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The roles of comorbidity and trauma exposure and its timing in shaping HPA axis patterns in depression

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ABSTRACT

Background: Much work has documented hypothalamic pituitary adrenal (HPA) axis abnormalities in major depressive disorder (MDD), but inconsistencies leave this system's role in the illness unclear. Comparisons across studies are complicated by variation in co-morbidity (Posttraumatic Stress Disorder-PTSD, anxiety disorders), exposure to trauma, and timing of trauma (child vs. adult). Here, we examined the impact of these factors on HPA axis profiles in depression.

Methods: We recruited 5 groups of participants: MDD (n = 14), comorbid MDD + PTSD following adulthood trauma (MDD + PTSD-Adult; n = 12), comorbid MDD + PTSD following childhood trauma (MDD + PTSD-Child; n = 18), comorbid MDD + social anxiety disorder (MDD + SAD; n = 12), and non-depressed control participants who were sex and age matched to patients (combined total n = 36). HPA axis function was assessed using three challenges: stress reactivity via the Trier Social Stress Test (TSST), feedback sensitivity via a dexamethasone suppression test (DST), and central drive via a metyrapone challenge (MET). We compared hormonal responses between patient groups and their respective non-depressed controls.

Results: MDD + PTSD-Child showed low cortisol levels at baseline, and reduced adrenocorticotropic hormone (ACTH) levels at baseline and throughout the TSST. MDD-only, MDD + PTSD-Adult, and MDD + SAD did not differ from non-depressed controls in HPA axis responses to the TSST. Controlling for childhood trauma severity, the reduced baseline levels in MDD + PTSD-Child were no longer significant and significantly reduced baseline cortisol levels emerged for MDD + PTSD-Adult. No diagnostic group effects were detected with DST and MET. Childhood maltreatment subtypes were associated with unique HPA axis responses to TSST and MET.

Conclusion: Comorbidity and trauma exposure, as well as their timing and type, contribute to inconsistencies in the depression literature and must be included in efforts to clarify the role of the HPA axis in MDD.

1. Introduction

The hypothalamic pituitary adrenal (HPA) axis is a critical stress response system. Its stress reactivity is modulated by developmentally shaped regulatory processes that occur at various levels. These include: (1) central drive that flows through and from the hypothalamus and is constrained by glucocorticoid-mediated feedback inhibition, (2) pituitary sensitivity to both hypothalamic drive via corticotropin releasing hormone (CRH) and feedback inhibition from adrenally-released glucocorticoids, and (3) adrenal sensitivity to pituitary-released adrenocorticotropic hormone (ACTH). The system also has its own intrinsic, centrally-driven circadian cycle of activity. Decades of research have documented dysregulation of the HPA axis in Major Depressive Disorder (MDD) including increased stress reactivity, as shown in increased HPA responses to the Trier Social Stress Test (TSST), reduced sensitivity to feedback inhibition, as shown by escape from suppression

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in the dexamethasone suppression test (DST), and increased central drive, as shown by elevated ACTH levels after an evening metyrapone (MET) challenge (an 11-βhydroxylase inhibitor of cortisol synthesis; Nestler et al., 2002; Pariante and Lightman, 2008; Young et al., 1994). However, the literature has been plagued by heterogeneous outcomes and failures to replicate results. Interpretation of findings may have been complicated, partly, because comorbidity is common in depressed samples and study samples vary in exposure to or timing of traumatic experiences.

Depression is frequently comorbid with anxiety disorders. Approximately sixty percent of individuals with MDD also meet criteria for an anxiety disorder (Kessler et al., 2003) – with substantial clinical consequences. For example, depressed patients with an anxiety disorder have greater depression severity, worse treatment outcomes, and greater chronicity over time (Hirschfeld, 2001; Merikangas et al., 2003). Anxiety comorbidity in depressed individuals also impacts neuroendocrine functioning. For example, depressed patients with a comorbid anxiety disorder (primarily social anxiety disorder) showed increased HPA stress reactivity, while patients with either depression or anxiety, but not both, showed normal HPA responses to psychosocial stress (Young et al., 2004a, 2004b; Young et al., 2000). While primary anxiety disorders such as panic disorder, generalized anxiety disorder, specific phobias and social anxiety disorder show less robust HPA axis dysregulation (reviewed by Elnazer and Baldwin, 2014), depressed patients with comorbid anxiety most often express the neuroendocrine patterns of depression (reviewed by Young et al., 2004a, 2004b). This changes, however, when depression is comorbid with Posttraumatic Stress Disorder (PTSD).

Depression and PTSD both show HPA axis abnormalities but in divergent directions. MDD has been associated with HPA axis hyperactivity, while PTSD is associated with reduced HPA axis activity as evidenced by lower basal cortisol levels and enhanced negative feedback sensitivity (De Kloet et al., 2006); though inconsistencies are common (Handwerger, 2009; Klaassens et al., 2012). The neuroendocrine profile when depression and PTSD are comorbid remains unclear. There is some evidence that the PTSD profile may dominate – for example, depressed patients showed HPA axis hyperactivity, but HPA axis hypoactivity was found in depressed patients with comorbid PTSD (Oquendo et al., 2003). Also, daily cortisol output and post-DST cortisol levels were lower in MDD + PTSD, relative to non-depressed controls, but comparable to those with PTSD (Morris et al., 2012). Nevertheless, exceptions exist (Griffin et al., 2005; Heim et al., 2000), and inconsistencies may be driven by other important factors at play (Zaba et al., 2015), including sample variations in trauma exposure and timing of trauma (e.g., child vs. adult).

Mood, anxiety, and trauma or stress-related disorders are all associated with trauma exposures (McQuaid et al., 2001) and childhood adversities (Kessler et al., 1997) – factors known to shape HPA axis functioning (Morris et al., 2012). The HPA dysregulation seen in depressed patients with comorbid PTSD could be a function of some complex interplay between PTSD, MDD, something unique to trauma-associated depression, or the trauma exposure itself, perhaps influenced by the timing of that exposure (e.g., early life stress; Heim et al., 2000). Given that HPA axis dysregulation is so often seen in depression and PTSD, and the system is critically shaped by developmental exposures that can both alter its adult functioning (Baumeister et al., 2014) and contribute to risk for the disorders, teasing apart the factors shaping HPA axis dysregulation in comorbid samples may be of considerable value. Clinically, patients with histories of maltreatment during childhood are at greater risk for psychiatric comorbidity (Dvir et al., 2014; Kessler et al., 2010; Kuhlman et al., 2017; Putnam et al., 2013), and clinical treatment responses are undermined by both co-morbidity and trauma histories (Harkness et al., 2012; Williams et al., 2014). Deeper understanding of the roles of trauma history and co-morbidity in shaping neurobiology that contributes to pathophysiology may help us better target interventions to the specific combinations of symptom profiles, developmental histories, and neurobiology brought to us by each patient.

This study examined the impact of co-morbidity (PTSD; Social Anxiety Disorder-SAD) and trauma exposure and its timing on HPA axis stress reactivity (TSST), feedback sensitivity (DST), and central drive (MET) in depressed patients. We hypothesized that the relationship between MDD and HPA axis alterations would be complex – influenced by co-morbidity, trauma exposure, and trauma timing. Specifically, we predicted that individuals with MDD, in the absence of co-morbidity and trauma, would not differ from non-depressed controls in stress reactivity, but would show escape from suppression on DST (increased post-DST cortisol levels) and enhanced ACTH response to metyrapone. We hypothesized that childhood trauma exposure would increase stress reactivity and produce compensatory changes in basal secretion and feedback sensitivity, as indicated by enhanced negative feedback and normal ACTH response to metyrapone compared to controls. We predicted that adult trauma would only sensitize the stress response system, so that the MDD + PTSD-Adult group would show enhanced HPA response to the TSST, feedback resistance to DST, and enhanced ACTH response to metyrapone, relative to controls. Lastly, we expected that individuals with MDD comorbid with early onset SAD would show increased stress reactivity and enhanced negative feedback (decreased post-DST cortisol levels) and normal response to metyrapone relative to controls.

2. Methods

2.1. Overview of experimental design

We recruited five groups, including 1) depressed participants (MDD-only; n = 14), 2) depressed participants with a comorbid diagnosis of PTSD from adulthood trauma (MDD + PTSD-Adult; n = 12), 3) depressed participants with a comorbid diagnosis of PTSD from childhood trauma (MDD + PTSD-Child; n = 18), 4) depressed participants with a comorbid diagnosis of an early life onset social anxiety disorder (MDD + SAD; n = 12), and 5) non-depressed healthy control participants who were sex and age matched to patients (combined total n = 36). We chose a healthy control group and opted against the inclusion of a trauma-exposed but symptom free (resilient) control group because of our focus on the impact of trauma and its timing on the HPA axis in depression, not in PTSD by itself. All participants underwent three neuroendocrine challenge paradigms, including a Trier Social Stress Test (TSST) to assess stress reactivity, a low dose Dexamethasone Suppression Test (DST) to assess negative feedback sensitivity, and an evening metyrapone challenge (MET) to assess HPA axis central drive. Because of expected recruitment challenges based on prior efforts, we did not anticipate being able to successfully recruit patient groups that were fully matched to each other on critical variables like age and gender. Because both of these variables do impact the HPA axis outcomes of interest, and we were not content with statistical control in analyses, our pre-planned analysis strategy was to compare each patient group directly to a control group that was specifically and precisely matched to them, testing predictions about each group's differences from their matched non-depressed controls. We did not compare all groups to each other in an initial omnibus analysis. We examined some differences between specific patient groups in post-hoc analyses (see analysis section). The hypotheses stated in the introduction and the primary planned analyses precisely reflected the plans laid out in the grant application that procured funds for this study. Participants were paid $400 for completing all study procedures. This study was approved by an Institutional Review Board and all participants signed written consent.

2.2. Participant recruitment and evaluation

All patient participants met MDD criteria using the Diagnostic and

Statistical Manual of Mental Disorders, fourth edition (DSM-IV). The comorbid patient groups additionally met DSM-IV criteria for an anxiety disorder (PTSD or early onset SAD). They were recruited through local flyers and online advertisements and from those applying for treatment in mood and anxiety subspecialty clinics or a general outpatient program in the Department of Psychiatry. Non-depressed control participants were recruited through local and online advertisements. All interested participants were telephone screened and those meeting basic eligibility criteria were invited for an in-person evaluation. At evaluation, they were screened for medical disorders via medical history and laboratory tests (blood chemistry profile, CBC and urine drug screen). Exclusion criteria were age less than 18, any serious medical illnesses (including anemia, diabetes mellitus, nutritional problems, surgery, head trauma, prior organic brain injury or major physiological disturbances during the past three months), any history of psychosis, any history of DSM-IV alcohol or drug dependence, any drug or alcohol abuse in the past 2 years, regular alcohol use during the past 6 months of > 7 cans of beer per week or equivalent, any use of street drugs during the past 2 years, positive urine drug screen (which included opiates) at evaluation, consumption of more than 500 mg of caffeine per day, daily tobacco use, active suicidal ideation, and major chronobiological disruption or phase shift during the preceding month. Due to exercise effects on the HPA axis, participants were excluded if they were completely sedentary or vigorously athletic. All were within +35 % / -10 % of normal weight based on body mass index (BMI) categories. Medication exclusions included any use of barbiturates, phenytoin, carbamazepine, meprobamate, glutethimide, alpha-methyl dopa, corticosteroids (oral), codeine, cyproheptadine, exogenous estrogen, progesterone or oral contraceptives in the past 3 months and any use in the past month of topical or inhaled steroids or herbal antidepressants (e.g., St John's Wort or S-adenosyl-methionine, SAMe). Patient participants were free of psychotropic drugs including antidepressants for at least 2 weeks, with no use of fluoxetine in the past 6 weeks. Control subjects had no history of any use of prescribed psychiatric medications.

A Structured Clinical Interview for DSM-IV Disorders (SCID) was administered to all potential participants at evaluation. Patient participants all met DSM-IV criteria for MDD and were in an active episode with a Hamilton Depression Rating Scale (HAM-D) score of > 14. The *MDD only* group had no history of PTSD and no history of DSM-IV qualifying childhood physical or sexual abuse. They also could not meet any lifetime anxiety diagnosis (except Generalized Anxiety Disorder or Specific Phobia) and no other DSM-IV diagnosis within the past 5 years. To qualify for the comorbid groups, patients also had to meet DSM-IV criteria for PTSD or SAD. Participants with Obsessive-Compulsive Disorder were excluded. Patients in the *MDD + PTSD-Adult group* had to have current symptoms of PTSD by the Clinician-Administered PTSD Scale for DSM-IV (CAPS; mean = 65, range = 50–84), and no history of DSM-IV qualifying traumatic event before the age of 18. The *MDD + PTSD-Child group* had to have sexual, physical, or emotional abuse or neglect in childhood and meet criteria for PTSD onset before the age of 16. Subsequent adult traumas, which may have re-activated symptoms, were permitted. The *MDD +SAD group* also met DSM-IV diagnostic criteria by SCID for current social anxiety disorder with onset before puberty (to serve as a "control" for an early onset anxiety disorder that was not a consequence of early exposure to a Criterion A trauma). They also had to have at least a score of 34 on the Social Interactional Anxiety Scale (SIAS).

Non-depressed control participants were evaluated by the SCID for Non-Patients (SCID-NP) and an adaptation of the Family Informant Schedule and Criteria (FISC) structured interview. They were required to be free of any current or past DSM-IV diagnoses (except Specific Phobia) and have no first-degree relatives with any anxiety disorder (except Specific Phobia), psychotic illness, or primary major affective disorder. Non-depressed control participants were matched by sex and age (within 5 years) individually to recruited patients (n = 36, controls could be matched to more than 1 patient participant).

2.3. Interview and self-report measures

In addition to the SCID-IV (SCID-NP for control participants), CAPS (only assessed in MDD + PTSD-Child and MDD + PTSD-Adult participants; Blake et al., 1995) and modified FISC interviews, participants also completed the following at evaluation: Beck Depression Inventory (BDI; Beck et al., 1996), Hamilton Depression Rating Scale (HAM-D; Hamilton, 1986), Brief Symptom Inventory (BSI-anxiety, BSI-depression; Derogatis and Melisaratos, 1983), the Childhood Trauma Questionnaire (CTQ; Bernstein and Fink, 1998), and the Social Interactional Anxiety Scale (SIAS; Mattick and Clarke, 1998). Exposure to clinically meaningful childhood maltreatment subtypes was assessed using validated cut-points (Walker et al., 1999). Participants with scores for emotional abuse ≥10, emotional neglect ≥15, physical abuse ≥8, physical neglect ≥8, or sexual abuse ≥8 were included in the maltreated group for that subtype.

2.4. Neuroendocrine challenge paradigms

Each participant was studied over a 7-day period, involving two laboratory study visits for a TSST challenge and an evening MET challenge as well as a home saliva cortisol DST (total of 3 days). Efforts were made to counterbalance the order of the neuroendocrine challenge paradigms with at least one rest day (normal activity) included between paradigms to minimize carry-over effects. However, issues related to scheduling, phase of menstrual cycle, and availability of space in the research unit could not always be overcome, so full counterbalancing sometimes had to be sacrificed to accommodate other critical issues. However, this did not meaningfully impact results (see Section 3.2).

Saliva samples were collected in salivettes (Sarstedt). On laboratory visits, blood samples for ACTH and cortisol were drawn into appropriate tubes (EDTA or heparin) via indwelling catheter, placed on ice immediately, and spun within 10 min.

Trier Social Stress Test (TSST). Participants arrived in the laboratory at 2:30PM and had an intravenous catheter inserted for blood collection. At 3:30PM, they moved to another room for the TSST, a public speaking task (social evaluative stressor) involving a mock job interview and mental arithmetic (Kirschbaum et al., 1993). They were introduced to two "panelists" (1 male and 1 female) who were described as the task administrators and evaluators. The task was introduced via a pre-recorded tape as a mock job interview during which the panelists would be evaluating performance and non-verbal indicators of anxiety. Participants had 3 min in front of the panelists to prepare a presentation to promote their candidacy for a job that was tailored to their interests. They were told their performance would be videotaped and further evaluated. After they completed the 5-minute job talk, they were given a second unexpected task, i.e. to subtract 13 serially from 1022. They were told to stop and begin again whenever an error was made. This continued for another 5 min. Beginning at 3:00PM, blood was drawn every 15 min for ACTH and cortisol (60, 45, 30, 15 min prior to TSST start). Blood was also drawn immediately before TSST instructions were given (TSST start, time 0), at the start of the speech task (+5 min post TSST start), at the beginning and end of the math task (+10 and +15 min post TSST start), as well as in 10-minute intervals after completion of the task (+25, 35, 45, 55, 65 min post TSST start).

Dexamethasone Suppression Test (DST). Baseline saliva cortisol was collected at home (or work, for 4PM sample) at wake-up, 4PM and bedtime for 2 days (pre-DST). On the evening of the second day of basal saliva sampling, dexamethasone (0.25 mg) was taken at bedtime. The dosage of 0.25 mg was chosen so we could examine both increased and decreased sensitivity to dexamethasone. While a 0.5 mg dosage gives near maximal suppression of salivary cortisol in healthy participants

(Pariante et al., 2002), a 0.25 mg dosage allows for good but submaximal suppression in saliva. This allows us to see *increased* dexamethasone suppression, and to also still see "escape" from suppression as more classically examined in the depression literature. This enabled us to test the specific hypothesis that the MDD + PTSD-Child group would show "enhanced" cortisol suppression relative to controls.

Efforts were made to reach participants on the evening of the second baseline day, via call or text, to enhance compliance with the bedtime dexamethasone dose. Participants then collected saliva samples for cortisol the following day (post-DST) at wake-up, 4PM and bedtime.

Evening Metyrapone (MET) Challenge. Participants reported to the laboratory at 3PM. An intravenous line was inserted, and they accommodated to the study environment. Metyrapone (750 mg) was administered at 4PM and 7:30PM. Blood samples were drawn every 30 min from 4PM until 10PM. The laboratory context was carefully controlled to minimize distractions or inconsistencies across subjects, and standardized snacks were provided at controlled times as previously (Young et al., 2004a, 2004), which has been successful in minimizing risk of nausea or emesis from the drug. The approach and dosing schedule used allow near maximal restraint of cortisol release with minimal subjective side effects.

2.5. Hormonal assays

Assays were conducted to quantify ACTH in plasma and cortisol in both plasma and saliva. Plasma was separated from blood by centrifugation at 1200 rpm for 10 min at 4 °C. Both plasma and saliva were stored frozen at −80 °C until assayed. A rapid, highly sensitive, and precise semi-automated chemiluminescent assay was conducted using Immulite (Siemens, Inc.), according to the manufacturer's directions (Siemens Healthcare Diagnostics, Tarrytown, NY). The intra-assay variability was < 5%, and the inter-assay variability was 5–7 % for both cortisol and ACTH detection.

2.6. Statistical analysis

Plasma and salivary cortisol values were transformed using Box-Cox transformation (Miller and Plessow, 2013). ACTH values were transformed using log transformation.

TSSTAnalyses were performed in SAS (v. 9.4) with the mixed procedure (Proc Mixed) using restricted maximum likelihood estimation (REML) to address our small sample size. Repeated measures in the mixed modeling framework had an autoregressive covariance structure. Post hoc analyses were performed on significant group-by-time interactions by slicing the interaction in Proc Mixed. *A priori* analyses examined HPA axis responses to the TSST between each diagnostic patient group and their respective non-depressed control group. To further examine the contributions of diagnostic group versus childhood trauma severity, we re-ran these models controlling for CTQ scores. We also compared HPA axis responses among specific patient groups to examine the effects of timing of trauma (MDD + PTSD-Adult vs. MDD + PTSD-Child) and whether early trauma exposure vs. an early onset anxiety disorder had unique effects (MDD + PTSD-Child vs. MDD + SAD). Finally, post hoc analyses compared hormonal responses to the TSST between all patients combined and their combined control group. Partial eta squared (ηp^2) was calculated as a measure of effect size for significant findings using the SAS macro developed by Tippey and Longnecker (2016).

DST ANOVA analyses were performed in SPSS. We compared patients (each diagnostic group separately in *a priori* analyses as well as all patients combined in post hoc analyses) to non-depressed controls in post-DST salivary cortisol values at awakening and at 4PM, as well as in percent cortisol suppression at awakening and 4PM, by subtracting post-DST levels from pre-DST levels (calculated as the mean of the levels on the two pre-DST collection days) and converting to a percent of pre-DST levels (Savic et al., 2012).

MET Analyses were performed in SAS (v. 9.4) as described above for the TSST. We first tested whether metyrapone administration successfully suppressed cortisol and enhanced ACTH across the challenge. We then confirmed that there were no diagnostic group differences between patients and respective matched non-depressed controls in cortisol suppression across the challenge. Finally, we tested whether diagnostic patient groups (each diagnostic group separately in *a priori* analyses as well as all patients combined in post hoc analyses) differed from non-depressed controls in ACTH levels (overall and change over time) from the first sample at which ACTH significantly increased (5:30PM) following metyrapone administration through the end of the challenge.

Childhood maltreatment subtypes. Post hoc analyses of the association between childhood maltreatment subtypes and responses to the TSST, DST, and MET were conducted separately using repeated measures ANOVA. Each of these models estimated mean differences in ACTH or cortisol as a function of clinically meaningful exposure to each of the 5 maltreatment subtypes assessed on the CTQ, while controlling for BMI, female sex, age, depressive symptoms (BSI-D), and anxiety symptoms (BSI-A). Effect sizes were calculated using Cohen`s *d*.

3. Results

3.1. Sample characteristics

The total patient group did not differ from the total control group in age, gender, race or BMI (Table 1). Similarly, each diagnostic group (MDD, MDD + PTSD-Adult, MDD + PTSD-Child, MDD + SAD) did not differ in these characteristics from their specifically matched non-depressed control group (all *ps* > .05, Table 1). Diagnostic groups also did not differ from each other in age, gender, race or BMI (*p* values range from 0.22 to 0.76). As expected, patients had significantly greater symptoms of depression and anxiety, and greater exposure/severity of childhood trauma (all *ps* < .01; Table 1). The four patient groups did not significantly differ from each other in severity measures of depression (BDI: $F_{3,\ 52} = 1.25$, $p = .30$; HAM-D: $F_{3,\ 52} = 1.31$, $p = .28$, BSI-D: $F_{3,\ 52} = 1.51$, $p = .22$). As expected, the groups did differ in anxiety (BSI-A: $F_{3,\ 52} = 6.27$, $p = .001$), such that all 3 comorbid groups had more anxiety than the MDD-only group (BSI-A: all *ps* < .01). Patient groups also differed from each other as expected in frequency/severity of childhood trauma ($F_{3,\ 52} = 13.04$, $p < .001$), such that the MDD + PTSD-Child group had significantly greater CTQ scores compared to the other patient groups (all *ps* < .01). The patient groups also differed as expected in social anxiety (SIAS: $F_{3,\ 52} = 4.03$, $p = .01$), such that the MDD + SAD group had greater social anxiety relative to each of the other groups (all *ps* < .05).

3.2. Order effects of neuroendocrine challenge tests

As mentioned above, efforts were made to counterbalance the order of the neuroendocrine challenge paradigms, though logistical and practical considerations resulted in some deviations from perfect counterbalancing. No significant order effects were seen for hormonal responses to DST and MET (all *ps* > .05). Similarly, no significant order effects were seen for ACTH responses to the TSST (order: $F_{2,\ 87} = 2.59$, $p = .08$, order-by-time interaction $F_{16,\ 696} = 1.12$, $p = .33$). The only significant order effects seen were on cortisol responses to the TSST (order $F_{2,\ 87} = 0.62$, $p = .54$, order-by-time interaction $F_{16,\ 696} = 2.03$, $p = .01$). However, careful analysis showed that this was not due to stress reactivity itself, which was our main TSST outcome variable, but was due to first visit effects that lead to higher baseline levels, which is something we have seen in much larger studies (unpublished data). With control for baseline levels, the order effect on reactivity was no longer significant (order $F_{2,\ 86} = 2.20$, $p = .12$, order-by-time interaction $F_{16,\ 668} = 1.59$, $p = .07$, baseline cortisol $F_{1,\ 86} = 78.39$, $p < .001$, baseline cortisol-by-time interaction $F_{8,\ 688} = 7.49$,

Table 1
Descriptive group statistics of demographic information and mental health measures.

Category	Variable	MDD (n = 14)	Controls for MDD (n = 12)	MDD + PTSD-Adult (n = 12)	Controls for MDD + PTSD-Adult (n = 12)	MDD + PTSD-Child (n = 18)	Controls for MDD + PTSD-Child (n = 16)	MDD + SAD (n = 12)	Controls for MDD + SAD (n = 12)	All Patients (n = 56)	Controls for All Patients (n = 36)
Demographics	Age	37.50 (17.59)	36.75 (17.04)	27.92 (7.42)	27.42 (7.90)	29.28 (10.39)	29.94 (11.58)	31.42 (13.57)	30.50 (13.12)	31.50 (12.96)	29.75 (12.06)
	Gender (percent female)	57 %	58 %	50 %	58 %	67 %	69 %	50 %	50 %	57 %	61 %
	Race (percent Caucasian)	64 %	75 %	83 %	67 %	67 %	69 %	83 %	50 %	73 %	69 %
	Body mass index (BMI)	24.75 (3.35)	23.68 (3.19)	24.71 (3.09)	23.98 (3.29)	26.18 (4.57)	26.53 (4.22)	25.11 (4.86)	25.81 (4.40)	25.28 (4.02)	24.66 (4.23)
Mental Health	BDI	20.86 (5.40)**	1.08 (1.88)	25.75 (11.89)**	0.25 (0.62)	26.06 (9.96)**	0.44 (0.89)	27.00 (8.14)**	0.17 (0.39)	24.89 (9.21)**	0.58 (1.27)
	HAM-D	16.93 (1.54)**	0.25 (0.62)	17.75 (2.34)**	0.08 (0.29)	16.39 (1.97)**	0 (0)	16.92 (1.31)**	0 (0)	16.93 (1.86)**	0.11 (0.40)
	BSI-D	1.69 (0.79)**	0.03 (0.06)	2.15 (0.85)**	0 (0)	2.39 (0.94)**	0.04 (0.17)	2.18 (1.13)**	0.06 (0.19)	2.12 (0.95)**	0.03 (0.12)
	BSI-A	0.65 (0.46)**	0.03 (0.06)	1.93 (0.90)**	0.01 (0.05)	1.40 (0.75)**	0.01 (0.04)	1.47 (0.92)**	0 (0)	1.34 (0.87)**	0.01 (0.05)
	CAPS (only for MDD/PTSD)	–	–	65.25 (11.52)	–	54.06 (14.07)	–	–	–	57.24 (14.07)	–
	CTQ	36.50 (9.37)**	28.08 (2.71)	35.42 (7.95)**	28.17 (2.62)	59.28 (15.12)**	28.81 (2.74)	45.17 (13.31)**	29.58 (3.32)	45.45 (15.63)**	28.58 (2.87)
	SIAS	34.50 (14.42)**	12.17 (6.19)	37.08 (16.96)**	9.92 (4.74)	32.67 (18.81)**	11.93 (5.76)	51.75 (8.78)**	9.92 (6.67)	38.16 (16.89)**	11.00 (5.97)

Notes: BDI = Beck Depression Inventory; HAM-D = Hamilton Depression Rating Scale; BSI-D = Brief Symptom Index - Depression Subscale; BSI-A = Brief Symptom Index - Anxiety Subscale; CAPS = Clinician Administered PTSD Scale; CTQ = Childhood Trauma Questionnaire; SIAS = Social Interactional Anxiety Scale.
**Indicates statistically significant difference compared to respective controls for this group $p < .01$.

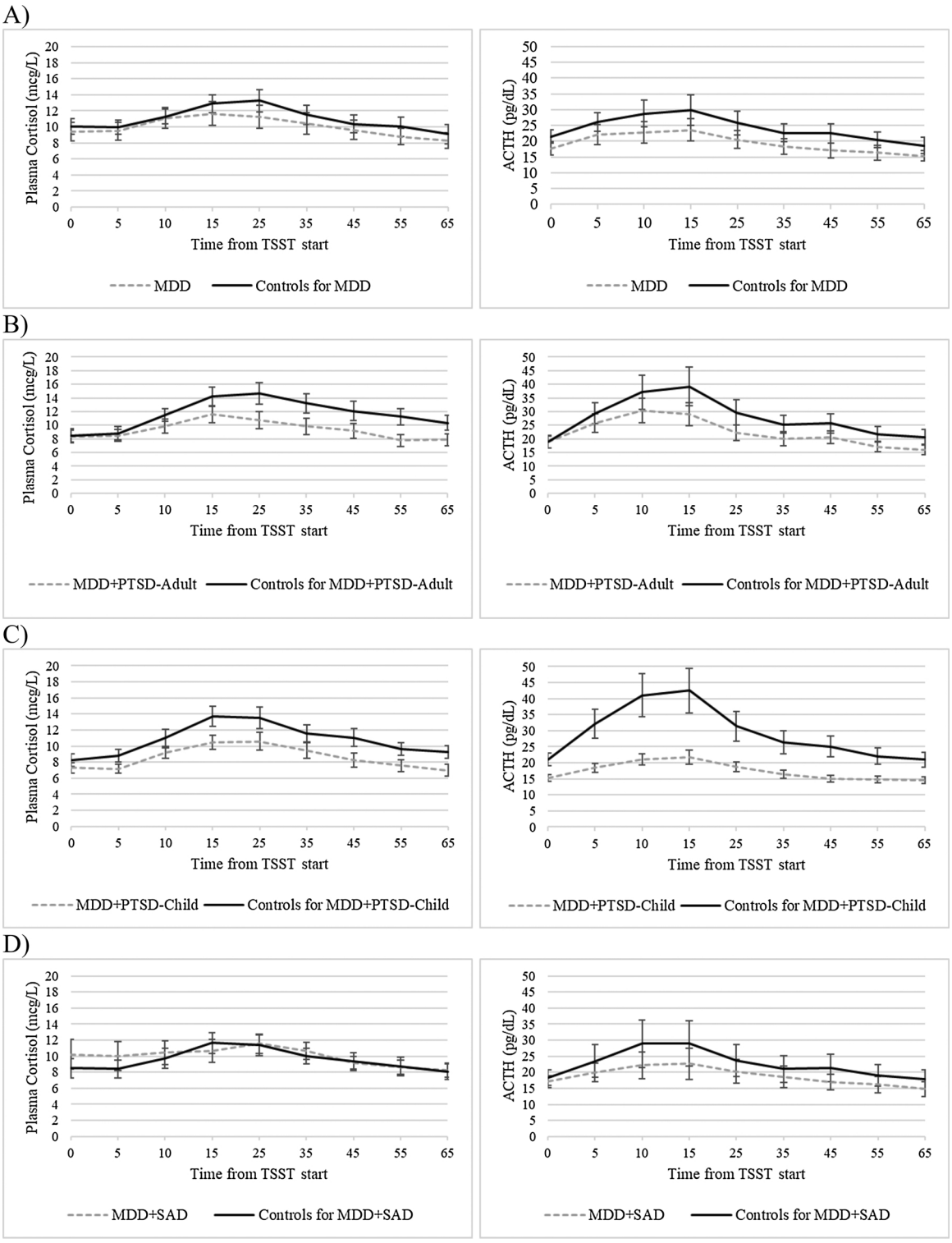


Fig. 1. Mean (± SE) of untransformed plasma cortisol (left panels) and ACTH levels (right panels) to the TSST between patients with MDD (Panel A), MDD + PTSD-Adult (Panel B), MDD + PTSD-Child (Panel C), and MDD + SAD (Panel D) and their non-depressed controls.

$p < .001$). Taken together, order of neuroendocrine challenge tests did not impact hormonal responses to neuroendocrine challenge tests.

3.3. TSST results

3.3.1. Unconditional model

We first modeled plasma cortisol (box-cox transformed) and ACTH (log-transformed) responses to the TSST (unconditional model). The TSST elicited significant changes in hormonal responses over time (Cortisol: $F_{8,712} = 31.86$, $p < .001$; ACTH: $F_{8,712} = 37.84$, $p < .001$). Cortisol rose significantly from 5 to 15 min following the stressor (Bonferroni corrected post-hoc analyses, $ps < .001$) and fell significantly from 25 − 65 min following the stressor ($ps < .05$). ACTH increased significantly from 0 − 10 min post-stressor ($ps < .001$) and declined from 15 − 55 min post-stressor ($ps < .01$).

3.3.2. Effects of patient groups vs. non-depressed controls

Next, we compared each diagnostic patient group to their respective age and sex matched non-depressed controls. Mean and standard error of untransformed hormonal data is presented in Fig. 1.

Patients with **MDD-only** did not differ from their matched controls in baseline hormone levels (Cortisol: $F_{1,23} = 0.54$, $p = .47$; ACTH: $F_{1,23} = 1.70$, $p = .21$) or hormonal responses across the TSST (Cortisol: $F_{8,184} = 0.87$, $p = .54$; ACTH: interaction $F_{8,184} = 0.36$, $p = .94$). To further examine the contributions of MDD versus childhood trauma, we re-ran these models controlling for CTQ scores. Findings did not change when controlling for CTQ scores (Cortisol: group effect on baseline $F_{1, 22} = 0.23$, $p = .64$, CTQ effect on baseline $F_{1, 22} = 0.06$, $p = .81$, group-by-time interaction $F_{8, 176} = 0.69$, $p = .70$, CTQ-by-time interaction: $F_{8, 176} = 0.46$, $p = .88$; ACTH: group effect on baseline $F_{1, 22} = 1.50$, $p = .23$, CTQ effect on baseline $F_{1, 22} = 0.07$, $p = .80$, group-by-time interaction $F_{8, 176} = 0.29$, $p = .97$, CTQ-by-time interaction $F_{8, 176} = 0.35$, $p = .94$).

Similarly, patients with **MDD + PTSD-Adult** did not differ from matched controls in their baseline hormone levels (Cortisol: $F_{1,21} = 2.66$, $p = .12$; ACTH: $F_{1,21} = 1.18$, $p = .29$) or hormonal responses across the TSST (Cortisol: $F_{8,168} = 1.39$, $p = .20$; ACTH: $F_{8,168} = 0.87$, $p = .54$). Given links and complex interactions between adult and childhood stress exposure and their combined impacts, we re-ran these models controlling for CTQ scores. With CTQ added, these models in fact revealed lower cortisol levels at baseline in the MDD + PTSD-Adult patients compared to their matched controls (Cortisol: group effect on baseline $F_{1,20} = 6.22$, $p = .02$, $\eta_p^2 = 0.03$, CTQ effect on baseline $F_{1,20} = 3.96$, $p = .06$, group-by-time interaction $F_{8,160} = 1.60$, $p = .13$, CTQ-by-time interaction $F_{8,160} = 1.57$, $p = .14$; ACTH: group effect on baseline $F_{1, 20} = 2.00$, $p = .17$, CTQ effect on baseline $F_{1, 20} = 0.92$, $p = .35$, group-by-time interaction $F_{8, 160} = 1.75$, $p = .09$, CTQ-by-time interaction $F_{8, 160} = 1.95$, $p = .06$).

MDD + PTSD-Child patients showed reduced cortisol (baseline levels) and ACTH (both baseline values and levels over time) during the TSST relative to matched controls (Cortisol: group effect on baseline $F_{1,30} = 4.36$, $p = .045$, $\eta_p^2 = 0.01$, group-by-time interaction $F_{8,240} = 1.19$, $p = .30$; ACTH: group effect on baseline $F_{1,30} = 11.03$, $p = .002$, $\eta_p^2 = 0.03$, group-by-time interaction $F_{8,240} = 1.98$, $p = .049$, $\eta_p^2 = 0.05$). Posthoc analyses (Bonferroni corrected for the number of actual comparisons; alpha = .0056) revealed that MDD + PTSD-Child had significantly lower levels of ACTH than matched controls at 5 min ($p = .0011$), 10 min ($p = .0002$), 15 min ($p = .0002$), 25 min ($p = .0047$), and 45 min ($p = .0028$). To further examine the contributions of diagnostic group versus childhood trauma severity, we re-ran these models controlling for CTQ scores. The patient-control group differences in baseline hormone levels and levels over time were no longer significant (Cortisol: group effect on baseline $F_{1,29} = 0.25$, $p = .62$, CTQ effect on baseline $F_{1,29} = 0.85$, $p = .36$, group-by-time interaction $F_{8,232} = 0.92$, $p = .50$, CTQ-by-time interaction $F_{8,232} = 1.21$, $p = .29$; ACTH: group effect on baseline $F_{1,29} = 0.48$, $p = .49$, CTQ effect on baseline $F_{1,29} = 2.62$, $p = .12$, group-by-time interaction $F_{8,232} = 0.81$, $p = .60$, CTQ-by-time interaction $F_{8,232} = 2.17$, $p = .03$).

Patients with **MDD + SAD** did not differ from their matched controls in their baseline hormone levels (Cortisol: $F_{1,21} = 0.07$, $p = .79$; ACTH: group $F_{1,21} = 0.31$, $p = .59$) or hormone levels across the TSST (Cortisol: $F_{8,168} = 1.08$, $p = .38$; ACTH: $F_{8,168} = 0.30$, $p = .96$). The findings did not change when controlling for CTQ (Cortisol: group effect on baseline $F_{1, 20} = 0.12$, $p = .73$, CTQ effect on baseline $F_{1, 20} = 0.05$, $p = .82$, group-by-time interaction $F_{8, 160} = 1.02$, $p = .42$, CTQ-by-time interaction $F_{8, 160} = 1.61$, $p = .13$; ACTH: group effect on baseline $F_{1, 20} = 0.59$, $p = .45$, CTQ effect on baseline $F_{1, 20} = 3.17$, $p = .09$, group-by-time interaction $F_{8, 160} = 0.18$, $p = .99$, CTQ-by-time interaction $F_{8, 160} = 1.03$, $p = .41$).

Post hoc tests showed that all diagnostic patient groups combined did not differ from their combined respective matched controls in their HPA axis response to the TSST (Cortisol: group effect on baseline $F_{1,85} = 1.46$, $p = .23$, group-by-time interaction $F_{8,680} = 0.96$, $p = .47$; ACTH: group effect on baseline $F_{1,85} = 2.76$, $p = .10$, group-by-time interaction $F_{8,680} = 0.67$, $p = .72$). The findings did not change when controlling for CTQ (Cortisol: group effect on baseline $F_{1, 84} = 0.28$, $p = .60$, CTQ effect on baseline $F_{1, 84} = 0.78$, $p = .38$, group-by-time interaction $F_{8, 672} = 0.42$, $p = .91$, CTQ-by-time interaction $F_{8, 672} = 0.60$, $p = .78$; ACTH: group effect on baseline: $F_{1, 84} = 0.24$, $p = .62$, CTQ effect on baseline $F_{1, 84} = 2.74$, $p = .10$, group-by-time interaction $F_{8, 672} = 0.52$, $p = .84$, CTQ-by-time interaction $F_{8, 672} = 1.64$, $p = .11$).

3.3.3. Effects between patient groups

We also compared hormonal TSST responses among specific diagnostic patient groups to examine effects of timing of trauma (by comparing MDD + PTSD-Child vs. MDD + PTSD-Adult) and whether early trauma exposure vs. early onset anxiety is unique in its effects (by comparing MDD + PTSD-Child vs. MDD + SAD). MDD + PTSD-Child patients presented with lower ACTH levels across the TSST compared to MDD + PTSD-Adult patients (ACTH: group effect on baseline $F_{1,27} = 1.02$, $p = .32$, group-by-time interaction $F_{8,216} = 2.44$, $p = .02$, $\eta_p^2 = 0.07$) – with no differences in cortisol levels at baseline or over time (Cortisol: all $ps > .36$). MDD + PTSD-Child and MDD + SAD did not differ in HPA activity during the TSST (all $ps > .18$).

3.4. DST and MET results

3.4.1. DST

There were no significant group differences in the DST (also see Supplemental Fig. 1). MDD-only, MDD + PTSD-Adult, MDD + PTSD-Child, and MDD + SAD did not significantly differ from their respective controls in post-DST values at awakening (all $ps > .20$), at 4PM (all $ps > .20$), or in percent cortisol suppression at awakening (all $ps > .10$) or at 4PM (all $ps > .20$). Similarly, all patient groups combined did not differ from combined controls in post-DST values at awakening ($p > .20$), 4PM ($p > .20$), or in percent cortisol suppression at awakening ($p > .20$) or at 4PM ($p > .20$).

3.4.2. MET

Metyrapone successfully suppressed cortisol production (time $F_{12,1128} = 93.26$, $p < .001$) and enhanced ACTH levels (time $F_{12,1080} = 17.39$, $p < .001$). As expected, Bonferroni corrected post-hoc analyses demonstrated that after metyrapone was administered at 4PM, there was a significant decrease in cortisol from 4−5:30PM ($ps < .001$). Cortisol levels remained suppressed until a second metyrapone administration at 7:30PM, at which time cortisol levels steadily declined until 9PM ($ps < .01$) and remained suppressed until the last sample was collected at 10PM. ACTH levels increased from 5:30−6:30PM ($ps < .02$) and remained elevated until 10PM.

None of the diagnostic patient groups (MDD, MDD + PTSD-Adult, MDD + PTSD-Child, and MDD + SAD) significantly differed from their respective matched controls in baseline cortisol levels (all $ps > .41$) or cortisol levels following metyrapone administration (all $ps > .43$), confirming that metyrapone successfully suppressed cortisol in all groups, as expected. Notably, each diagnostic group vs. their respective matched control group as well as all patients vs. combined controls did not significantly differ in ACTH (baseline levels and levels over time) in response to the metyrapone challenge (all $ps > .20$; also see Supplemental Figure 2).

3.5. Role of childhood maltreatment subtypes

We then conducted post hoc analyses testing the role of childhood maltreatment subtypes in the response of the HPA axis to our challenges, controlling for diagnosis-related symptom severity (see Table 2 and Supplemental Figure 3). HPA axis reactivity to the TSST was amplified by exposure to emotional neglect in childhood, such that greater

Table 2
Within-subject effects of childhood maltreatment subtypes on neuroendocrine responses to the Trier Social Stress Test (TSST), the dexamethasone suppression test (DST), and the metyrapone challenge (MET) on ACTH and cortisol over time.

	TSST		DST	MET
	ACTH *F*	Cortisol *F*	Cortisol *F*	ACTH *F*
Time	0.71	2.39*	1.19	9.68***
Female	1.60	0.45	0.13	0.78
Age	0.21	0.76	0.45	1.31
BMI	1.42	2.79**	0.68	2.82***
Depressive symptoms (BSI)	1.33	1.39	0.28	0.30
Anxiety symptoms (BSI)	0.72	0.27	0.14	0.39
Emotional abuse	1.08	1.63	0.64	0.99
Emotional neglect	2.16*	2.84**	0.78	2.54**
Physical neglect	1.07	0.46	1.53	0.87
Physical abuse	0.27	0.22	0.52	2.70***
Sexual abuse	1.13	0.30	1.34	0.18

Note: ***$p < .001$, **$p < .01$, *$p < .05$, +$p < .10$.

emotional neglect was associated with a steeper slope of increase in ACTH ($d = 0.30$, $F_{8,72} = 2.16$, $p = .029$) and a steeper slope of increase in cortisol ($d = 0.34$, $F_{8,72} = 2.84$, $p = .004$) following stressor-exposure in the TSST. No other maltreatment subtypes were associated with differences in ACTH or cortisol responses in the TSST (all $ps > .11$). Emotional neglect was also significantly associated with an attenuated increase in ACTH following metyrapone administration ($d = 0.32$, $F_{8,72} = 2.54$, $p = .003$). In contrast, physical abuse exposure was associated with larger increases in ACTH following metyrapone administration ($d = 0.33$, $F_{8,72} = 2.70$, $p = .001$). No other maltreatment subtypes were associated with differences in ACTH or cortisol responses to metyrapone administration (all $ps > .19$). No maltreatment subtypes were associated with the cortisol response to the DST. It may be notable that individuals with a sexual abuse history in childhood exhibited greater suppression of cortisol following dexamethasone administration ($d = 0.64$, $F_{8,72} = 9.79$, $p = .002$), which was no longer significant when controlling for anxiety symptom severity.

4. Discussion

This study examined the impact of co-morbidity, trauma exposure, and trauma timing on HPA axis patterns in depression. We compared HPA axis responses to three neuroendocrine challenge tests (TSST: stress reactivity; DST: negative feedback sensitivity; MET: central drive) between non-depressed controls and patient groups who either had MDD-only, MDD comorbid with PTSD from adulthood trauma (MDD + PTSD-Adult), MDD comorbid with PTSD from childhood trauma (MDD + PTSD-Child), or MDD comorbid with an early onset social anxiety disorder (MDD + SAD). We failed to replicate prior evidence of increased stress reactivity (TSST response), increased feedback sensitivity (escape from dexamethasone suppression), or increased central drive (increased ACTH response to metyrapone) in our carefully selected MDD-only group (Burke et al., 2005; Carroll et al., 1981; Nestler et al., 2002; Pariante and Lightman, 2008; Young et al., 1994). This could be due to small samples sizes, though current results were consistent with our own previous evidence of normal HPA responses to the TSST in patients with depression (Young, Abelson, et al., 2004; Young et al., 2000) and with work by others (Mielock et al., 2017; Wichmann et al., 2017). We were also unable to replicate the previously reported neuroendocrine alterations using our full sample of depressed patients (n = 56) with mixed comorbidity and varied trauma histories, but all with MDD. However, when we separately examined comorbidity and trauma exposure/timing in planned analyses, some intriguing group differences relative to their respective controls were evident in the context of the psychosocial stress activation paradigm (TSST).

Most importantly, reduced HPA axis activity (blunted baseline levels and reduced ACTH responses) was seen in the context of the stress paradigm in depressed patients with comorbid PTSD from childhood trauma (MDD + PTSD-Child). This supports the growing impression that early life trauma can lead to diminished reactivity to psychosocial stress, as supported by recent meta-analytical evidence (n = 4292; Bunea et al., 2017), even in the presence of MDD (Mielock et al., 2017), creating a "PTSD-like" neuroendocrine profile in patients with MDD-PTSD comorbidity (Oquendo et al., 2003). The attribution of reduced HPA activity in the comorbid group to early trauma exposure was supported by the fact that the diagnostic group differences disappeared when CTQ scores were added as a covariate and, while the group effect for ACTH disappeared, a significant CTQ-by-time interaction emerged when CTQ scores were added to the model. The explainable variance appeared primarily attributable to the childhood trauma scores rather than to diagnostic category. Depressed patients with comorbid PTSD from childhood trauma also had significantly reduced ACTH responses to the TSST relative to depressed patients with comorbid PTSD from adult trauma. This provides additional evidence that early trauma exposure exerts unique effects, highlighting the fact that timing of trauma exposure "matters" to the HPA axis. Overall, these data highlight the idea that diagnostic categories are less salient to this system's functioning in adulthood and that, to some degree, prior HPA axis work – focused on HPA "abnormalities" in each DSM diagnosis – were perhaps somewhat misguided. It also supports a dimensional approach, as suggested by Research Domain Criteria (RDoC), and incorporation of a neurodevelopmental perspective – with its concept of sensitive periods – within that framework (Casey et al., 2014; Mittal and Wakschlag, 2017).

Embracing an RDoC-type perspective, we conducted a post-hoc set of analyses that collapsed across diagnostic groups, accounted for severity of symptoms of anxiety and depression dimensionally, and further dissected childhood maltreatment into subtypes. We found that even dimensionally measured symptoms had little impact on adult HPA axis function, but different childhood maltreatment *subtypes* were associated with unique alterations in HPA axis responses to our challenges. This is consistent with the idea that different types of abnormalities in the child-rearing environment can differentially shape long-term functioning of the axis (Kuhlman et al., 2017). The specific differential effects of trauma subtypes seen here – particularly neglect enhancing HPA responses to psychosocial stress but reducing central drive – need to be replicated in studies specifically designed and adequately powered to more definitely test hypotheses based on these preliminary post hoc results. However, these data suggest that neither diagnosis nor symptom severity seem to drive the HPA axis alterations detected in this study of DSM categorical diagnoses, and that type of trauma exposure, as well as its timing, needs to be taken into account in future studies of the HPA axis. It is useful to add, however, that the CTQ focuses on abuse and neglect perpetrated by caregivers. There is accumulating evidence that many forms of adversity (Kuhlman et al., 2015), such as abuse perpetrated by peers (Knack et al., 2011; Ouellet-Morin et al., 2020) and socioeconomic adversity (Li et al., 2007) are related to alterations in the HPA axis, and warrant further investigation as contributors to the developmental neurobiology of the HPA axis and its role in psychopathology.

The MDD patients with comorbid PTSD from adult trauma (MDD + PTSD-Adult) appeared similar to our MDD-only group, and neither group showed elevated basal levels, increased stress reactivity, escape from dexamethasone suppression, or enhanced central drive relative to their controls. These null findings could be explained by low power to detect effects. However, in the MDD + PTSD-Adult group, when CTQ scores were added to the model (and removed variance linked to trauma exposure from the error term), there was some indication for a possible diagnostic group effect, with lower cortisol levels at baseline in the MDD + PTSD-Adult group. Though this emergence of a group effect is complex to interpret and preliminary, given the small sample size, it

is consistent with prior work showing a "PTSD-like" profile of hypocortisolemia in MDD comorbid with PTSD (Oquendo et al., 2003). It is possible that the comorbidity of PTSD and MDD in the context of adult trauma exposure may also be associated with reduced HPA axis activity. Our data cannot shed light on whether this is due to the adult trauma exposure, the development of PTSD, or is a pre-existing vulnerability factor. However, early trauma exposure is important even in this context, suggesting that it can possibly mask group differences as well as account for others, and must always be measured and analytically addressed in any HPA axis studies of psychopathology and its pathophysiology.

The MDD + SAD comorbid group did not significantly differ from non-depressed control participants in hormonal responses to the TSST, consistent with other research (Yoon and Joormann, 2012), but not our own data (Young et al., 2004a, 2004b). The inclusion of the MDD + SAD group as a partial "control" for MDD comorbid with an "early onset" anxiety disorder (other than PTSD) was of some but limited value. It suggested that the MDD + PTSD-Child group effects were perhaps due to the early trauma leading to PTSD, specifically, and not just the presence of anxiety symptoms more generally during development. However, this conclusion is weak because we did not see significant differences between the MDD + PTSD-Child and the MDD + SAD groups themselves, and only have the fact that the PTSD group was altered relative to its own non-depressed controls, whereas the SAD group was not. Furthermore, the type of trauma/stress differs across the PTSD-Child and SAD comorbid groups, and we do not have information about potentially differential timing. We have already seen that the nature of developmental challenges and their timing may be important and will require more detailed attention in future studies.

Limitations and strengths. The small cell sizes when comorbid groups were analyzed separately is a major limitation. Our failure to replicate previously reported MDD abnormalities in feedback sensitivity and central drive (Carroll et al., 1981; Young et al., 1994) could well be at least partly due to the resultant lack of power. Our failure to replicate previous meta-analytic findings of compensatory changes in feedback sensitivity (DST) in depressed patients with PTSD (Morris et al., 2012) could also be due to the small groups. Anecdotally, the MDD + PTSD-Adult group was the one most difficult to recruit as MDD that is comorbid with adult onset PTSD in the absence of a DSM-IV qualifying traumatic event in childhood may be uncommon. The MDD + PTSD-Child group, which produced the most interesting findings, did have the largest sample size, though it was still small. Conclusions about subgroups, especially negative findings, must be interpreted with caution. Due to the small numbers, we also could not examine subtypes of depression, like "melancholia," which may be more likely to show hypercortisolemia and DST escape (Juruena et al., 2018; Lamers et al., 2013). Depression severity was also limited in our sample. The lack of power due to small group sizes was partially a consequence of and perhaps somewhat mitigated by the careful selection process and strict entry criteria that included extensive medication and other exclusions. This resulted in patient groups that were "clean" and clinically distinct, as confirmed by psychometric assessment (see Section 3.1, Sample Characteristics).

Despite weaknesses, we did achieve the primary aim of the study, which was to examine the presence and timing of trauma exposure and determine whether that helped explain the complex findings in the HPA axis literature on MDD, PTSD and their comorbidity. Using carefully selected patient groups, matched non-depressed control samples, and established neuroendocrine challenge paradigms under controlled laboratory conditions, we added useful support to growing evidence that early trauma is likely critically shaping adult HPA axis activity. At least some of the findings once attributed to psychopathology are likely due to exposure to childhood trauma, regardless of symptoms. The presence of early trauma exposure in MDD-PTSD comorbidity likely accounts for the "PTSD-like" pattern of reduced HPA axis seen in this group. Our data also suggest that the *type* of early life trauma also contributes to the specific type of HPA axis dysregulation seen in adulthood. In other words, type of trauma, as well as its timing, needs to be taken into account in future work.

Conclusions and future implications. There is little surprise, from this vantage point, that the history of HPA axis research in psychiatry has been so muddled by inconsistencies. The data presented here suggest that those inconsistencies may be partly due to complex interactions between comorbidity, exposure to traumatic events, and the timing and type of those exposures. This is consistent with emerging models of pathophysiology that support polygenic risk and resilience factors, likely involving distinct genes that contribute to risk or resilience to traumatic exposures and perhaps distinct genes that shape symptom expression. The nature and timing of traumatic/stress exposures, as well as the genetic "milieu" in which they occur, probably shape the emergence of adult psychopathology and its particular symptomatic expression. This likely occurs via epigenetic mechanisms involving altered regulatory control of the HPA axis and associated changes in hippocampal-prefrontal circuits where glucocorticoid receptors are densely distributed (Baumeister et al., 2014; Papst and Binder, 2020; Pariante and Lightman, 2008). Genetic variations of the FK506-binding protein 51 (FKBP5/FKBP51) gene, CRH receptor gene, the serotonin transporter gene, and the dopamine receptor gene are of particular interest given their documented interaction with early environmental factors to shape both adult stress reactivity and risk or resilience to a range of psychiatric symptoms (Binder et al., 2008; Hornung and Heim, 2014; King et al., 2016; Tamman et al., 2019; Tyrka et al., 2009; Zaba et al., 2015; Zannas et al., 2016). At this point, it seems clear that the HPA axis is important in the pathophysiology of at least some of our disorders, and ongoing study of it is warranted. It remains unclear though, when it is "just" *marking* brain changes occurring in the context of gene-environment interactions and when it is *shaping* the brain changes that ultimately contribute to symptom expression, in the context of a given genetic milieu and the epigenetic processes generated by the developmental experiences (and their specific timing) to which that genetic milieu is exposed.

The data presented here suggest that comorbidity and childhood trauma may contribute to inconsistencies in the HPA-depression literature, and that all future HPA axis research must include measures of early life stress and trauma. The role of the HPA axis in depression and other psychiatric disorders will likely remain confused and confusing until we can measure both the developmental factors that shape adult functioning of the system and the genetics variants that interact with those factors to create psychopathological risk.

5. Contributors

Stefanie E. Mayer led initial data analyses, wrote the first draft of the manuscript and was instrumental in editing the manuscript. Melissa Peckins and Kate R. Kuhlman conducted final data analyses, wrote the results section, and edited the manuscript. Nirmala Rajaram oversaw hormonal assays and edited the manuscript. Nestor L. Lopez-Duran provided critical guidance and input for data analysis and interpretation, as well as manuscript editing. Elizabeth A. Young designed the study, obtained NIH funding, and oversaw recruitment and data collection until she passed away in 2009. James L. Abelson was a co-investigator on the original grant and assumed the principal investigator role after Dr. Young`s untimely death. He completed participant recruitment and data collection, gave instrumental input throughout data analysis and interpretation, and substantially contributed to manuscript editing. All authors contributed to and have approved the final manuscript.

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Declaration of Competing Interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2020.104776.

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