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Investigation into the cross-correlation of salivary cortisol and alpha-amylase responses to psychological stress

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Summary Stress is a multidimensional construct. To accurately represent stress physiology, multiple stress measures across multiple stress-related systems should be assessed. However, associations may be masked given that different systems underlie different time courses. Salivary cortisol and alpha-amylase (sAA) are reliable biological stress markers of the sympathetic nervous system (SNS) and the hypothalamus pituitary adrenal (HPA) axis, respectively. Studies examining the link between sAA and cortisol levels in response to stress have produced inconsistent results. Here, we investigated whether the covariance of stress-induced sAA and cortisol release is dependent on the distinct temporal dynamics of the two stress markers. A total of 50 male participants were exposed to a psychological laboratory stressor with high frequency (2-min interval) saliva sampling in two independent studies. Synchronized time series of sAA and cortisol measures before, during and after stress induction were obtained. Cross-correlation analysis was applied to test for the association of sAA and cortisol levels at various stages relative to the onset of the stressor. Positive and negative cross-correlations between lagged pairs of sAA and cortisol measures were found in both studies. The strongest correlation was found for sAA preceding cortisol release by 13.5 min ($r = .27$, $p < .001$). With a smaller effect size cortisol also significantly preceded sAA by 13.5 min ($r = -.16$, $p < .001$). We suggest that sAA and cortisol stress responses are reliably associated at various time lags throughout a stressful situation. As a possible connection site between HPA axis and SNS that may underlie sAA-cortisol associations, we discuss CRF neurons of the hypothalamus involved in sympathetic regulation.

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1. Introduction

Stress is defined as a state in which an organism's homeostasis is threatened (real or perceived) by potentially adverse physical or psychological states. The organism's principal metabolic peripheral effectors of the stress system are the catecholamines, norepinephrine and epinephrine, which are regulated by the sympathetic nervous system (SNS), and the glucocorticoids, which are regulated by the hypothalamic–pituitary–adrenal (HPA) axis (reviewed in Chrousos, 2009).

The role of salivary cortisol (the most potent human glucocorticoid) as a reliable biological stress marker is recognized for several decades now (reviewed in Kirschbaum and Hellhammer, 1989, 2000). Upon HPA axis stimulation, a cascade of neuro/hormones is released: corticotropin releasing factor (CRF) from the hypothalamus stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which stimulates the release of cortisol from the adrenal cortex. Kirschbaum and Hellhammer (2000) outline the characteristic slow profile of cortisol activation following a psychosocial laboratory stressor, the Trier Social Stress Test (TSST; Kirschbaum et al., 1993): cortisol levels gradually increase until a peak is reached at approximately 20–30 min after stressor onset. Hormone levels fall back to baseline values within 1 h post-stress.

The salivary enzyme alpha-amylase (sAA), which is mainly involved in the digestion of starch in the oral cavity, has received increasing attention as a stress marker of the SNS within the past ten years (reviewed in Nater and Rohleder, 2009). Although sAA is not a direct by-product of the SNS, there exist several studies showing the involvement of the autonomic nervous system (ANS), particularly its sympathetic branch, in the sAA secretion process. Batzri and Selinger (1973) and Batzri et al. (1973) were first to reveal that beta-adrenergic receptors caused the secretion of sAA on slices of the parotid gland in rats. Examining differential contributions of the two ANS branches, Anderson et al. (1984) found that sympathetic stimulation led to the secretion of parotid saliva characterized by low salivary flow rate and high sAA concentrations. In contrast, parasympathetic stimulation induced a rich saliva flow and substantially lower sAA concentrations. In an early human study, Speirs et al. (1974) provoked a sympathetic response and associated sAA increase by immersing participants into cold water. Several specific and unspecific beta-adrenergic blockers (e.g. atenolol, metroprolol, propranolol, timolol) were shown to decrease sAA concentrations (Laurikainen et al., 1988; Nederfors and Dahlof, 1996; Nederfors et al., 1994; Speirs et al., 1974). Given that its release is governed by the ANS, an increase in sAA levels during stress – when autonomic activation is high – can be expected. In 1979, Gilman et al. first found increased levels of sAA in response to intense physical exercise (Gilman et al., 1979). Almost twenty years later, Chatterton et al. (1996) revived the interest in the salivary enzyme as a stress marker by demonstrating that both physical and psychological stress triggered sAA release. In the following years, numerous studies reported increased levels of sAA in response to a variety of psychologically stressful conditions (e.g. academic examinations, the TSST, the cold pressor task; reviewed in Nater and Rohleder, 2009). Some of the these studies also found an association of sAA release and different indicators of sympathetic activity like plasma norepinephrine (Chatterton et al., 1996; Rohleder et al., 2004), left ventricular ejection time (Bosch et al., 2003) and the low frequency/high frequency ratio (Nater et al., 2006). Consistent with the more or less immediate stress-induced activation of the SNS, alpha-amylase levels were shown to peak earlier (between 5 and 10 min after onset of a stressor) and return to baseline faster (10 min post-stress) (Granger et al., 2007; Nater et al., 2006) than cortisol levels.

Because stress is a multidimensional construct, multiple measures of stress across multiple stress-related systems should be assessed to accurately represent stress physiology (Lovallo and Thomas, 2000). However, associations may be systematically masked given that different stress-related systems underlie different time courses. In general, the time dimension of stress responses has been claimed to be an important, but often ignored aspect: Understanding the interactions and feedback-loops within the time courses of different stress-related systems will facilitate the detection of dissociations between these systems—information, which may be of relevance for the early detection of health risks (Eriksen et al., 1999). Eriksen's claim holds true for SNS and HPA axis. Hence, the goal of the current research was to explore the correlation patterns between sAA and cortisol using a cross-correlational approach, based on which conclusions can be drawn about the time course of SNS-HPA axis coordination. There are several potential sites of interaction among SNS and HPA axis that could explain a positive sAA-cortisol correlation (which we would expect based on the physiological differences in the timing of the two systems' stress responses). The CRF and the locus coeruleus-norepinephrine sympathetic systems, for example, seem to participate in a positive, reverberatory feedback loop such that activation of one system activates the other as well (for a review see Chrousos and Gold, 1992). Also, epinephrine has the potential to augment cortisol secretion (Mokuda et al., 1992). Previous studies on the relationship between sAA and cortisol have produced inconsistent results. Grillon et al. (2007) found a strong positive correlation between the two stress markers after the TSST. Schoofs et al. (2008) and van Stegeren et al. (2008) found only weak positive correlations after the TSST and the cold pressor task. Nater et al. (2006, 2005) found non-correlations after the TSST. One reason for this inconsistency may be that the different time courses of SNS and HPA axis were not taken into consideration. If bivariate sAA-cortisol correlations are calculated, a low association is likely to occur. We therefore applied cross-correlation analysis to synchronized time series of sAA and cortisol measures obtained from fifty male participants who underwent the TSST in two independent studies. The studies were conducted to characterize the temporal link between sAA and cortisol response profiles in general, to be able to investigate whether the normal time link is dysregulated in special populations suffering from increased stress sensitivity (to be published elsewhere). The cross-correlation approach allowed us to calculate correlations at various system-specific stages relative to the onset of the stressor and thus consider the distinct temporal dynamics of each stress marker. We hypothesized to find positive cross-correlations at nonzero lags (when correlating sAA-cortisol pairs at time intervals different from zero). Corresponding to their kinetic profiles, sAA levels were hypothesized to predict cortisol levels approximately 20 min later.

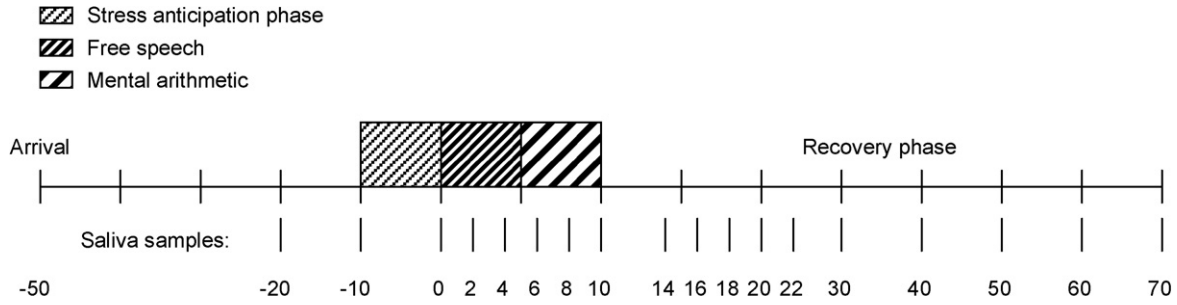

Figure 1 Timeline and schema of the testing procedure.

2. Materials and methods

2.1. Participants

Male participants between 18 and 30 years of age were recruited for two independent studies by posting ads on the electronic billboard of the McGill University website. Given a potential influence on sAA or cortisol activity, information about recreational drug use, medical and psychological history were assessed in an extensive telephone interview. Regular recreational drug users (cannabis within the past two months, any other recreational drug within the past year) and habitual smokers (more than five cigarettes per week) were excluded from the study. Individuals reporting chronic illness (including current psychological disorders) or taking medication that may influence HPA axis activity were excluded from the study. Altogether, 20 participants (mean age 21.83 years; SD 4.27) were included in study 1, and 30 participants (mean age 22.81 years; SD 3.03) were included in study 2 (conducted using the identical protocol as in study 1). All participants gave written consent to the studies, which were approved by the local Research Ethics Board.

2.2. Trier social stress test

Participants were exposed to the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), the most frequently administered psychological paradigm to stimulate an endocrine stress response in a laboratory setting. The TSST is a social evaluative and mentally challenging task, which was shown to provoke the most robust HPA axis stress response when compared to several other laboratory stressors (Dickerson and Kemeny, 2004). The TSST was also shown to increase the activity of sAA in a pattern resembling that of norepinephrine (Rohleder et al., 2004). To establish a common baseline, and control for the pre-test exposure to food, stress and physical exercise before starting the testing procedure, participants had a little snack upon arrival at the laboratory after which they rested for approximately 60 min (without eating or drinking anything but water).

2.3. Assessment and analysis of cortisol

Cortisol was sampled using the salivette collection device (Sarstedt Inc., Quebec City, QC, Canada) and stored at −20 °C until analysis. Saliva samples were taken in 10-min intervals before the TSST (at −20, −10 and 0 min), in 2-min intervals during the TSST and the following 12 min (at +2, +4, +6, +8, +10, +14, +16, +18, +20 and +22 min, with the exception of the +12 min sample) and in 10-min intervals thereafter (at +30, +40, +50, +60 and +70 min) (Fig. 1). The +12 min measure was skipped due to a lack of time for proper sampling when bringing participants back to their resting rooms immediately after the TSST. As recommended for the collection of salivary cortisol and alpha-amylase (Rohleder and Nater, 2009), participants were instructed to place the saliva collection swabs in their mouths and to refrain from chewing for exactly two min. Since cortisol secretion is characterized by a strong circadian rhythm with peak levels after awakening (Pruessner et al., 1997), testing was performed between 1 pm and 5 pm. Cortisol activity (nmol/l) was determined using a time-resolved fluorescence immunoassay (Dressendorfer et al., 1992), with intra- and interassay variabilities of less than 10% and 12%, respectively.

2.4. Assessment and analysis of salivary alpha-amylase

We sampled sAA from the same salivette collection devices as cortisol. Therefore, measuring time points were identical. Alpha-amylase activity was determined using an enzyme kinetic method. First, saliva was diluted 1:200 in assay diluent. 8 μl of the diluted saliva were then pipetted into individual wells of a 96-well micortiter plate. After adding 320 μl of substrate solution (2-chloro-4-nitrophenyl-αD-maltotrioside), the plate was incubated for three min at 37 °C and 500 rpm in a microtiter plate incubator. The enzymatic action of alpha-amylase on this substrate yields 2-chloro-p-nitrophenol (CNP), which can be spectrophotometrically measured at 405 nm. Optical density of CNP was determined exactly at the 1-min and 3-min marks. The change in optical density was calculated by subtracting the measure of the first from the second reading. Considering several factors (absorbance difference per min, total assay volume, sample volume, dilution factor, millimolar absorptivity of CNP and the plate specific light pass), the activity of sAA was calculated and expressed in U/ml (Lorentz et al., 1999; Winn-Deen et al., 1988).

2.5. Statistical analysis

Alpha-amylase and cortisol data were not normally distributed. Therefore, to test whether the TSST produced significant sAA and cortisol stress responses, Wilcoxon signed-rank tests of

the respective maximum responses versus the pre-stress measure (the average of −20 and −10 min measures) were computed per study. To test for covariance of sAA and cortisol measures at different time intervals, cross-correlation analysis was conducted separately for both study samples. The first assumption in time series analysis is that the series are stationary, i.e. that they are normally distributed and that mean and variance are constant over a long period of time (Brockwell and Davis, 1991). To achieve stationarity, the respective mean has to be subtracted from each time series. Positive cross correlation coefficients will consequently result from two measures lying above the mean, and negative cross correlation coefficients will result from one measure lying above and one below the mean. Thus, in order to remove mean level differences between participants, sAA and cortisol measures were centered around their respective within-subject mean. To obtain an increased number of equispaced measurement time-points, the sequence in each data vector was resampled such that two measures per min were generated using a linear interpolation of adjacent measures. Per participant, this provided us with time series of two measures per min for both sAA and cortisol. The maximum number of lags was computed following Schlotz et al.'s (2008) approach. Our original time series consisted of $m = 18$ measures (where m indicates the number of repeated measures) over a period of 90 min. As cross-correlations are influenced by decreasing numbers of corresponding measures when the lag increases, the lag limit criterion was set as overlap/$m > 0.5$, which resulted in a maximum number of eight lags, and ten overlapping measures at minimum and maximum lags. It was thus ensured that the number of overlapping measures was always higher than the number of non-overlapping measures on each side of the lagged series (until ten overlapping and eight non-overlapping measures on each side were reached). The mean time interval between original measures was (90 min/(18−1)) = 5.294 min. The cross-correlation lag limits for the created time series were computed as the maximum number of lags (eight) multiplied by the mean time interval between measures (5.294 min), rounded down to the next integer, which resulted in lag limits of ±42 min. Cross-correlation coefficients were computed participant-wise such that per participant one cross-correlation function describing the covariance of sAA and cortisol measures at every lag was obtained. With the resulting lag limits of ±42 min and two measures per min, we obtained cross-correlation coefficients for altogether 169 lags per participant. Cross-correlation functions were then averaged over all participants for each study. Finally, both study samples were combined and cross-correlation functions were averaged over all participants of the combined sample. For each of the resulting three mean cross-correlation functions, the mean cross-correlation coefficients were plotted against the time lags. For each time lag of 30 s within each mean cross-correlation function, a signed rank test examining the null hypothesis − that the median cross-correlation coefficient equaled zero − was computed. All analyses were performed with the Predictive Analytics Software (PASW) version 17.

3. Results

Due to several successive missing values in the sAA data, one participant from study 2 was excluded from all calculations.

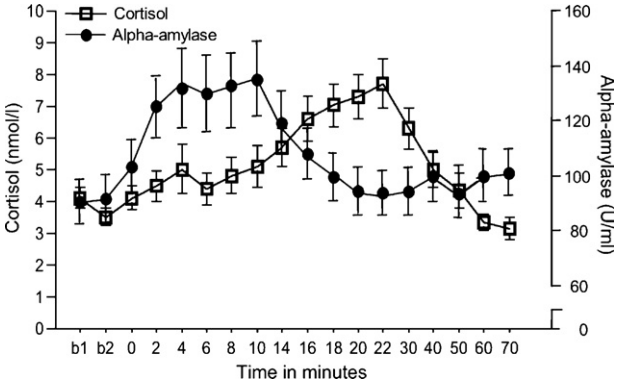

Figure 2 Mean alpha-amylase (U/ml) and cortisol (nmol/l) levels with standard errors for the 18 original measurement time-points in the combined sample ($n = 49$).

Alpha-amylase levels peaked at +4 min in study 1 and at +10 min in study 2. Cortisol levels peaked at +22 min in both studies. Compared to the respective mean baseline levels, these peak stress responses were significant for sAA (study 1: $z = -3.32$, $p = .001$; study 2: $z = -3.64$, $p < .001$) and cortisol (study 1: $z = -3.14$, $p = .002$; study 2: $z = -3.84$, $p < .001$) in both studies. Fig. 2 shows mean sAA and cortisol levels for the 18 original measurement time-points in the combined sample ($n = 49$).

The average cross-correlation functions and significance levels for study 1 ($n = 20$), study 2 ($n = 29$) and the combined sample ($n = 49$) are shown in Fig. 3a–c. The time ranges with significant cross-correlations and the respective peak cross-correlation coefficients, significance levels and time-points per time range are shown in Table 1. Overall, the two studies yielded the same pattern of results. As expected, we found significant positive cross-correlations between sAA and cortisol at positive time lags in both studies. Positive cross-correlations at positive time lags signify that changes in sAA preceded same-direction changes in cortisol release. In the combined sample, sAA levels predicted cortisol levels 5–24 min later. Peak positive correlations ($r = .27$, $p < .001$) were observed at 13.5 min, which means that the average positive association of all possible pairs of sAA with cortisol was highest when cortisol followed sAA by 13.5 min. According to Cohen (1988), a correlation coefficient of .27 represents a medium-sized effect, which explains approximately 10% of the total variance in the data. Additionally, we found significant negative cross-correlations between sAA and cortisol at negative time lags in both studies. Negative cross-correlations at negative time lags signify that changes in cortisol preceded opposite-direction changes in sAA release. In the combined sample, cortisol levels predicted sAA levels 4–28 min later, with peak negative correlations ($r = -.16$, $p < .001$) at −13.5 min. Thus, the average negative association of all possible pairs of sAA with cortisol was highest when sAA followed cortisol by 13.5 min. Note that this effect was much smaller, with cortisol explaining only 2.5% of the total variance. Only in the combined sample, significant negative cross-correlations were also found at positive time lags. Changes in sAA levels predicted opposite-direction changes in cortisol levels 36–42 min later, with peak correlations ($r = -.09$, $p = .017$) at 41.5 min. This negative correlation

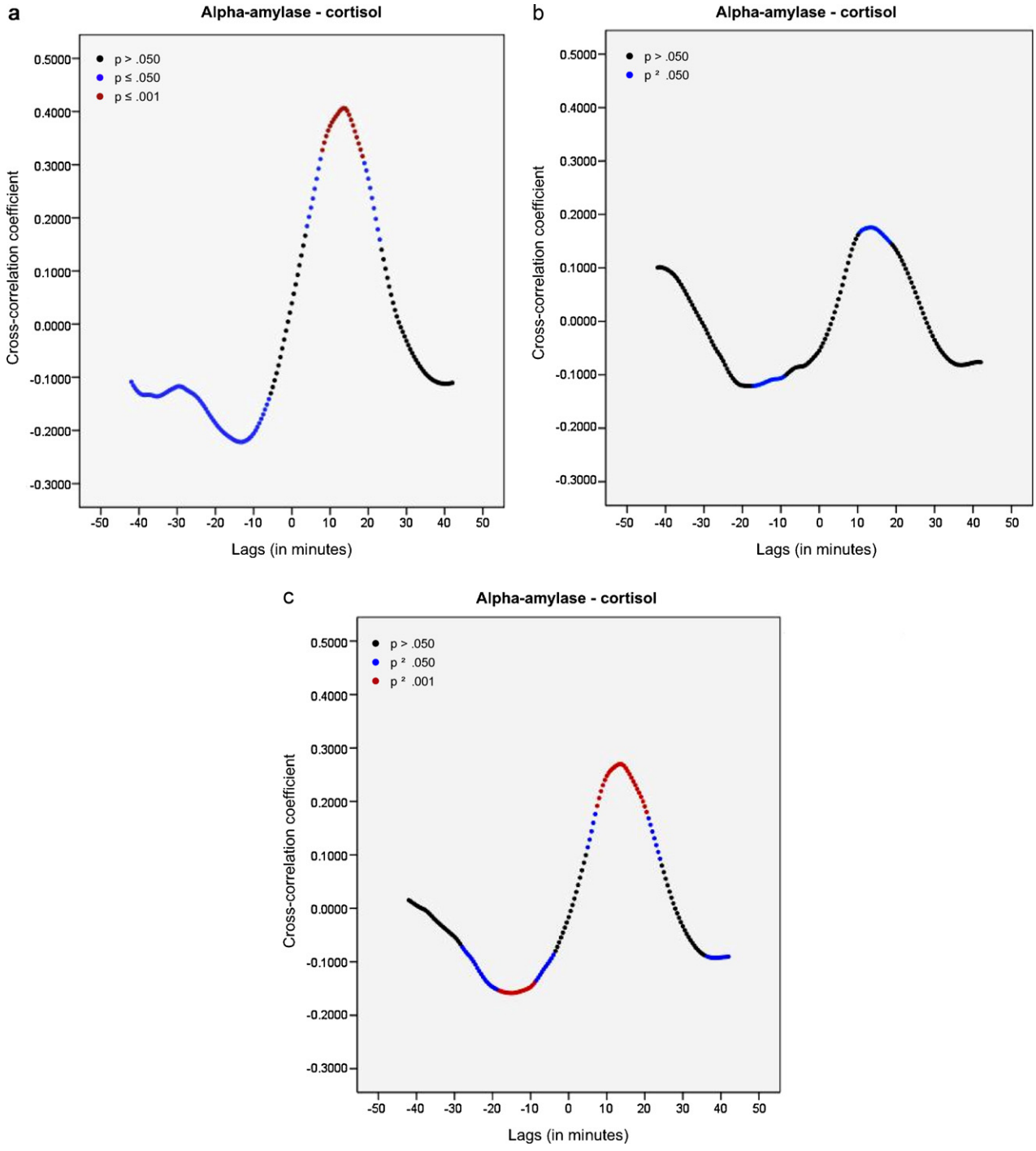

Figure 3 (a) Average cross-correlations and significance levels of salivary alpha-amylase (sAA) and cortisol plotted against time lags in study 1 ($n = 20$). Positive lags indicate that the variable first named in the heading precedes the second variable. The cross-correlation coefficient of $r = .41$ at a lag of 13.5 min indicates that sAA levels throughout the experiment are most strongly associated with cortisol levels 13.5 min later. (b) Average cross-correlations and significance levels of salivary alpha-amylase (sAA) and cortisol plotted against time lags in study 2 ($n = 29$). Positive lags indicate that the variable first named in the heading precedes the second variable. The cross-correlation coefficient of $r = .18$ at a lag of 14 min indicates that sAA levels throughout the experiment are most strongly associated with cortisol levels 14 min later. (c) Average cross-correlations and significance levels of salivary alpha-amylase and cortisol plotted against time lags in the combined sample ($n = 49$). Positive lags indicate that the variable first named in the heading precedes the second variable. The cross-correlation coefficient of $r = .27$ at a lag of 13.5 min indicates that sAA levels throughout the experiment are most strongly associated with cortisol levels 13.5 min later.

Table 1 Time ranges with significant cross-correlations between alpha-amylase and cortisol, and the respective peak cross-correlation coefficients per time range.

	Time range (lag in min)		Maximum		
			Cross-correlation coefficient	*p*	Lag (min)
Study 1 (*n* = 20)	Negative lags	−42 to −6	−.22	.002	−13.5
	Positive lags	+4 to +23	.41	<.001	13.5
Study 2 (*n* = 29)	Negative lags	−16.5 to −9.5	−.11	.024	−11
	Positive lags	+10.5 to 18.5	.18	.025	14
Combined sample (*n* = 49)	Negative lags	−28 to −4	−.16	<.001	−13.5
	Positive lags	+5 to +24 and	.27	<.001	13.5
		+36 to +42	−.09	.017	41.5

coefficient represents a small effect size explaining around 1% of the total variance (Cohen, 1988).

4. Discussion

The role of salivary cortisol as a meaningful and reliable stress marker indicative of HPA axis function has been recognized for more than twenty years (reviewed in Kirschbaum and Hellhammer, 1989, 2000). The salivary enzyme alpha-amylase — indicative of SNS function — has received increasing attention as a stress marker within the past ten years (reviewed in Nater and Rohleder, 2009). However, associations of sAA and cortisol have not been consistently found. One reason could be that their distinct temporal dynamics were not taken into consideration. Using cross-correlation analysis applied to synchronized time series of the two stress markers, we showed that at different time lags, stress-induced increases in levels of sAA were reliably correlated with cortisol levels in two independent studies. In detail, the two studies revealed a pattern of sAA and cortisol associations where sAA levels moderately predict cortisol levels around 14 min later (with 10% explained variance), followed by cortisol levels weakly and inversely predicting alpha-amylase levels around 14 min later (with 2.5% explained variance), and finally sAA levels weakly and inversely predicting cortisol levels around 42 min later (with 1% explained variance).

The positive association we found at positive time lags reflects the kinetic profiles of stress-induced sAA and cortisol release shown in previous TSST studies and matches the expectations of the physiological differences in the timing of SNS and HPA axis stress responses. Sympathetic activation is initially achieved through neural stimulation and is more or less immediate. Measurable increases in organ or system function tied directly to SNS activation (e.g. heart rate) can be detected within a minute after stressor onset. Catecholaminergic activity intensifies and extends the duration of neural stimulation. HPA axis-mediated effects, which cumulate in the release of cortisol, are slower to act and recover. Changes in HPA axis activity are not detectable for several minutes. Once initiated, they may persist for hours (Whiteside et al., 2000). CRF neurons of the hypothalamus sending fibers to the brain stem and locus coeruleus (both of which are involved in sympathetic regulation) represent a possible connection site between HPA axis and SNS that may explain the positive sAA/cortisol cross-correlation at positive time lags. The neurotransmitter role of CRF is supported by the widespread distribution of CRF-immunoreactive terminals and binding sites in the brain (DeSouza, 1987; DeSouza et al., 1985; Sakanaka et al., 1987; Swanson et al., 1983). Consistent with this idea, central CRF administration mimics many autonomic and behavioral stress responses (i.e. a rises in epinephrine and norepinephrine plasma levels, cardiovascular function, locomotor activation), even in hypophysectomized animals (Brown et al., 1982; Owens and Nemeroff, 1991; Valentino et al., 1993), while central administration of CRF antagonists prevents those very responses (Britton et al., 1986; Brown et al., 1985; Kalin et al., 1988). The positive sAA/cortisol cross-correlation at positive time lags can also be explained from the opposite perspective given that norepinephrine stimulates CRF (Calogero et al., 1988; Cunningham et al., 1990), and epinephrine stimulates cortisol release (Mokuda et al., 1992).

The negative association we found at negative time lags was unexpected. However, although HPA axis and SNS clearly work in coordination to mediate the physiologic response to a perceived stressor, the exact nature of their coordination (e.g. additive or interactive; opposing or complementary) is under debate (Granger et al., 2007). In accordance with a role of glucocorticoids in restraining sympathoneural outflows, typical uncontrollable glucocorticoid-increasing stressors like footshock or activity stress with food restriction in rats have been shown to cause transient decreases in brain norepinephrine levels (Anisman et al., 1981; Irwin et al., 1986; Tsuda et al., 1982). Research in adrenolectomized rats showed that endogenous glucocorticoids restrain responses of catecholamine turnover, synthesis, release, reuptake and metabolism in sympathetic nerves during immobilization stress (Kvetnansky et al., 1993). In humans, one week of treatment with 20 mg of prednisone, a dehydrogenated analogue of cortisol, reduced sympathetic nerve activity and plasma norepinephrine levels in healthy participants (Golczynska et al., 1995), whereas increased plasma catecholamine levels have been observed in patients with stress-related disorders characterized by hypocortisolemia (Fries et al., 2005). Findings from Pavcovich and Valentino (1997) imply a potential physiological mechanism underlying the negative feedback effect of glucocorticoids on sympathoneural outflows. The authors demonstrated that — like its

neurohormone action — the putative neurotransmitter action of CRF is negatively regulated by circulating glucocorticoids. In detail, adrenolectomy was shown to enhance tonic and stress-induced CRF releases within the LC, and alter the postsynaptic CRF sensitivity of LC neurons. We suggest that the negative sAA/cortisol cross-correlation at negative time lags may reflect the negative feedback effect of glucocorticoids on sympathoneural outflows.

The negative association we found at positive time lags was also unexpected. Given the positive alpha-amylase/cortisol cross-correlation, however, we can assume that stress-induced cortisol release was the higher, the higher the preceding sAA release was. In healthy participants, as examined here, it would moreover be expected that high levels of cortisol trigger an accordingly strong inhibition of subsequent hormone release (Miller and O'Callaghan, 2002). In other words, we suggest that the negative sAA/cortisol cross-correlation at positive lags may reflect cortisol's negative feedback properties on HPA axis activity, and thus its own release. In order to disentangle the timeline of positive and negative associations between sAA and cortisol, future studies will need to independently investigate cross-correlations at baseline, in response to stress and during stress recovery.

One limitation of the presented studies is that the number of original observations was relatively small (n = 18). In order to allow for the independent investigation of cross-correlations between sAA and cortisol at different stages of the stress response, future studies will have to increase measurement time-points, especially at baseline and during stress recovery. However, we felt that for the method of sampling saliva, we were at the upper end of what was feasible in terms of sampling interval. A second limitation is that sampling saliva in two-minute intervals somewhat changed the "normal" procedure of the TSST. Having to talk while keeping a saliva collection swab in the mouth, for example, might have interfered with the speech portion of the test. However, participants reported no difficulties with talking. Also, distribution and collection of salivettes were performed by a research assistant. If anything, the additional sampling and observer presence in the room might have increased the stressfulness of the situation. Third, a much discussed issue in determining the validity of sAA as a measure of SNS activation is whether amylase is independent of saliva flow rate. Whereas the stimulation of salivary proteins is generally ascribed to sympathetic activity, the stimulation of saliva flow rate is mainly mediated by parasympathetic nerves (Anderson et al., 1984; Garrett, 1987). In this regard, Rohleder et al. (2006) could demonstrate that stress-induced increases in sAA levels were correlated with increases of amylase output but not with increases of flow rate. These results indicate that saliva flow rate is not a confounder of stress-induced sAA activation, and that valid sAA measurements can be obtained by the use of salivettes. Eventually, it is important to note that apart from the distinct temporal dynamics of alpha-amylase and cortisol, various factors like measurement errors, nonlinear associations and different thresholds of stress sensitivity contribute to reducing covariance.

An accurate representation of stress physiology is only possible if multiple measures of stress across multiple stress-related systems are considered (Lovallo and Thomas, 2000). However, different stress-related systems follow different time courses, and associations may be systematically overlooked when correlating at lag zero only. Here, we could show covariance of stress-induced sAA and cortisol release at nonzero lags in two independent studies. Our data confirm that cross-correlation analysis represents a valuable approach to explore the time course of two physiological stress measures in relation to one another. Understanding the normal pattern of SNS-HPA axis associations in healthy individuals will facilitate the detection of dissociations linked to disease—information which can be utilized to improve the early identification of health risks (Eriksen et al., 1999). Future studies should collect simultaneous time-series of sAA, heart rate, cortisol and subjective-psychological data. In combination with the assessment of clinical and psychological variables, this approach will allow the systematic investigation of physiological and psychoendocrine dissociations in the presence of psychopathology.

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Conflict of interest

None declared.

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