The relationship between the cortisol awakening response and cortisol reactivity to a laboratory stressor

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Abstract

Objectives. The cortisol awakening response (CAR) and cortisol reactivity to an acute laboratory stressor both involve steep increases in cortisol secretion, are associated with preparing the body to deal with stressors ahead, and alterations in both have been linked to negative clinical and health outcomes. However, these two aspects of our biological stress response have rarely been directly compared, and the extant research focuses on or state, rather than trait CAR. Given the similar roles of the CAR and cortisol reactivity, and their relationship to psychopathology, it is important to understand if trait CAR and cortisol reactivity to acute stressors are related, and whether a blunted CAR may be predictive of blunted cortisol reactivity across an acute laboratory stress task.

Design. Cross-sectional. Participants completed the Trier Social Stress Test (TSST) the week after daily assessment of the CAR.

Methods. Salivary cortisol secretion across the TSST was compared to the CAR, sampled across 5 weekdays at waking (S1) and 30 minutes past waking, for 54 female participants.

Results. A smaller CAR, lower peak cortisol, and blunted CAR increase were all significantly related to a steep rise and flattened slope of recovery in cortisol secretion following the TSST. Additionally, lower S1 was predictive of a blunted rise in cortisol secretion from baseline to immediately post-task.

Conclusion. There was a significant relationship between trait CAR and cortisol secretion across the TSST. The results provided mixed support for hypotheses. A blunted CAR was associated with impaired recovery in cortisol secretion following the TSST, but, surprisingly, a rapid rise in cortisol peaking immediately following the stress task. .

The Relationship Between the Cortisol Awakening Response and Cortisol Reactivity to an Acute Laboratory Stressor

Two important facets of the functioning of the Hypothalamic Pituitary Adrenal (HPA) axis, a main part of our biological stress response system, include a sharp peak in cortisol secretion immediately after waking (Cortisol Awakening Response: CAR), and cortisol reactivity to acute environmental or laboratory stimuli. Both the CAR and cortisol reactivity involve elevations in salivary cortisol secretion, but due to their biological differences and a tendency in the literature to focus on distinct aspects of HPA axis functioning, they have rarely been compared directly (Kidd, Carvalho, & Steptoe, 2014; Kudielka & Wüst, 2010; Wetherell, Lovell, & Smith, 2015). Both the CAR and cortisol reactivity have been linked to anticipation of stressors, either of the day ahead or a stressful event, and marshaling resources to deal with stressors (Adam, Hawkley, Kudielka, & Cacioppo, 2006; Powell & Schlotz, 2012; Wetherell et al., 2015). Additionally, both have been related to psychosocial, clinical and health outcomes (Adam et al., 2010; Dienes, Hazel, & Hammen, 2013). Finally, the CAR has been linked to naturalistic stress reactivity on both the day prior and day of waking (Doane & Adam, 2010; Gartland, O'Connor, Lawton, & Bristow, 2014; Rohleder, Beulen, Chen, Wolf, & Kirschbaum, 2007; Stalder, Evans, Hucklebridge, & Clow, 2010). However, trait CAR has not been examined in relation to cortisol secretion (specifically rise and recovery) across an acute laboratory stress task. Both trait CAR and recovery in cortisol following acute laboratory stress tasks have been linked to major depression (Adam et al., 2010; Burke, Davis, Otte, & Mohr, 2005; Dienes et al., 2013; Vrshek-Schallhorn et al., 2013). Therefore, an examination of their relationship may aid our understanding of biological stress processes and psychopathology.

The CAR is a spike in cortisol secretion of 50-156%, distinct from the diurnal cortisol rhythm, that occurs approximately 30-45 minutes after waking (Clow, Thorn, Evans, & Hucklebridge, 2004; Stalder et al., 2016). The exact nature of the CAR, the purposes it serves for the body and what variability in the CAR

means, has received increasing attention within the past 10 years, but remains unclear (Stalder et al., 2016). The CAR is under the control of not only the HPA axis, but also the superchiasmatic nucleus (SCN) (Clow et al., 2004). During the transition from sleep to conscious awakening, sub-cortical areas of the brain undergo processes of swift deactivation, while neocortical networks are stimulated (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010). Researchers have suggested this process of neo-cortical arousal is implicated in the stimulation of memory representations, awareness of life conditions, and anticipation of life demands, which in turn, stimulate activity in the HPA axis (Mikolajczak et al., 2010; Wilhelm, Born, Kudielka, Schlotz, & Wüst, 2007).

Researchers have proposed that the CAR involves reactivity to the stress of waking (Wilhelm et al., 2007) or that it marshals the body's resources to deal with the stress of the day (Chida & Steptoe, 2009; Fries, Dettenborn, & Kirschbaum, 2009). The later hypothesis has received support given the CARs relationship to memory, awareness and anticipation, and its relationship to daily mood and acute naturalistic stressors (Doane & Adam, 2010; Gartland et al., 2014; Rohleder et al., 2007; Stalder et al., 2010). According to Powell and Schlotz (2012), this *CAR anticipation hypothesis*, (the "boost hypothesis") (Adam et al., 2006), posits that the CAR is adaptive to the extent that it provides the energy and resources needed to meet immediate daily demands. However, research regarding whether a larger CAR is related to healthy daily responding, and whether it has a stronger relationship to prior day mood and stress remains mixed.

Biological and cognitive resources are also marshaled through HPA-axis activation in response to acute threat from the environment, or acute stress reactivity. One of the most reliable and widely used acute psychosocial laboratory stress tasks is the Trier Social Stress Test (TSST: Kirschbaum et al., 1993). The TSST is a social evaluative stress task that involves speaking and performing a difficult math task in front

of a panel (see Methods). The TSST leads to 2-3 fold elevations in cortisol secretion from baseline in 70-85% of participants and had been administered in thousands of sessions worldwide (Allen et al., 2017; Kudielka, Hellhammer, & Kirschbaum, 2007; Kudielka & Wüst, 2010). A sharp spike in salivary cortisol peaking approximately 10-30 minutes post stressor, followed by a gradual recovery, is the hallmark pattern for cortisol reactivity (Allen et al., 2017). Cortisol elevations directly influence alertness, heart rate, and metabolic production and contribute to a shutdown of non-essential systems such as the immune system and reproduction in the short term to deal with the stressor (McEwen & Wingfield, 2003). Cortisol elevations in response to an acute laboratory stress task, such as the TSST, may therefore be related to cortisol elevations in preparation for the stress of the day (CAR).

Four studies directly examined the relationship between state CAR and cortisol reactivity to an acute laboratory stress task. Schmidt-Reinwald and colleagues (1999) reported no correlation between the CAR and the TSST (assessed on the same day) in 22 young adults. Kidd et al. (2014) reported greater cortisol reactivity (to behavioral laboratory stressors) significantly predicted greater diurnal cortisol secretion, but irrespective of waking levels and the CAR (assessed on one day the following week) in 446 men and women. In keeping with predictions of a relationship between the CAR and acute cortisol reactivity, Quirin and colleagues (2008) found a negative correlation between the CAR (assessed across two days within two weeks of the stress test) and cortisol reactivity to an aversive, uncontrollable noise stimulus. Most relevant to the current study, Wetherell et al., (2015) compared diurnal cortisol secretion, including the CAR, on a day where participants completed the TSST, to diurnal cortisol secretion on an average day, in 23 young adults. They reported a significantly greater CAR on the day when the TSST was anticipated.

As can be seen from this limited picture, few studies have directly compared state CAR and acute stress reactivity, fewer still have examined state CAR in relation to cortisol secretion across the TSST, and no studies have compared trait CAR and cortisol secretion across the TSST. Quirin et al., (2008) and Kidd et al. (2014) used uncontrollable noise and behavioral stressors, which do not combine the two factors shown to elicit the strongest cortisol response, social evaluation and uncontrollability (Dickerson & Kemeny, 2004). The two studies that have examined the relationship between the CAR and the TSST did so on the same day (Schmidt-Reinwald et al., 1999; Wetherell et al., 2015), which does not allow for an examination of the relationship of trait CAR to the TSST, and may lead to anticipation effects which may alter both the CAR and cortisol reactivity (Wetherell et al., 2015). Hellhammer and colleagues (2007) reported that single day CAR is determined by state factors, whereas two to six days of CAR sampling, including weekdays, is needed to establish trait CAR. Although the CAR has been linked more strongly to state variables than trait variables (Doane, Chen, Sladek, Van Lenten, & Granger, 2015), both trait CAR and altered cortisol secretion across the TSST have been associated with trait variables such as psychopathology (depression,) and conscientiousness (Adam et al., 2010; Dienes et al., 2013; Gartland et al., 2014; Vrshek-Schallhorn et al., 2013). Additionally, the extant research on the relationship between the CAR and TSST uses summary measures of cortisol secretion across the TSST. Recovery in cortisol secretion following the TSST has been linked to depression (Burke et al., 2005) and therefore examining rise and recovery separately may be valuable.

The current study compares the CAR (across 5 days of sampling) to rise and recovery in cortisol secretion across the TSST predicting that trait CAR and cortisol reactivity to the TSST will be related based on their similar functionality in preparing the body to deal with stress. Specifically, blunted cortisol secretion in response to the TSST (smaller rise and flattened slope of recovery) will be associated with blunted trait CAR, because individuals who typically secrete less cortisol in the morning may not be able to secrete as

much cortisol in response to acute stressors. This hypothesis is in keeping with the boost and CAR anticipation hypotheses which state that a larger CAR provides resources to meet the demands of the day ahead (Adam et al., 2006; Powell & Schlotz, 2012). Comparison of trait CAR and acute cortisol reactivity may increase our understanding of how both relate to psychosocial variables such as mood and stress, and clinical and health outcomes.

Methods

Sample

Participants were 57 women aged 17 to 23 (M=18.60, SD=0.90) recruited from the undergraduate subject pool at a large research university in the USA over one academic year. The sample was ethnically representative of the student population (see Table 1). Seventy-seven (26.3%) of the 293 participants contacted about the study did not meet exclusion criteria (regular smoking, regular stimulant medication use, current pregnancy, current anxiety disorder, any serious medical condition) and agreed to participate. Detailed clinical screening was conducted and 57 participants met inclusion criteria (no generalized anxiety disorder, dysthymic disorder, panic disorder, obsessive-compulsive disorder, psychotic symptoms, psychoactive substance use, post-traumatic stress disorder, anorexia, or bulimia nervosa). Fifteen participants met DSM-IV diagnostic criteria for a current major depressive episode. Three participants did not complete the TSST and were excluded from analyses (final N=54). These three participants did not differ on any demographic variables.

Measures

A phone interview was conducted and eligible participants were invited for an initial interview. The Beck Depression Inventory-II (BDI-II, Beck et al., 1996) and modified Structured Clinical Interview for DSM-IV (SCID: First et al., 1995) were administered by a master's level graduate student with 7 years of

experience in SCID administration. The BDI-II consists of 21 items rated on a 4-point scale from 0 to 3 with a score of 14-63 indicating depression ranging from mild to severe. Internal consistency has been measured at .93 and test-retest reliability over 1 week at .93 (Beck et al., 1996). The SCID is a commonly used semi-structured diagnostic interview with moderate to excellent inter-rater reliability (kappas ranged from .60-.83) (Lobbestael, Leurgans, & Arntz, 2011). The SCID was modified to focus on mood and anxiety disorders due to the emphasis of the original study.

Chronic stress and early adversity were assessed and controlled for because of their relationship to both cortisol and depression (Adam et al., 2010; Heim et al., 2000; Miller, Chen, & Zhou, 2007). Chronic stress was assessed using the UCLA Life Stress Interview (LSI; Hammen et al., 1987) administered by three trained masters-level graduate students. The LSI is modelled after the contextual threat procedures of Brown & Harris (1989) and involves assessment of ongoing stressful life events over the past six months in multiple domains. Standard probes (e.g. Close Friend: "Do you have a best friend? Who would that be? How has this relationship been going?") are followed by queries and behavioural probes on stability, proximity, conflict etc. Interpersonal domains (close friendships, social life, romantic relationships, and family relationships) were queried in detail in the current study due to their relationship to depression. Non-interpersonal domains (financial independence, work, individual health, family health, school and neighbourhood) were combined to form a questionnaire. Interviewers used specific anchors to objectively rate severity of chronic stress from 1 to 5 in each domain. Domains were summed to calculate a total chronic stress score. Intra-class correlations for independent judges ranged from .82 to .91 in past research (Davila, Hammen, Burge, Paley, & Daley, 1995). Stability and convergent validity have also been demonstrated (Daley, Hammen, & Rao, 2000; Hammen, Kim, Eberhart, & Brennan, 2009).

Early adversity was assessed using the Early Adversity Questionnaire (EAQ: Cohen et al., 2004). The EAQ is a semi-structured interview, designed to assess occurrence of adverse events in multiple domains prior to age 13 (separation and loss involving the primary caretaker(s), significant loss involving non-caretaker (i.e., sibling or close friend), death and life-threatening illness or injury to self or others, physical neglect, emotional abuse or assault, physical abuse or assault, witnessing violence, sexual abuse or assault and peer victimization). Presence/absence of each domain was indicated, with a brief written description of the event if present. The interviewer rated event severity on a 5-point scale (1=no adversity to 5=extreme impact). Adversity was considered present if severity was rated as 2 or higher on seven of the eight scales (or 4 or higher on the peer victimization scale). Intraclass correlations for the severity ratings ranged from .63 to 1.00 with a mean correlation of .86. A total adversity score was calculated by summing the number of domains where adversity was present.

Biobehavioural questionnaires. Participants reported any regular medication use, current oral contraceptive use, and date of their last menstrual period. Each participant was instructed not to drink or eat anything containing sugar or caffeine and to avoid brushing teeth and significant physical activity for one hour before sampling (TSST and CAR) as these factors may influence cortisol levels (Kudielka et al., 2007).

Cortisol Awakening Response. The CAR was measured across 5 weekdays of salivary cortisol sampling at awakening (before getting out of bed) and 30 min post-waking to capture peak secretion using Salivettes (Sarstedt Rommelsdorf INC, Germany). Diurnal cortisol was also collected at 8 and 11 hours post-waking but levels were not included in current analyses. Participants were instructed to record time of sampling. They were also asked to record time of waking, hours slept, and quality of sleep (1=very badly; 4=very well) on each sampling day. Electronic Medication Electronic Monitoring System (MEMS)

caps were given to a randomly selected 19 of the 54 participants (35.2%). MEMS caps monitor time and date that the bottle was opened to check reporting accuracy (to encourage compliance, all participants were told there was a chance they would be monitored, as suggested by Adam & Kumari, (2009)). Data was downloaded using MEMS software (MEMS View, version 161; Aprex Corporation). Six days of MEMS time-stamp data were unavailable due to reported error in sampling or failure of data capture from the MEMS caps. 178 of a possible 190 data points were available for analysis. 90.7% of MEMS-based intervals were within seven minutes of the self-reported interval. The mean difference for all 178 MEMS recorded samples from self-reported time of sampling was 5.98 minutes (SD=16.5) and for the 89 S1 (waking) samples, 5.62 minutes (SD=15.72). Upon comparison of MEMS S1 time with self-reported time of waking, one individual failed to comply with the CAR sampling protocol on three days, which were removed from further analysis.

The Trier Social Stress Test. Participants came to the lab on a weekday afternoon one week after CAR sampling between 16:00 and 18:00 hours to complete the TSST. Participants were told that they must deliver a five-minute impromptu speech as if to a judge and jury in response to being accused of shoplifting following a five-minute preparation period. They were then instructed to count backwards by 13 as quickly as possible, starting at 6233 for five minutes. If the participant gave an incorrect answer, they were corrected and told to start over. The experimenter explained that the audience were trained in behaviour analysis and would take notes, and that the participant would be video- and audio-recorded for verbal and non-verbal behaviour coding. Saliva samples were taken at baseline, immediately post-task, and 10, 25 and 40 minutes after the task. The current protocol differs from the original TSST, which involved a ‘‘job interview’’ before the ‘‘selection committee”. The shoplifting paradigm was first used by al’Absi et al., (1997). Buchanan, Bagley, Stansfield, & Preston (2012) tested both paradigms and found that the shoplifting paradigm elicited the greatest change in cortisol

secretion. The shoplifting paradigm also incorporates both social evaluative threat and uncontrollability components, which meta-analysis has shown to be needed to reliably elicit a cortisol response (Dickerson & Kemeny (2004).

Sample storage. Salivettes from CAR sampling were stored in a refrigerator and returned to the lab 2 days after sampling was completed, then placed in a freezer (-20 C) with the TSST samples. Salivettes were sent to Trier, Germany packed in dry ice to be assayed for cortisol. Assays were conducted using time-resolved immunoassay with fluorescence detection (DELFIA; Dressendorfer et al., 1992). Intra- and interassay coefficients of variance have been reported at below 12% for the lab.

Data Analysis Plan

A total of 5 days of CAR data for each of 54 individuals was collected for a possible 270 days. Three days of sampling were eliminated due to one individual who failed to comply with CAR sampling procedure (see MEMS section) and one day (two cortisol samples) to lack of cortisol data upon assay. Additionally, one individual was missing baseline and final cortisol across the TSST upon assay. Therefore, a total of 266 sampling days and 53 individuals were included in the final analyses.

Four CAR variables: S1, Peak, CAR Difference, and CAR Slope were entered as outcome variables in separate analyses. These variables were chosen based on expert recommendation for CAR assessment using two time points (Adam & Kumari, 2009; Stalder et al., 2016). The S1 variable often has an inverse relationship to the CAR and should be analysed separately or controlled for (Clow et al., 2010), and

measurements for the CAR should include “the dynamic” of post-wakening cortisol changes such as baseline-to-peak increase (CAR Difference). Peak and CAR Slope were also included to look at the highest point of secretion and change over time.

The TSST variables included two area under the curve variables (AUC); AUC with respect to ground or 0 (AUC_g) and AUC increase (AUC_i: most commonly used in TSST research), which measures change from baseline (Kudielka et al., 2007). Rise (from baseline to 10 minutes post task) and recovery (from 10 to 40 minutes) are the most commonly examined slopes in TSST research (Foley & Kirschbaum et al., 2010). However, our research has indicated that the peak in cortisol secretion can happen earlier, immediately following the task, possibly in response to anticipation of the experiment (Dienes et al., 2013). We have called these variables Rise Anticipation (baseline to post-task) and Recovery Anticipation (post-task to 40 minutes). The six TSST predictor variables included Rise Anticipation, Rise Task, Recovery Anticipation, and Recovery Task, AUCg, and AUCi.

The level-1 variables were: (1) wake-time and (2) sleep aggregate (the sum of hours of sleep, how well they slept, type of sleep quality) and the level-2 potential covariates were: (1) age, (2) psychological distress aggregate (the sum of total chronic stress + depression symptomatology + anxiety symptomatology + early adversity severity). The aggregate variables were summed as they were highly inter-correlated and when analysed individually produced a similar pattern of results to their aggregate, (3) MEMS, (4) menstrual cycle, and (5) medication (sum of BCP plus any mediation). The level-2 predictors were the 6 TSST parameters. Outcomes were the 4 morning cortisol estimates.

To examine the association between TSST reactive cortisol response and the waking response we ran a series of 2-level random effects MLMs following the modelling procedure recommended by Raudenbush

& Bryk (2002). For each of the 4 morning cortisol estimates we ran: (1) an *intercept-only null model* as the baseline to compare other models, (2) *level-1 only model* to explore how well level-1 variables predicted the outcome and retain any significant level-1 predictors for the final model, (3) *level-2 covariate selection model* to identify any significant level-2 covariates to add to the final model and (4) the *final model (level-1 and level-2).* Using this final model, we then ran 6 models, one for each of the TSST parameters, and included the significant predictors identified in the *level-1 only* and *level-2 covariate selection models*. The difference in deviance was used to explore improvement in model fit in nested models. All level-1 variables are group mean centered (except MEMS) and all level-2 variables grand mean centred. Models were estimated using REML and ran in HLM 7.02. Missing data were *listwise* deleted.

Results

Descriptive statistics for each sampling point for the CAR and TSST are presented in Tables 1 and 2. Descriptive statistics of level-1 and level-2 covariates are presented in Table 1. We ran 6 separate models for each of TSST parameters. The significant effects only are reported in Table 3.

Waking Cortisol (S1)

Intercept only Model. The null model was significant, indicating that waking cortisol differed from zero (β_{00}= 13.74, $SE_{robust=}$0.74, p<.001) and there was significant variance to be explained (χ2 (49)=124.42, p=.001). The ICC was .249 indicating that nearly 25% of the variance in wakening cortisol was attributable to variation at level-2 (Deviance=1643.972 with 2 parameters).

Level-1 only model. For the level-1 when both sleep and wake-time were entered simultaneously only wake-time was significant (β_{00}= 1.12, SE_{robust}=0.55, p=.047). The model with only wake-time included showed a marginal significant effect for wake-time (β_{00}= 1.03, SE_{robus}=0.53, p=.057). However, as this

model showed a significant reduction in deviance (Deviance=1629.592: $\chi 2$ (2)=14.379, p=.001) wake-time was retained.

Level-2 Covariates Selection Model. None of the level-2 covariates were significantly associated with waking cortisol.

Final Model. This model specifies wake-time as a level-1 predictor. The only significant effect was for TSST Rise Anticipation which was positively associated with waking cortisol. A large, rapid response to the TSST was associated with higher waking cortisol as predicted from the boost hypothesis. This model accounted for an additional 18% of the variance over the intercept-only model.

Peak Cortisol

Intercept only Model. The null model was significant showing that peak cortisol differed from zero (β_{00}= 20.01, $SE_{robust=}$1.10, p<.001) and there was significant variance to be explained ($\chi 2$ (49)=193.29, p<.001). The ICC of .392 indicated that just over 39% of the variance in peak cortisol was attributable level-2 (Deviance=1696.7919 with 2 parameters).

Level-1 only model. For the level-1 when both wake-tine and sleep aggregate were entered simultaneously neither were significant (*p*s=.404 & .914 respectively) nor was the reduction in Deviance ($\chi 2$ (5)=9.10, p=.104). Thus, no level-1 variables were retained.

Level-2 Covariates Selection Model. Aggregated medication was significantly associated with peak cortisol (β_{00}= -5.77, $SE_{robust=}$1.53, p<.001: Deviance =1679.164158). Thus higher peak cortisol was associated with taking less medication.

Final Model. This model specifies no level-1 predictor and one level-2 covariate (medication aggregate). TSST Recovery Task was negatively associated with the peak morning cortisol as was the aggregate medication. Again, this finding is consistent with the boost hypothesis in that higher peak cortisol in the

morning was associated with a more negative, or steeper, slope of recovery following the TSST. This model accounted for an additional 12% of the variance over the intercept-only null model.

Diff Cortisol

Intercept only Model. The null model was significant showing that diff cortisol differed from zero (β_{00}= 6.30, $SE_{robust=}$1.26, p<.001) and there was significant variance to be explained ($\chi 2$ (49)=177.38, p<.001). The ICC was .359 indicating that nearly 36% of the variance in diff cortisol was attributable to variables at level-2 (Deviance=1778.303527 with 2 parameters).

Level-1 only model. For the level-1 when both sleep and wake-time were entered simultaneously only wake-time was significant (β_{00}= -1.67, SE_{robust}=0.69, p=.020). The model with only wake-time included showed a significant effect for wake-time (β_{00}= -1.51, SE_{robust}=0.65, p<.025). The model showed a significant reduction in deviance (Deviance=1760.038722: $\chi 2$ (2)=18.26, p=.001), thus wake-time was retained.

Level-2 Covariates Selection Model. Aggregated medication was significantly negatively associated with the diff cortisol response ($\beta_{=}$-4.87, $SE_{robust=}$2.12, p<.001: Deviance=1764.193354).

Final model. This model specifies wake-time as a level-1 predictor and medication aggregate as a level-2 covariate. There were significant negative effects for TSST Rise Anticipation and TSST Recovery Task. Greater morning cortisol secretion was associated with a steeper slope of recovery, as predicted, but a blunted rise in cortisol. The model for TSST Recovery Task accounted for an additional 17% of the variance over the intercept-only null model. The model for TSST Rise Anticipation also accounted for an additional 17% of the variance over the intercept-only null model.

Cortisol Slope

Intercept only Model. The null model was significant showing that cortisol slope differed from zero (β_{00}= 12.54 , $SE_{robust=}$2.49, p<.001) and there was significant variance to be explained (χ2 (49)=182.383 p<.001). The ICC was .369 indicating that nearly 37% of the variance in cortisol slope was attributable to variables at level-2 (Deviance= 2083.469571 with 2 parameters).

Level-1 only model. For the level-1 when both sleep and wake-time were entered simultaneously only wake-time was significant (β_{00}= -2.96, SE_{robust}=1.34, p=.032). The model with only wake-time included showed a significant effect for wake-time (β_{00}= -2.96, SE_{robust}=1.34, p<.032). The model showed a significant reduction in deviance (Deviance=2068.364847: χ2 (2)=15.10, p=.001), thus wake-time was retained.

Level-2 Covariates Selection Model. Aggregated medication was significantly associated with the cortisol slope response ($\beta_{=}$-9.88, $SE_{robust=}$4.09, p<.020: Deviance=2062.531861).

Final model. This model specifies wake-time as a level-1 predictor and medication aggregate as the level-2 covariate. We ran 6 separate models for each of TSST parameters. The significant effects are reported in Table 3. There were significant negative effects for TSST Rise Anticipation and TSST Recovery Task. A steeper rise in morning cortisol was associated with a steeper slope of recovery, as predicted by the boost hypothesis, but a blunted rise in cortisol prior to the TSST. The model for TSST Recovery Task accounted for an additional 13% of the variance over the intercept-only null model. The model for TSST Rise Anticipation also accounted for an additional 13% of the variance over the intercept-only null model.

Discussion

Results indicate that there may be a relationship between trait CAR and cortisol reactivity across the TSST captured by looking separately at rise and recovery in cortisol secretion. Blunted increase and lower peak cortisol in the morning were associated with a flattened slope of recovery in cortisol secretion following the TSST. A flattened slope of recovery following the TSST has been called 'impaired'

recovery and has been associated with major depressive disorder in past research (Burke et al., 2005). Therefore these results are in keeping with the boost, and CAR anticipation, hypotheses (Adam et al., 2006; Powell & Schlotz, 2012). However, greater S1 (waking cortisol), and blunted CAR increase were significantly related to a steep, rapid rise in cortisol from baseline to immediately post-task across the TSST. At first glance these findings seem to contradict the boost hypothesis and require further exploration. The TSST variables accounted for a large portion of the variance in the CAR variables, indicating a strong relationship between the TSST and the CAR.

Blunted trait CAR was associated with an unusual pattern of cortisol secretion across the TSST involving a blunted or impaired slope of recovery, but contrary to prediction a rapid increase in cortisol leading to an early peak immediately post-task (see Figures 1-2). This finding was consistent across three CAR outcome variables with the exception of S1 which makes sense given the inverse relationship between CAR and S1 (Stalder et al., 2016)). The rapid rise in cortisol, only until post-task and not the usual 10 minutes post-task, has rarely been discussed in past literature. It has been called rise in cortisol in response to task anticipation, as it represents HPA axis activation roughly in the time period before the task begins (cortisol takes 10-30 min to appear in saliva)(Dienes et al., 2013). Response to anticipation of stress, rather than the stressor itself, can have a significant impact on the body (O'Donovan et al., 2012) and has been associated with risk for depression (Dienes et al., 2013). It is possible that individuals may marshal a strong initial response, but are unable to maintain the stress response (hence the early peak) which may leave the individual without the biological resources needed to deal with the stressor itself. Therefore, blunted trait CAR would be associated with a "sensitive" stress response and impaired recovery in cortisol secretion.

Quirin et al. (2008) reported a negative correlation between peak morning cortisol) and cortisol immediately post-task in contrast to the results of the current study. Results are consistent with the negative findings of both Schmidt-Reinwald et al. (1999) and Kidd et al. (2014) as there was no relationship between the AUCs of cortisol secretion across the TSST and any of the CAR variables. Finally, the results are partially consistent with the study conducted by Wetherell et al., (2015) who reported that greater peak of cortisol secretion and CAR AUCi were associated on a day on when the TSST was anticipated, in comparison with a typical day. We did not find any relationship with the AUCi across the TSST, but we did find that greater peak CAR was associated with a steep slope of recovery.

The current study has several strengths. It is the first study to examine the relationship of trait CAR to rise and recovery in cortisol secretion separately across an acute laboratory stress task. Methodologically, the CAR was assessed over five days to measure trait CAR. Also MEMS caps were used in a subsample of CAR measurement in accordance with expert consensus (Stalder et al., 2016).

The study also has several limitations. A small sample size may have limited power. Also, generalizability may have been affected by the inclusion of depressed and at-risk individuals. However, depression status did not appear to affect the current results. Further limitations include the lack of a waiting period before TSST baseline, the exclusion of males, and limited age range. A 2-3 fold elevation in cortisol secretion is expected in 75% of people in response to the TSST and we found this elevation in 38.9% of participants (see Table 2) (Allen et al., 2017). This result may be due to the absence of a 20-minute pre-task rest period. Also, females may have a later peak in cortisol secretion post-waking (45 minutes) which may have not been captured by peak sample (Pruessner et al., 1997). The sample size did not allow for assessment of non-responders and responders (20.4% of our sample had 3 or more days without a cortisol increase) as has been suggested in past research (Thorn, Hucklebridge, Evans, &

Clow, 2006). Timing of waking itself is an important factor in cortisol research and objective measurement of wake-time using actigraph watches is ideal (Stalder et al., 2016). Finally, MEMS caps were used for only 35.2% of the sample due to resource limitations.

In conclusion, our findings indicated that there may indeed be a relationship between trait CAR and cortisol reactivity to a psychosocial laboratory stressor. Both patterns of cortisol reactivity involve the marshaling of resources to deal with a stressor, both involve a sharp spike in cortisol secretion and both have been related to psychopathology. The results are in partial support of the boost hypothesis as blunted trait CAR was predicted by impaired recovery in cortisol secretion following the TSST. However, rapid rise in cortisol to immediately post-task also predicted blunted CAR, perhaps due to an overly sensitive response followed by impaired shutdown. This secretory pattern requires additional exploration and explication. Further research is needed, especially longitudinal research, to determine whether the relationship between trait CAR and cortisol reactivity continues over time, whether a breakdown in this relationship may be related to psychopathology, and whether this relationship is affected by psychosocial variables such as daily mood and stress.

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Tables

Table 1. Descriptive statistics of level-1 and level-2 variables.

Level-1 Variables	**Mean (SD)**	**Minimum**	**Maximum**
S1	13.63(8.78)	0.97	49.54
Peak Cortisol	20.15(10.94)	0.72	57.67
CAR Difference	6.48(12.58)	-34.7	52.68
CAR Slope	12.93(24.55)	-67.2	105.4
Hours Slept	6.52(1.76)	1	13
Typical Sleep	4.94(1.78)	0	10
Quality Sleep	2.88(0.67)	1	4
*Wake Time	8.47(1.13)	3.40	14.27
Level-2-variables	**Mean(SD)**	**Minimum**	**Maximum**
Rise Anticipation	12.11(16.11)	-17.05	67.46
Rise Task	9.43(14.34)	-25.35	64.02
Recovery Anticipation	-4.30(6.75)	-23.50	17.58
Recovery Task	-6.49(12.85)	-44.71	41.15
AUCg	647.95(342.79)	157.52	1849.38
AUCi	197.49(302.17)	-425.98	1314.32

Age	18.76(1.05)	17	22
Chronic Stress Index	21.31(3.41)	15	29
Early Adversity Severity	1.57(1.39)	0	6
BDI-II	8.56(7.76)	0	35
Dichotomous Level-2 Variables	**Percent**		
Oral Contraceptive Use	22.2		
Medication Use	9.3		
*MEMS Caps	35.2		
Menstrual Cycle (follicular)	42.6		
Ethnicity			
Asian	33.3		
Latino	24.1		
Black	18.5		
Indian	1.9		
Middle Eastern	7.4		
Pacific Islander	3.7		
Other	5.6		

* significantly affected CAR outcome variables included in the final models

Table 2. Descriptive statistics at each sampling point for the TSST

	Mean (SD)	**Minimum**	**Maximum**
Baseline	7.07(4.17)	2.22	20.58
Post-task	11.4(7.03)	2.39	30.39
10 min	12.2(8.43)	2.75	45.44
25 min	10.5(7.09)	1.72	37.24
40 min	8.82(5.38)	1.74	25.09

Table 3. Models showing the relationships between TSST variables and CAR variables (separate models run for each TSST variable)

	Coefficient	***Se (Robust)***	***p***
Outcome: *Waking Cortisol (S1)*			
Intercept π_0			
Waking Cortisol β_{00}	13.74	0.75	< .001
TSST Rise Anticipation β_{01}	0.14	0.04	< .001
Slope π_1			
Wake Time β_{10}	1.04	0.52	= .055
Outcome: *Peak Cortisol*			
Intercept π_0			
Peak Cortisol β_{00}	20.02	0.92	< .001
TSST Recovery Task β_{01}	-0.18	0.05	= .001
Medication Aggregate β_{02}	-4.72	1.27	< .001
Outcome: *CAR Difference*			
Intercept π_0			
Diff Cortisol β_{00}	6.28	1.12	< .001

TSST Rise Anticipation β_{01}	-0.22	0.07	= .002
Medication Aggregate β_{02}	-4.90	1.85	= .011
Slope π_1			
Wake Time β_{10}	-1.50	0.65	=.025
Outcome: *CAR Difference*			
Intercept π_0			
Diff Cortisol β_{00}	6.28	1.11	< .001
TSST Recovery Task β_{01}	-0.22	0.09	= .015
Medication Aggregate β_{02}	-3.29	1.59	= .044
Slope π_1			
Wake Time β_{10}	-1.54	0.65	=.023
Outcome: *CAR Slope*			
Intercept π_0			
Cortisol Slope β_{00}	12.49	2.15	<.001
TSST Rise Anticipation β_{01}	-0.40	0.12	= .002
Medication Aggregate β_{02}	-10.07	3.87	= .012
Slope π_1			
Wake Time β_{10}	-2.57	`1.19	= .037
Outcome: *CAR Slope*			
Intercept π_0			
Cortisol Slope β_{00}	12.51	2.18	<.001
TSST Recovery Task β_{01}	-0.42	0.16	= .013
Medication Aggregate β_{02}	-6.83	3.05	= .030
Slope π_1			
Wake Time β_{10}	-2.73	1.21	= .028

Figures

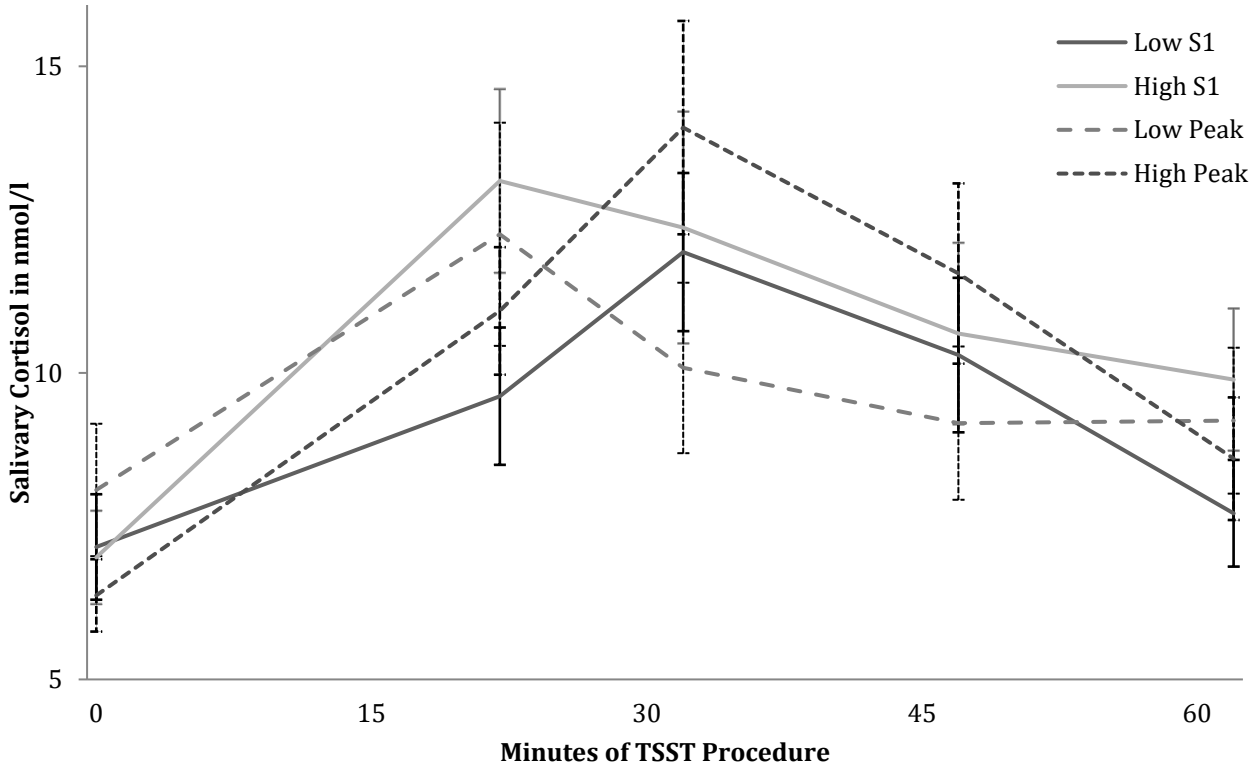

Figure 1. Mean split of S1 and Peak cortisol secretion across 5 days at each sampling point across the Trier Social Stress Test.

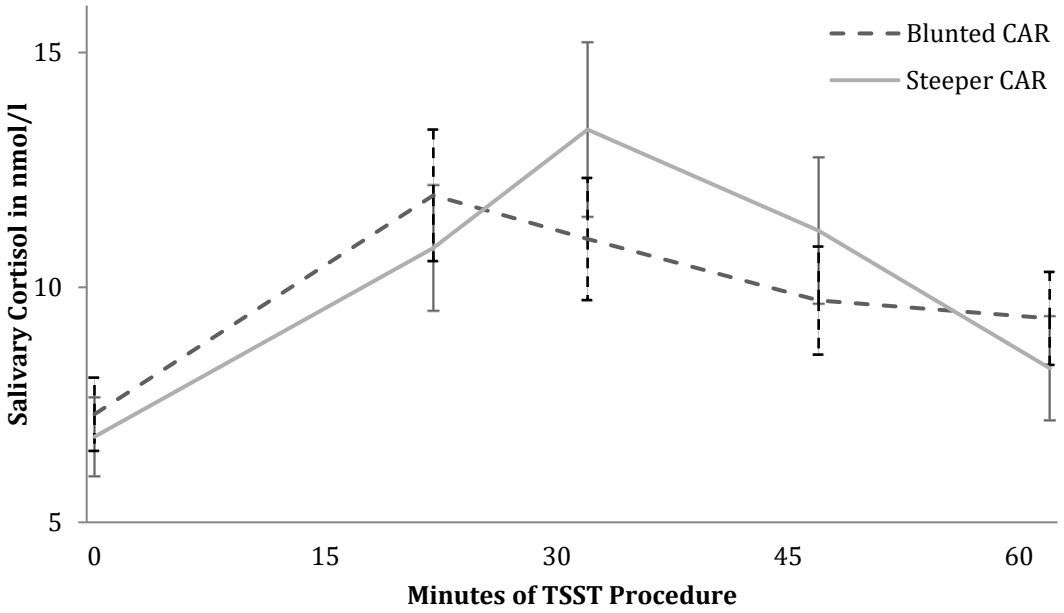

Figure 2. Mean split of CAR Slope across 5 days at each sampling point across the Trier Social Stress Test.