, $p = .035$, and Peak, $F(3, 151) = 2.83$, $p = .041$ (post hoc comparison showed that MDD/NSSI-repeated had significantly lower cortisol than MDD/NSSI-subthreshold or MDD), and for AUCi, $F(3, 151) = 6.69$, $p < .001$ (post hoc comparisons showed that MDD/NSSI-repeated had less of an increase than MDD/NSSI-subthreshold or HC) (results provide upon request).

We also considered if other aspects of self-harm showed a similar pattern to NSSI by accounting for both NSSI and suicide attempts (SA). In this five-group analysis we compared HC, MDD/NSSI/SA, MDD/NSSI, MDD/SA, and MDD (for this analysis, the depressed group represents those with no history of repeated NSSI or SA). Significant group differences were noted for AUCg, $F(4,158) = 2.47$, $p = .047$. Post hoc contrasts showed that cortisol levels of MDD/NSSI/SA, which were the lowest levels across all the groups, were significantly lower than MDD. Of note, the MDD/NSSI (without SA) did not significantly differ from the MDD or HC groups (results provided upon request).

Finally, as noted with superscripts in the far left column of Table 2, we considered a series of supplemental analyses (while also controlling for the standard covariates of age, sex, BDI-II scores, and the time of day of the TSST) to explore a wide array of possible confounds and robustness checks that may account for the main analyses for the MDD/NSSI, MDD, and HC groups. The primary findings remained significant or marginally significant even after accounting for these additional covariates of IQ, comorbidities, awakening time, and duration of illness in the supplemental analyses. By contrast, the analysis controlling for medication and the number of days between visits only showed evidence of group differences for TSST AUCg. Results of these supplemental analyses can be provided upon request.

5. Discussion

A critically important function of threat system signaling is to preserve the individual from psychological and bodily harm. Continued efforts are needed to enhance our knowledge of stress regulation when alterations in self-preservation instincts may have taken place, such as when individuals engage in self-harm. This study used a multi-level methodology to examine HPA function in a clinical sample of adolescents with NSSI. The results add to existing evidence showing a pattern of blunted physiological reactivity under stress conditions in depressed adolescents who engage in NSSI. Follow-up analyses provided evidence that this pattern of HPA functioning in the context of the TSST was evident in females and males, in those that have a history of both NSSI and suicide attempts, and remained significant even when accounting for a number of possible confounds (e.g., sex, age, BDI-II, time of TSST in addition to comorbid conditions, IQ, and duration of illness). In contrast to the patter of blunted physiological response to a social stressor, there was evidence that depressed adolescents (both those who do and do not engage in repeated NSSI) expressed higher behavioral stress reactions than controls.

A primary goal of this study was to advance our understanding of stress system functioning by examining cortisol levels of adolescents with depression with or without a history of NSSI in the context of a social stressor. There are likely to be both physiological and psychological mechanisms for these alterations in stress responding. At a psychological level, repeated exposure to painful or fearful experiences leads to a down-regulation of self-preservation instincts (Joiner, 2005). It is also possible that these adverse experiences provide an explanatory physiological mechanism, as revealed by the process by which stress responses “get under your skin.” The findings of this study are largely consistent with a prominent allostatic load model of stress system functioning, reflected in a shift toward hyporeactivity (Juster et al., 2010). Our results show a blunted response to psychosocial stress for those who repeatedly self-injure, as compared to the trajectory analyses for controls that show a linear increase followed by a linear decrease with corroborating evidence from findings for the AUCi summary index that captures the increase from initial cortisol levels. Our findings add to the results of the two other studies that evaluate adolescents who engage in self-harm in the context of the TSST (Kaess et al., 2012; Plener et al., 2017). Preclinical studies have noted similar patterns. For example, socially reared monkeys who self-injure show lower cortisol levels when confronted with acute stressors (Tiefenbacher et al., 2005). Evidence from other clinical populations, including those diagnosed with Borderline Personality Disorder (e.g., Scott et al., 2013) and those with histories of abuse or trauma (e.g., Wichmann et al., 2017), show this similar blunting pattern. Further, there is one study that shows that the developmental progression of a shift from hyper to hypo arousal of the HPA axis may take place from childhood into adolescence, as suggested by a longitudinal study on girls with a history of abuse (Trickett et al., 2010).

The results of this study highlight the importance of accounting for NSSI in the context of depression. It is possible that some of the past failures to detect group differences for adolescent depression may be attributed to not accounting for NSSI as a possible moderator. There is some degree of consensus in the field that depressed adults have poorer feedback regulation in the context of social stressors (Burke et al., 2005), but more recent reviews have also highlighted an array of conflicting findings with some research failing to identify group differences between depressed and healthy controls, particularly in adolescence (see reviews by Ciufolini et al., 2014; Guerry and Hastings, 2011). Similarly, some of our earlier published work on a subset of this sample failed to show differences between depressed and healthy control adolescents in cortisol in the context of the TSST (Klimes-Dougan et al., 2014). In this larger sample that accounted for NSSI, group differences emerged, showing significantly lower cortisol levels (e.g., AUCg, Peak) in MDD/NSSI than MDD. Beauchaine and colleagues (2015) also considered variation in stress responding in adolescents with NSSI in the context of depression. They found lower blood cortisol levels post dexamethasone suppression test for those who reported suicidal ideation and self-inflicted injury. Importantly, these findings may inform the global initiatives that are underway to delineate the corresponding biological underpinnings of mental illness (Research Domain Criteria; Insel et al., 2010).

Expanding on previous research, the slightly larger sample of adolescents with NSSI also allowed for a series of follow-up analyses to begin to better characterize what factors are likely to account for this blunted reactivity in the context of the TSST. Past research has typically focused on predominately female samples when considering HPA axis functioning in for those who engage in self-harm. Attention to sex is important given that there are sex differences in salivary cortisol in

responses to social stressors. In contrast, with regard to self-harm, similar patterns were found for males and females and there was no evidence of an interaction between group and sex. While the limited power was likely to account for the relatively few significant findings when analyses were conducted separately for the males and females (particularly for males total $n = 53$, with only 8 participants in the MDD/NSSI group), this preliminary evidence suggests a pattern of lower cortisol responding for both female and male adolescents who engage in NSSI.

Other exploratory findings suggest that the severity of self-harm, as indexed by the frequency of engagement and the type of self-harm, may be important to consider. Our results suggest that those who have experimented with NSSI (subthreshold) do not share a pattern of low cortisol that is characterized by those with repeated NSSI. Additionally, those with more severe self-harm, those with a history of repeated NSSI *and* suicide attempts, had the lowest cortisol levels within the context of a stressor. Our results were largely consistent with the growing evidence that this blunted pattern of stress reactivity is evident in a range of self-harm behaviors, including suicide attempts (Lindqvist et al., 2008), and more explicitly within the HPA axis in the context of the TSST with those at risk for a suicide (McGirr et al., 2010) Importantly, group differences, including those considering the frequency and severity of self-harm, were evident even when accounting for an index of symptom severity (BDI-II). However, more work will be needed to reconcile these findings with the evidence that adult depressed patients who show a pattern of DEX nonsuppression (higher cortisol) are at the highest risk for death by suicide in adults (Coryell and Schlesser, 2001). Longitudinal studies are needed to determine whether the pattern of lower cortisol reported here in adolescents with depression and repeated NSSI is predictive of future suicidal behavior, which would implicate this pattern as an important biological risk marker that could guide targeted intervention to prevent adolescent suicide.

This study provided an opportunity to use a multi-level approach to assess stress system functioning at the physiological and behavioral levels in the context of the TSST. While blunted physiological reactivity to psychosocial stress was associated with depressed adolescents who engage in NSSI, both depressed groups (combined MDD/NSSI and NSSI) were rated by an observer to express more stress than controls $F(152) = 5.154$, $p = .028$, with the highest levels observed in MDD/NSSI (Table 2, Fig. 2). Perhaps this is because individuals who engage in NSSI are particularly self-deprecating following stressful social situations, focusing more on the negative mistakes they may have made (Nock et al., 2008). It is also possible that they come into a situation with particularly negative biases, as Kaess et al (2012) found that participants with NSSI experienced negative emotion prior to but not during the TSST.

Preliminary evidence from our study also suggests that correspondence across the systems (experience, expression, physiology) differs across groups. Because emotions have communicative functions, in some situations it is adaptive to convey the feelings of stress one is experiencing (Lepore et al., 2000), as shown in the association of experienced and expressed stress in HC and MDD. By contrast, our results showed tentative evidence of less of a correspondence between experienced and expressed stress for MDD/NSSI. At the same time the MDD/NSSI group showed some evidence of correspondence across physiological (Peak) and behavioral (expression) systems, perhaps suggesting that even though those who self-harm may not be aware of their emotions, some aspects of stress may be quite aptly conveyed to others. While it is possible that overall patterns of discordance may point to limited emotional awareness, it is also possible that NSSI may be a way of diverting attention away from negative thoughts or a form of dissociation from traumatic reminders (McKenzie and Gross, 2014). These results are sparse and preliminary, and future research should continue to apply a multi-level approach in an effort to more fully understand across system functioning.

While study strengths include a multi-level approach to assess a comparatively large sample of adolescents with NSSI, there are a number of study limitations. This study is part of a larger series of studies in which we evaluated the stress response system using neuroimaging, neurocognitive assessments, and physiological responding to advance our understanding of the pathophysiology of adolescent depression (Cullen et al., 2014; Klimes-Dougan et al., 2014). To characterize NSSI, we relied on clinical ratings and notes from the KSADS-PL based on the report of the adolescent and their parent. Our focus on repeated NSSI with four or more episodes was similar to others who have used repetitive self-injury defined as five or more episodes of NSSI as is noted in the DSM-5 (American Psychiatric Association, 2013). Research that incorporates validated assessment tools to assess NSSI in the context of stress responding have been used by some studies (e.g., Beauchaine et al., 2015) and should be used more widely in the future. Relatedly, more extensive measurement of NSSI would allow researchers to better examine the features of NSSI that account for the blunted patterns of stress responding.

It is also important to account for possible confounding factors. Our study controlled for the potential impact of gender, age, depressive symptoms, time of the assessment, and used procedures to minimize other possible confounds (e.g., participants were instructed to not eat for an hour before the visit). The BDI-II provided tentative evidence that group differences of cortisol and expressions of stress were present even when accounting for the severity of depressive symptoms, which is particularly important given that the interval between diagnosis and TSST was quite variable (although there was a similar interval across the MDD/NSSI, MDD, and HC groups, $p > 0.20$). There was little evidence that other factors that could influence allostatic load, such as duration of illness, age of onset or GAF that were reported for the depressed adolescents, accounted for the reported findings. While future studies should limit the assessment interval, as it is possible that a deteriorating or remitting course of depression may have influenced the results, the follow-up analyses suggested group differences remained even when accounting for this factor.

Assuring that samples are collected under similar conditions is an ongoing challenge in the field. Our results suggested that group differences were evident even when efforts were made to account for IQ, the presence of other comorbid disorders that are commonly associated with NSSI, or medication use. In some cases it was difficult to determine if limited power was the primary factor accounting for the findings (e.g., sex, medication status). Future studies should account for the effects of medication (limited because only in the depressed group), as there was some evidence to suggest that by controlling for this factor, the group differences may be minimized for the TSST. Additionally, it is not clear to what extent other factors (e.g., time of awakening, season of the year, phase of the menstrual cycle, the use of oral contraceptives, BMI, or smoking) may have influenced findings. While the sample size was considerably larger than past research, there were still constraints when exploring additional explanatory variables or multiple possible confounds.

Finally, it is not clear to what extent these results pertaining to self-harm are relevant to a broader group of individuals who are not diagnosed with depression. The proportion of NSSI (40% of those diagnosed with MDD) in our sample is in line with other clinical samples. Given that this pattern of stress response is similar across studies using different criteria for NSSI and different recruitment procedures, we are increasingly confident that alterations in HPA axis functioning are evident across different populations who engage in NSSI. Future efforts should consider broader criteria for recruitment given that NSSI is now considered a disorder under study in the DSM-5 and evident across a range of disorders.

6. Conclusions

This study used multi-level assessments (experience, expression and physiological indexes of stress functioning in the TSST) in depressed

adolescents who did and did not engage in repeated NSSI. The findings shed light on the heterogeneity of the biological underpinnings of depression, and suggest that it is important to consider those who engage in self-harm as a potentially distinct subgroup among adolescents with depression. The larger sample also allowed for exploratory follow-up analyses to consider the role demographic factors (e.g., sex), explore critical aspects of self-harm (e.g., frequency of NSSI, suicide attempt), and rule out some confounds (e.g., comorbid conditions). It will be important to replicate these findings, but also to use longitudinal research to characterize developmental mechanisms and clinical outcomes and to identify biological predictors that could guide targeted intervention. Understanding the course of these biological disruptions, when they begin and how they evolve over time, will be critical to guide decisions about the optimal timing for interventions. Applications of this work are potentially relevant to personalization, for some research is starting to consider HPA axis functioning as predictors of psychotherapy or psychopharmacological treatment response in adolescents (e.g., Gunlicks-Stoessel et al., 2013; Klimes-Dougan et al., 2018). Overall, research geared toward testing interventions that are optimally timed to target an individual's biological disruptions is most promising to ultimately restore healthy neurodevelopment and prevent a host of deleterious outcomes.

Conflict of interest

Bonnie Klimes-Dougan - no conflict of interest
Erin Begnel – no conflict of interest
Brandon Almy – no conflict of interest
Michelle Thai – no conflict of interest
Melinda Westlund Schreiner – no conflict of interest
Kathryn Cullen – no conflict of interest

Acknowledgements

The authors would like to first and foremost thank the adolescents and families who volunteered to participate in this study in an effort to contribute to scientific efforts to understand and elevate the suffering associated with depression. The study was funded by a grant to Dr. Klimes-Dougan from the Deborah E. Powell Center for Women's Health at the University of Minnesota, and by grants to Dr. Cullen including the National Institute of Mental Health (K23MH090421), the National Alliance for Research on Schizophrenia and Depression, the University of Minnesota Graduate School, and the Minnesota Medical Foundation.

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