Mind the Gap between Feeling Bad and Feeling Dead:

Stress but not Death Reminders Elicit Endocrine Responses

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Abstract

Stressors and mortality salience share considerable conceptual overlap. Thus, we examined the impact of a standard mortality salience and a standard stress manipulation on the activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis via endocrine measures of stress; a neutral control condition completed the design. The results revealed that stress elicits increased salivary α-amylase and salivary cortisol reactions; however, no endocrine reactions were found in the mortality salience and the control conditions. To the contrary, we did not find any differences regarding positive and negative affect between any conditions. Implications for social and health psychology are being discussed.

Key Words: stress, threat, mortality salience, salivary α-amylase, salivary cortisol

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The question of how people deal with existential threat is as old as psychology, but experimental research on this topic was missing for a long time. However, after *9/11* and with increasing terrorism awareness in the Western world, existential threat research increased. This rise was closely related to the development of the terror management theory thirty years ago (TMT; Pyszczynski, Solomon, & Greenberg, 2015). TMT did not only offer explanations why awareness of the own mortality (mortality salience; MS) leads to particular psychological reactions but also provided an experimental paradigm allowing to investigate this topic. Derived from the work of the anthropologist Ernest Becker (e.g., Becker, 1973), TMT posits that if humans become aware of the inevitability of their own demise (i.e., mortality salience), this gives rise to the potential of paralyzing anxiety. This anxiety leads to a variety of specific coping mechanisms—like world-view defenses—by which people try to achieve symbolic immortality.

The core idea of TMT, that people feel threatened by environmental factors and try to cope with them in more or less specific ways, shares substantial overlap with a different and hitherto nearly unrelated psychological domain: stress research. In fact, stress research posits that all organisms strive for a life in homeostatic balance. This balance is continuously challenged throughout life and may be disturbed by extrinsic threats that are called stressors (Chrousos & Gold, 1992). Subsequent to these feelings of stress, individuals try to adapt and reestablish homeostasis by a variety of different coping mechanisms.

Albeit being grounded in different research traditions, similarities between both TMT and stress research are manifold. For example, “stress is assumed to occur whenever a demand exceeds the regulatory capacity of an organism, particularly in situations that are unpredictable

and uncontrollable" (Starcke & Brandt, 2012, p. 1232). This assumption is backed up by many studies showing that the effects of stress inductions are highest if the stressor is perceived as uncontrollable (Dickerson & Kemeny, 2004). Similarly, MS inductions are more effective if individuals do not perceive their demise as controllable (e.g., Fritsche, Jonas, & Fankhänel, 2008). Thus, the (un-)controllability of an event is an important boundary condition for the effectiveness of stressors and threats. Further similarities between stressors and threat become evident by looking at the coping behavior. For example, Arndt et al. (2013) demonstrated that MS leads to an intensification of smoking behavior comparable to the intensification of smoking behavior following stress inductions (McKee et al., 2011).

Despite these similarities there are also differences with respect to the assumed processes underlying threatening events and subsequent behavioral reactions. Whereas TMT assumes that mostly cognitive and affective reactions follow external hazards (i.e., world-view defense based on potential for anxiety; Pyszczynski et al., 2015), stress research makes highly specific assumptions about psychophysiological reactions following stress, including a highly adaptive endocrine response (Chrousos & Gold, 1992). Thus, besides physiological measures (like heart rate variability or blood pressure; e.g., Vrijkotte, van Doornen, & Geus, 2000), endocrine parameters are commonly used in stress studies to measure the activation of the sympathetic nervous system (SNS) and of the hypothalamic–pituitary–adrenal axis (HPA axis). The activations of the SNS—measured via salivary α-amylase (sAA; e.g., Nater & Rohleder, 2009)—and the HPA axis—measured via salivary cortisol (e.g., Dickerson & Kemeny, 2004)—are crucial to cope with stressful events. In an attempt to bridge between these research traditions, Tritt, Inzlicht, and Harmon-Jones (2012) "suggest that priming participants to ponder their own mortality creates feelings of uncertainty about their goals and to their future. Such feelings of

uncertainty signal an orientating response […] and associated release of noradrenaline, cortisol, and other stress hormones […]" (p. 724).

Interestingly, however, it is widely unknown whether these stress reactions are actually related to MS, because in thirty years of TMT research, studies focused mostly on the affective and cognitive reactions towards MS (Pyszczynski et al., 2015). That is, to the best of our knowledge, only one study addressed the question of whether physiological stress parameters (i.e., pulse rate, pulse volume and skin resistance) are related to MS inductions (Rosenblatt, Greenberg, Solomon, Pyszczynski, & Lyon, 1989)—finding no differences between a MS and a control condition. Even more striking, there seems to be no research addressing MS and endocrine parameters like cortisol and sAA. Thus, an investigation addressing the influence of MS on endocrine parameters would not only help to deepen the understanding of the biological side of death anxiety, but would also help to determine differences and similarities of stress and MS on a theoretical level.

In the present study, we therefore aimed to elucidate the relation between stress and existential threat by investigating the impact of a standard MS manipulation (Rosenblatt et al., 1989)—in comparison to a standard stress manipulation (Schwabe, Haddad, & Schächinger, 2008)—on the activation of the SNS and the HPA axis. Whereas SNS activation is considered to be a first-wave response that is elicited rapidly, the activation of the HPA axis is considered to be a second-wave response that is elicited rather slowly (Charmandari, Tsigos, & Chrousos, 2005). To examine these first- and second-wave reactions, we therefore assessed sAA and salivary cortisol, respectively. By doing so, we hope to improve our understanding of how MS affects the endocrine system and to clarify how MS is related to stress on a psychophysiological level. Please note that materials, data, and the analysis script for this study are available on https://osf.io/8enqh/.

Method

Participants and Design

Thirty-six male participants (M_{Age} = 24.9, age range = 19–32) participated in the study. Participants were recruited at the local university via email distribution lists and posters at the university. In order to participate, certain criteria (male, no physical, mental or chronic illnesses; no regular tobacco, alcohol or drug use; body mass index below 27) had to be met, because these variables were shown to influence cortisol levels in prior studies (for a review see Kudielka, Hellhammer, & Wüst, 2009). These inclusion criteria were queried by telephone screenings prior to the start of the investigation. During these screenings participants were scheduled for three sessions and informed that 90 minutes before their appointments strenuous physical activities, the consumption of liquids, except for water, and food should be avoided. At the end of their last session participants received 40 € or course credits for their participation.[1]

The study was conducted in a 3 (condition: MS vs. control vs. stress) x 7 (time of measurement) within-participants design. To avoid sequence effects, the order of conditions was counterbalanced, while participants were randomly distributed to these different orders. We sampled N = 36 participants to achieve a power of $1-\beta \geq .95$ for detecting a medium sized effect or bigger ($d \geq 0.5$). All data analyses were conducted after the a priori determined sample size was reached. We report all measures, manipulations and exclusions.

Materials and Procedure

The study was conducted between 1 and 6 p.m. in order to minimize fluctuations in cortisol levels induced by the circadian rhythm of cortisol (Kudielka et al., 2009). During their first appointment, participants received a general information sheet concerning contents of the study. They had to confirm that they fully understood the given information. It was emphasized that it was possible to quit the experiment any time without any disadvantages. After signing the

agreement to participate, the first session started. All sessions were identical except for different manipulations and audiobooks (see below).

Sessions lasted approximately 75 minutes: Each session started with a questionnaire to assess food, drink, alcohol, tobacco and drug consumption in the hours prior to data collection as well as sport activity the day before; participants were rescheduled if the criteria for participation were not met. After that, the experimenter described how to give a saliva sample, and the first saliva sample was collected (Baseline). Subsequently, one of the experimental manipulation was conducted and participants were asked exactly 7 minutes after the Baseline to provide the second sample (Time 1). Afterwards, participants were seated in front of a computer and asked to put headphones on. Further instructions were provided via the headphones or the computer screen using E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA).

In order to conduct the study like a prototypical MS experiment (Burke, Martens, & Faucher, 2010), participants completed two filler questionnaires after the manipulations. The most common delay questionnaire in TMT research is the Positive and Negative Affect Scale (PANAS, Watson, Clark, & Tellegen, 1988) which queries participants about their present mood with 10 positive and 10 negative mood items. In addition to the PANAS, we decided to include a second filler survey: the Need for Cognitive Closure Scale (NCC; Webster & Kruglanksi, 1994). The German version of the questionnaire consists of 16 items which measure the motivation to seek certainty (Schlink & Walther, 2007). Because stress and MS reactions have both been linked to uncertainty (Monat, Averill, & Lazarus, 1972; Van den Bos, 2009), the NCC was administered as an innocuous questionnaire in order to investigate if physiological responses to stress and MS might be accompanied by a heightened need to achieve cognitive closure.

Subsequent to the delay tasks, we decided not to include any measures on psychological coping behavior (i.e., world-view consistent behavior), in order to avoid any buffering effects of

these behaviors that possibly might influence physiological responses. Thus, after finishing the questionnaires participants waited for a request to provide the next saliva sample (Time 2) which was prompted exactly 10 minutes after Time 1.

Thereafter, participants listened to one of three different audio books about German landscapes. The order of the presentation of audiobooks was counterbalanced across participants and experimental conditions. By choosing and editing these audiobooks, we were able to avoid arousal inducing contents and topics which might relate to threats during the waiting period. The audiobooks were interrupted after another 10 (Time 3), 25 (Time 4), 40 (Time 5), 55 (Time 6) minutes in order to prompt the participant to provide another saliva sample and were continued after the collection of each sample except for Time 6. In session one and two participants were thanked and reminded of their next appointment. In session three, participants were additionally asked about their religiosity and afterlife beliefs following Time 6; then, participants were debriefed and received money or a course credit.

Experimental Manipulations. The Mortality Attitudes Personality Survey (Rosenblatt et al., 1989) was used which is the most common MS manipulation used in TMT research (Burke et al., 2010). In this survey participants were asked to “Please briefly describe the emotions that the thought of your own death arouses in you.” and to “Jot down, as specifically as you can, what you think will happen to you when you physically die and once you are physically dead.”. In the control condition, the participants were asked the same questions but with regard to watching television. The questionnaire was administered in a paper and pencil version and participants were given six minutes to answer these questions.

For the stress control condition, the socially evaluated cold pressor test (SECPT) was used which has been shown to reliably activate the HPA axis and the SNS (Schwabe et al., 2008). During the SECPT, participants were asked to put their dominant arm into cold water (0-2°C) for

a maximum of three minutes. They were informed, however, that they could take their arm out of the water at any time if they could not tolerate the pain anymore. Additionally, participants were told not to move and to look directly into a camera which would ostensibly record their facial expressions. While doing so, they were observed by an unfamiliar experimenter of the opposite sex who would remind them not to move or look in another direction if necessary. Due to this socially evaluative component, the SECPT has been proven to elicit the SNS and HPA axis reliably; conversely, a mere pain exposure by immersing the arm into cold water does not elicit the HPA axis (Schwabe et al., 2008). Before the SECPT was carried out, participants were given information and a consent form. Including the following preparations (seating of the participant, putting up the camera, time it takes participants to dry themselves), the SECPT took approximately six minutes. Thus, we were able to match the duration of all manipulations.

Measures. In order to assess salivary cortisol and sAA, samples were collected with the 'Salivette Cortisol' collection device (Sarstedt Inc., Nümbrecht, Germany), and subsequent to the experiment samples were stored in a -20°C freezer. A time-resolved fluorescence immunoassay was used (Dressendörfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992) in order to determine cortisol levels. The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% and 9.0%. Concentration of sAA was assessed with a chromogenic assay, using 2-Chloro-4-nitrophenyl-a-D-maltotrioside as a substrate (Lorentz, Gütschow, & Renner, 1999). The intra-assay coefficient of variation was between 3.5% and 6.3%, and the corresponding inter-assay coefficients of variation were between 5.5% and 7.6%.

Results

Because some samples didn't provide enough saliva, the assessment of salivary cortisol and/or sAA was not possible for all times of measurements for some participants. Consequently,

the data of these participants could not be analyzed (Cortisol: 4; sAA: 2). In addition, we had technical issues with E-Prime 2.0 in five sessions; thus, questionnaire data is not available for these sessions. We therefore report the resulting N for each analysis. Please note that we will use the Greenhouse-Geisser correction for the following analysis and report ε- and corrected *p*-values if Mauchly's test indicates a violation of the assumption of sphericity. Note that the endocrine parameters were not normally distributed, however, the results pattern stays stable when log transformed. Thus, the untransformed data is being presented for the sake of interpretability.

Cortisol

In order to test whether MS affects levels of salivary cortisol we conducted a 3 (condition: MS vs. control vs. stress) x 7 (time of measurement) repeated measures ANOVA ($N = 32$). The analysis revealed a significant main effect for time of measurement, $F(6, 186) = 17.87$, $\varepsilon = .35$, $p < .001$, $\eta^2 = .37$ and for our manipulation, $F(2, 62) = 13.17$, $\varepsilon = .65$, $p < .001$, $\eta^2 = .30$. Importantly, these effects were qualified by the significant interaction of both factors, $F(12, 372) = 16.77$, $\varepsilon = .16$, $p < .001$, $\eta^2 = .35$ (see Figure 1). That is, in the stress control condition participants showed higher cortisol levels dependent on the time of measurement. For further investigation of this interaction, pairwise comparisons (adjusted Bonferroni) were conducted. Higher cortisol levels in the stress condition, compared to the control and MS conditions, were found at Time 2, Time 3, Time 4, Time 5 and Time 6 (all ps < .043). Levels of salivary cortisol did not differ at any point of time between the control and MS condition (see Table A1 for detailed descriptive statistics).

[Insert Figure 1 near here]

α-amylase

For sAA, the measurement directly after the manipulation is the most crucial, because sAA is released fast during the first-wave SNS response. Therefore, a 3 (condition: MS vs. control vs. stress) x 2 (time of measurement) repeated measures ANOVA was conducted looking at Baseline and Time 1 ($N = 34$). This analysis revealed a significant main effect for time $F(1, 33) = 8.98$, $p = .005$, $\eta^2 = .21$ and revealed a significant two way interaction between time and condition, $F(2, 66) = 3.27$, $p = .039$, $\eta^2 = .09$ (see Figure 2). Pairwise comparisons (adjusted Bonferroni) revealed a significant decrease of sAA in the control (Baseline: $M = 159.98$; $SD = 119.22$; Time 1: $M = 118.96$; $SD = 76.66$; $p = .007$) and the MS conditions (Baseline: $M = 135.26$; $SD = 98.74$; Time 1: $M = 104.23$; $SD = 59.45$; $p = .006$) from Baseline to Time 1, but not in the stress control condition (Baseline: $M = 141.27$; $SD = 95.33$; Time 1: $M = 130.92$; $SD = 82.99$; $p = .256$). There was no significant main effect for condition $F(2, 66) = 1.97$, $p = .148$, $\eta^2 = .06$.

[Insert Figure 2 near here]

Questionnaires

In three separate one-way repeated measure ANOVAs, we analyzed the impact of the different conditions on the delay questionnares ($N = 31$). Consistent with the TMT literature (Pyszczynski et al., 2015), neither positive affect ($F(2, 60) = 1.07$, $p = .351$, $\eta^2 = .03$) nor negative affect ($F(2, 60) = 0.94$, $\varepsilon = .81$, $p = .380$, $\eta^2 = .03$) yielded significant effects for the PANAS subscales if the MS condition (positive: $M = 23.31$; $SD = 27.80$; negative: $M = -61.76$; $SD = 32.00$) was compared with the control condition (positive: $M = 22.56$; $SD = 30.82$; negative: $M = -56.76$; $SD = 40.11$). Surprisingly, however, there was also no observable difference

regarding the stress condition (positive: $M = 27.81$; $SD = 23.09$; negative: $M = -62.34$; $SD = 26.73$). In addition, we did not find an effect on the NCC scale, $F(2, 60) = 0.5$, $p = .612$, $\eta^2 = .02$ (MS: $M = -5.15$; $SD = 26.10$; Control: $M = -6.52$; $SD = 24.47$; Stress: $M = -7.50$; $SD = 27.27$).

Discussion

In the present research, we compared the effects of death reminders and stress on endocrine reactions in a sample of male participants. Despite manifold similarities between stressors and threat on a conceptual level, our results indicate that thinking about one's own demise does not affect the endocrine system: Our findings suggest that a standard MS manipulation (Rosenblatt et al., 1989) was not sufficient to activate the HPA axis and to elicit cortisol reactions. In the stress condition, however, the typical pattern of stress response with a peak in cortisol after 20 minutes was observed. Consistent with the data on salivary cortisol, we showed that a significant drop-off in sAA occurred in the MS and control conditions—but not in the stress condition. This indicates that, in comparison with the stress manipulation, the induction of MS is not sufficient to activate the SNS. Furthermore, in comparison with the control condition, the MS manipulation did not yield greater endocrine responses. Importantly, the different manipulations did not elicit affect differentially. Due to this divergence, we conclude that the Mortality Attitudes Personality Survey (Rosenblatt et al., 1989) as a mortality reminder does not seem to be sufficient to elicit endocrine reactions via the HPA axis and the SNS.

Regarding social psychology theorizing, it remains in question why such abstract death reminders might not elicit endocrine responses—at least in comparison to the control condition. Although the Mortality Attitudes Personality Survey is rather abstract and less intense compared to more vivid threats (e.g., news about terrorist attacks), this manipulation has been shown to elicit similar cognitive responses when compared to more intense threats. For example, abstract MS and the saliency of terrorist attacks increased faith in cultural worldviews and institutions in

laboratory and real life settings (e.g., Landau et al., 2004; Smith, Rasinski, & Toce, 2001). Consequently, it may be derived that a common mechanism contributes to these cognitive effects of more or less intense threat. However, despite these similarities on a cognitive level, by means of TMT it is not clear why a terrorist threat may elicit stress symptoms (Schuster et al., 2001), but a more abstract and less intense threat does not. Thus, future studies should investigate the boundary conditions that render threats effective to incite stress responses, and they should examine how death anxiety contributes to stress reactions.

One potential moderator of the effect concerns the duration of MS or stress exposure. Brief manipulations of MS, as the Mortality Attitudes Personality Survey (Rosenblatt et al., 1989), have consistently shown to elicit world-view defense as a part of coping behavior (Burke et al., 2010). Also, chronic exposure to MS seems to lead to chronic coping with world-view defense (Fernandez, Castano, & Singh, 2010). Likewise, there is evidence for stress reactions towards prolonged threat exposure, provided by Schuster et al. (2001). The authors show that extended exposure to threatening news might result in a heightened stress experience in comparison with brief exposure. By means of the present investigation, however, it remains unclear how prolonged experiences of abstract death reminders affect the endocrine system, and future research might address this topic.

Other relevant boundary conditions, which render threats effective to induce stress responses, may be derived from stress research. As already alluded to, perceived controllability is an important aspect which seems to be a common moderator of threat and stress responses (Dickerson & Kemeny, 2004; Fritsche et al., 2008). In fact, stressors and threats are most effective if they are perceived as uncontrollable. Thus, future studies may manipulate the perceived sense of control for stress and threat manipulations. For instance, MS may be rendered

less controllable by eliciting thought about an incurable illness or more controllable by thinking about suicide (Fritsche et al., 2008).

Another major difference of abstract MS manipulations compared to laboratory stress inductions is the social-evaluative nature of standard stress manipulations. In fact, the techniques which are most often used to induce stress, and which are well-known to elicit endocrine responses, possess a clear social-evaluative element (Dickerson & Kemeny, 2004). Thus, it is plausible to assume that threat may elicit endocrine responses if the contemplation of one's death is associated with a negative social evaluation. Future studies should decompose threat and stress manipulations reading their social and non-social features in order to provide a more specific understanding of their effect mechanisms.

In order to achieve these goals, future studies may incorporate additional measures to assess responses of the SNS. While this study focused on endocrine parameters – measuring salivary alpha-amylase and cortisol –, non-endocrine parameters, like blood pressure, heart rate variability, might be taken into account to investigate how MS affects the SNS. It is important to note that the manipulation used in the current investigation did not affect these measures in a study conducted in the beginnings of TMT research (Rosenblatt et al., 1989). Considering the theoretical and methodological advancement since the time when this study was conducted, future research may, however, use endocrine and non-endocrine measures to assess how and when MS affects psychophysiological processes. This could be especially insightful if one considers the proposed moderators.

In sum, this research provides first insights into differential endocrine responses elicited by standard laboratory stress and MS inductions. Nonetheless, further studies are necessary to improve our knowledge about the (dis-)similarities of existential threats and stressors, because this would provide implications for human health and work behavior as well as well-being (e.g.,

Cohen, Janicki-Deverts, & Miller, 2007; Goldenberg & Arndt, 2008; Zapf, 2002). If we understand in which way MS and stress are related and unrelated, this knowledge may fortify our understanding of effective coping behaviors. Especially in a modern world, in which stress levels and threat exposure increase (Cohen & Janicki-Deverts, 2012; Gibson, 2007), this will be crucial to comprehend individual's cognitive, affective and physiological reactions to an increasingly challenging life. This way, research about the relation on threat and stress has the potential to strengthen and promote human's health and well-being.

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Data availability statement

Materials, data, and the analysis script will be made available on https://osf.io/8enqh/. For review purposes, this information can be assessed via: https://osf.io/8enqh/?view_only=976bc4f4e8774c078af3a311db6e6a6f

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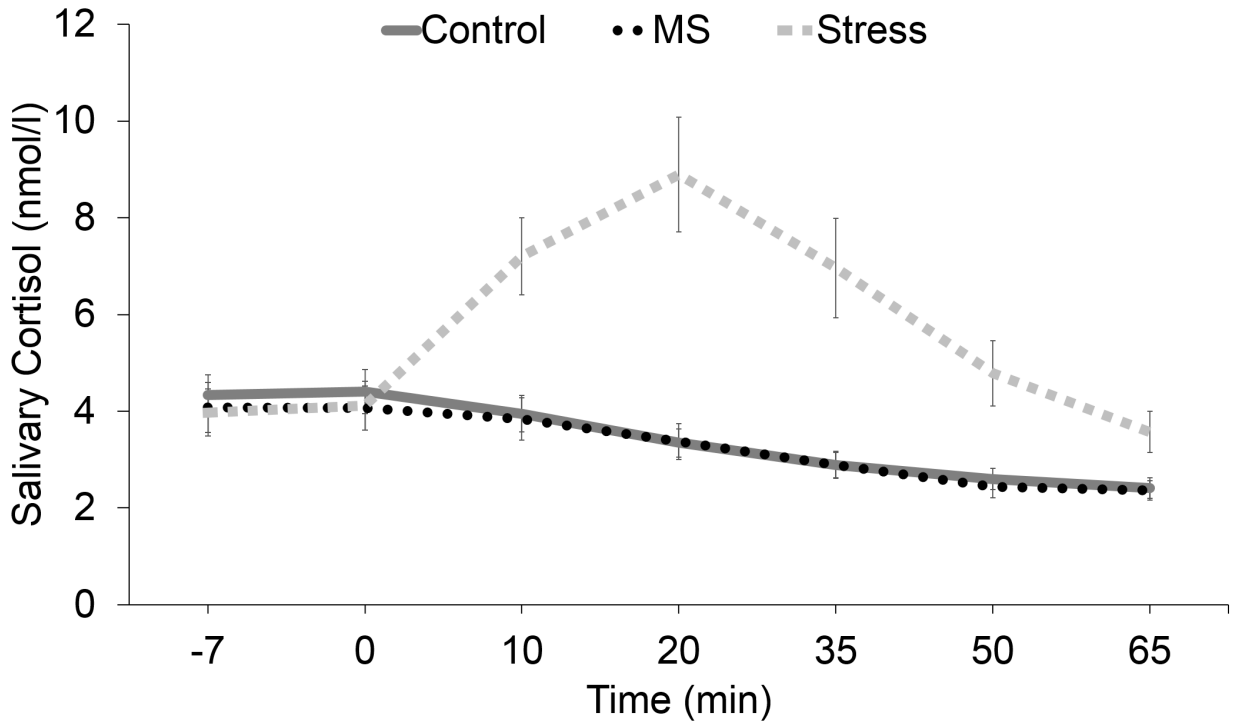
Foot Notes

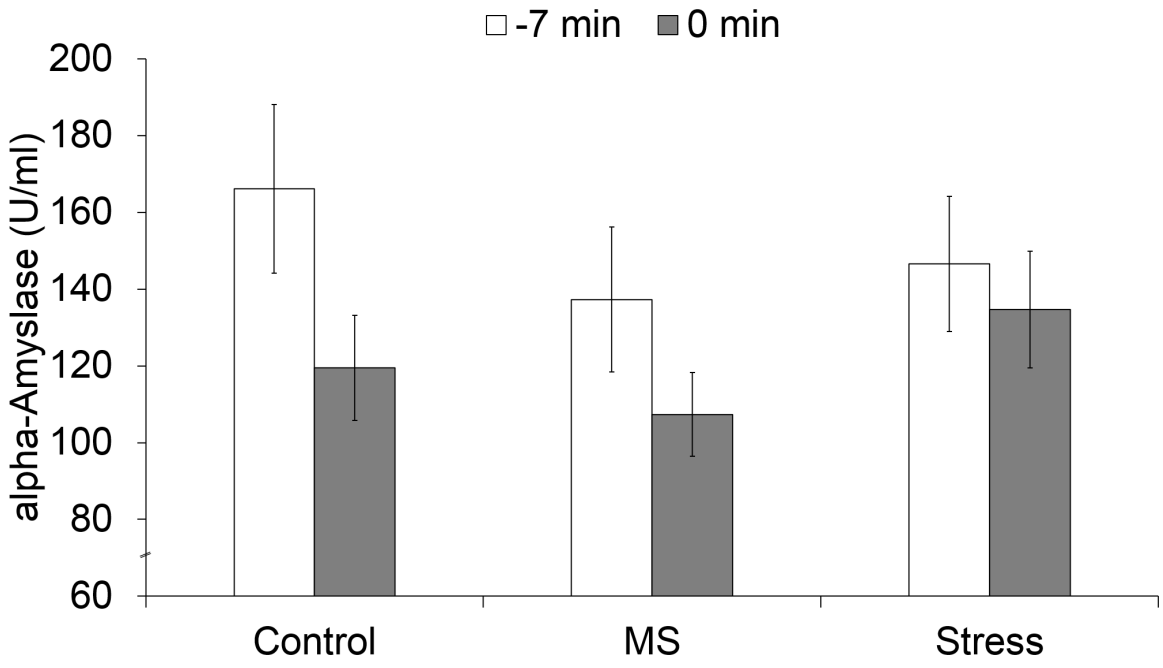
[1]We did not apply for ethical approval for this specific study. This is because the procedures (including the SECPT) have been approved before (and after) this study was conducted by the local ethics committee. By the time at which the study was planned and conducted, it was a common approach at our university to get ethical approval for the procedure instead of the particular study.

Figure Caption

Figure 1. Cortisol levels across times of measurement in the different conditions. Error bars represent standard errors.

Figure 2. α-amylase levels across at Baseline and Time 1 in the different conditions. Error bars represent standard errors.


Control
MS
Stress
Salivary Cortisol (nmol/l)
12
10
8
6
4
2
0
-7
0
10
20
35
50
65
Time (min)


-7 min
0 min
alpha-Amyslase (U/ml)
200
180
160
140
120
100
80
60
Control
MS
Stress

Appendix A

Table A1.

Means and standard deviations for salivary cortisol per condition at each time of measurement.

	Baseline	Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
Condition	*M(SD)*	*M(SD)*	*M(SD)*	*M(SD)*	*M(SD)*	*M(SD)*	*M(SD)*
Control	4.34(2.28)	4.40(2.52)	3.94(2.10)	3.34(1.60)	2.89(1.38)	2.60(1.23)	2.41(1.16)
MS	4.08(2.81)	4.07(2.52)	3.84(2.42)	3.37(2.01)	2.89(1.53)	2.44(1.26)	2.36(1.09)
Stress	3.97(2.71)	4.11(2.78)	7.21(4.41)	8.89(6.53)	6.96(5.67)	4.78(3.73)	3.57(2.28)