

Illuminating the Dark Side of Stress:
A mortality salience-based psychosocial stressor

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Abstract

Mental health professionals are concerned about the increasing prevalence of stress-related illnesses. Elevated levels of the hormone cortisol are implicated in these illnesses, and have consequences for affective, behavioural, and cognitive functioning. To better understand these consequences, it is imperative that neuroscientists develop laboratory-based protocols to study fluctuations in cortisol levels and, subsequently, relations between stress, cortisol, affect, and cognition. However, no existing protocol has induced robust and sustained cortisol responses in both men and women. We aimed to address this problem by developing a novel laboratory-based stress-induction protocol – the Mortality Salience Stress Test (MSST). Because contemplating one's own death creates an indiscriminate, ever-present potential to experience anxiety, we combined an existing stress-induction protocol with a mortality salience manipulation. We then measured salivary cortisol levels, heart rate and subjective anxiety in a sample of undergraduate students assigned pseudorandomly to one of four groups: Stress-Male ($n = 12$), Control-Male ($n = 11$), Stress-Female ($n = 10$), and Control-Female ($n = 11$). Those in the Stress groups were exposed to the MSST; those in the Control groups were exposed to an equivalent, non-stressful control condition. In both men and women, and relative to the control condition, the MSST produced significantly elevated salivary cortisol, heart rate, and subjective anxiety. However, some sex differences were observed with regard to magnitude of elevation and sustainability of cortisol responses. Future research should seek to better attenuate these sex differences and should directly compare the MSST with other stress-induction protocols.

Keywords: stress; salivary cortisol; HPA-axis; mortality salience; sex differences, heart rate, subjective anxiety

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Medical and mental health professionals are greatly concerned about the increasing prevalence of stress-related illnesses (Kudielka, Hellhammer, & Wüst, 2009). Consequently, it is imperative to investigate the biological bases of these illnesses (Kemeny, 2003). Empirical research has shown, consistently, that elevated levels of the stress hormone cortisol, resulting from overactivity of the hypothalamic-pituitary-adrenal (HPA) axis, are implicated in these illnesses (Dickerson & Kemeny, 2004; Kudielka & Wüst, 2010). Therefore, to investigate the effects of stress on human psychobiological, cognitive, and affective processes, and hence on human health, it is important to design studies featuring experimental stimulation of the HPA axis. Many existing laboratory-based stress-induction methods attempt to do this; however, each has limitations. The current study describes an improved laboratory-based psychosocial stressor.

Background

Physiological Responses to a Stressful Event

A *stressor* is any perceived threat to one's physical or psychological wellbeing (Kemeny, 2003). Prolonged exposure to stressors can result in impaired physical and mental health. In humans, stress affects three physiological systems: (a) the sympathetic nervous system, (b) the immune system, and (c) the HPA axis (Dickerson & Kemeny, 2004).

The HPA axis is of central concern in stress research: It is responsible for the release of cortisol into the urine, blood, and saliva of humans. Cortisol dysregulation is correlated with the presence of many stress-related diseases and disorders (Kudielka et al., 2009; Kudielka & Wüst, 2010). Hence, studying the precursors and effects of elevated cortisol, under controlled laboratory conditions, is an important step in developing measures to counteract such dysregulation.

Existing Laboratory-Based Stress-Induction Methods

Empirical studies (e.g., Kirschbaum, Pirke, & Hellhammer, 1993; Schwabe, Haddad, & Schachinger, 2008; Smeets et al., 2012) demonstrate that acute psychological stressors activate the HPA axis in that they result in elevated cortisol levels. Experimental research into the effects of stress on cognitive systems has utilised several different laboratory-based procedures in an attempt to elicit a physiological stress response. These studies have demonstrated, conclusively, that cortisol mediates the effects of stress on cognition.

The Trier Social Stress Test (TSST; Kirschbaum et al., 1993) and the Cold Pressor Test (CPT; Hines & Brown, 1932) are used frequently to induce stress in the laboratory. The TSST requires participants to deliver a speech and to perform a mental arithmetic task in front of a panel of judges; hence, it involves social-evaluative components and is a psychological means of inducing a stress response. The CPT, in contrast, requires participants to submerge their dominant hand in ice water for a few minutes; hence, it involves physiological induction of the stress response.

Although both procedures provoke physiological stress responses reliably, each is limited in certain ways. The CPT activates the sympathetic nervous system, but does not stimulate the HPA axis strongly, and is consequently less proficient at eliciting cortisol responses (McRae et al., 2006). In contrast, the TSST elicits cortisol responses more reliably than the CPT because it incorporates psychosocial evaluative threat, unpredictability, and uncontrollability (Dickerson & Kemeny, 2004). The TSST does not, however, produce a consistent, robust, and sustained cortisol response in all participants (Buchanan & Tranel, 2008; Kudielka et al., 2009). Numerous studies report that cortisol elevations are greater in men than in women after TSST exposure (Kudielka & Kirschbaum, 2005).

These sex differences are not unique to the TSST: There are clear sex differences in HPA-axis response patterns, and so, in reaction to psychosocial stressors, men consistently show a cortisol response almost twice that of women (Kudielka et al., 2009). One explanation for these differences involves the effect that menstrual cycle phase has on the magnitude of cortisol elevation in response to psychosocial stress (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Whereas women in the follicular phase of the cycle demonstrate considerably lower cortisol responses than men following TSST exposure, women in the luteal phase demonstrate similar responses to men. Furthermore, women using oral contraceptives show diminished cortisol responses to psychosocial stress (Kirschbaum, Pirke, & Hellhammer, 1995). Another explanation considers the possibility of sex differences in the psychological response to stress-induction methods. Stroud, Salovey, and Epel (2002) found that women demonstrated greater cortisol responses to social rejection challenges (e.g., task performance judged negatively by others), whereas men demonstrated greater cortisol responses to achievement challenges (e.g., the TSST).

To address some of these limitations of the TSST, Smeets et al. (2012) developed the Maastricht Acute Stress Test (MAST): a combination of the most successful physiological and psychological components of the CPT and TSST, respectively. Although the authors reported that the MAST elicited robust cortisol responses in comparison to the CPT and the

TSST, the study was limited in that these elevated responses were not sustained over time (i.e., they diminished sharply after 10 minutes). Another major limitation of Smeets et al.'s study was that their sample consisted of only male participants; hence, it did not demonstrate the effectiveness of the protocol in women.

du Plooy, Thomas, Henry, Human, and Jacobs (under review) also devised a stress-induction protocol, the Fear Factor Stress Test (FFST), that combines the TSST and the CPT. Participants are asked to imagine undergoing an audition for the reality television show *Fear Factor*. As part of the 'audition,' they are required to deliver a motivational speech, perform a verbal arithmetic task, and place one hand into a bucket of ice water. Preliminary data showed that, although the FFST did not elicit greater subjective anxiety responses, sympathetic activation or cortisol responses than the TSST, it did produce a sustained cortisol response over a longer period and in a greater proportion of participants. The FFST also produced a significant increase over the TSST in the number of women who demonstrated a sustained cortisol response. Hence, du Plooy et al. asserted that the FFST reduces the magnitude of (but does not eliminate) sex differences often seen post-TSST.

These findings suggest that the combination of physiological and psychological components in the MAST and the FFST, and not simply the occurrence of each component in isolation, produces an increased HPA-axis response, and therefore a more sustained cortisol response, than that seen following TSST exposure. This conclusion is consistent with Dickerson and Kemeny's (2004) assertion that laboratory-based stressors containing uncontrollable, social-evaluative, and physiological elements are associated with the largest and most sustained cortisol responses.

A continuing problem with these protocols, however, is that they lack ecological validity: They require participants to perform tasks that do not necessarily approximate real-world events or settings. For instance, delivering a job-interview speech is not a frequently encountered stressful experience, particularly in the undergraduate samples that comprise the typical TSST study. Similarly, stress associated with delivering a speech regarding one's suitability for an appearance on a television show is also not commonly encountered by most individuals. Improving ecological validity in experimental protocols is important for the application of laboratory-based conclusions to real-world settings (Orne & Holland, 1968).

Mortality Salience

Humans have self-awareness and an ability for symbolic thought that allows for the comprehension of imminent death (Tritt, Inzlicht, & Harmon-Jones, 2012). Landau, Solomon, Pyszczynski, and Greenberg (2007) suggest that this knowledge is the largest source

of debilitating anxiety in psychological functioning: From an evolutionary perspective, it conflicts directly with the dominant and primal drive for continued survival.

Mortality salience (MS) laboratory manipulations induce an awareness of participants' inevitable death and investigate subsequent effects on worldview defence (Arndt, Allen, & Greenberg, 2001; Burke, Martens, & Faucher, 2010). Although Arndt et al. (2001) showed that MS inductions result in physiological signs of subjective anxiety, MS research generally makes use of a black box approach that does not delineate biological mechanisms that may underlie social or cognitive changes following the experimental manipulation. Tritt et al. (2012), however, propose a neuroscientific understanding of MS effects. They suggest that model the resolution of death-related uncertainty is processed in brain-based anxiety systems. These systems produce raised levels of cortisol and other stress hormones in response to the experience of uncertainty.

Previous social psychological research has investigated the effects of MS manipulations on a range of human behaviour (see, e.g., Burke et al., 2010). However, there is no study, to our knowledge, that has investigated MS as a part of a stress protocol within a neuropsychological framework. Applying Tritt et al.'s (2012) notion that cortisol levels increase following MS manipulations, we investigated whether incorporating an MS manipulation into the FFST results in a more efficient psychosocial stressor.

Rationale, Specific Aims, and Hypotheses

Existing laboratory-based stress-induction protocols produce, in many participants, a physiological stress response via stimulation of the HPA axis. Although the combination of physiological and psychological stressors in the MAST and FFST elicits a more sustained HPA-axis response than the TSST and the CPT separately, there are a number of limitations of the FFST. These include (a) a lack of ecological validity, (b) sex differences in cortisol responses, and (c) a lack of robust and sustained cortisol responses in all exposed participants.

Because the uniquely human awareness of the inevitability of death is at odds with the desire for continued life, and creates an indiscriminate, ever-present potential to experience anxiety (Landau et al., 2007), we sought to investigate whether the addition of an MS manipulation to the FFST would improve the ecological validity of this stress-induction protocol. Moreover, to attenuate sex differences, we sought to reduce the achievement components present in existing protocols such as the TSST and FFST. Finally, because the concept of death is a highly pertinent and authentic threat to existence, we proposed that the

stressor described here would induce larger, and more sustained, cortisol responses in a greater proportion of participants.

Overall, we addressed the limitations of existing laboratory-based stress-induction protocols by investigating the efficacy of a novel protocol called the *Mortality Salience Stress Test (MSST)*. We hypothesised that, compared to participants exposed to a control condition, those exposed to the MSST would show (1) an elevated and sustained cortisol response across all measurement points during and post-manipulation, (2) elevated heart rate during the manipulation, with a decline immediately post-manipulation, and (3) elevated subjective anxiety during the manipulation, with a decline immediately post-manipulation. We also hypothesised that (4) there would be no significant sex differences on both physiological and self-report measures of stress.

Methods

Design and Setting

The study employed a 2 x 2 x 5 (Experimental Condition x Sex x Time) repeated-measures factorial design. Between-subject variables were Experimental Condition (Stress versus Control) and Sex (male versus female). The within-subject variable was Time; measurement points for this variable were once before the manipulation (baseline) and four times post-manipulation. Outcome variables included salivary cortisol levels, heart rate, and subjective anxiety.

Study procedures took place between 14h00 and 18h30 to control for cortisol's diurnal cycle. Studies utilising acute psychosocial stressors are best run in the late afternoon (i.e., when cortisol levels are at their lowest and most constant) because this is when changes in cortisol due to a stressor are most easily identifiable (Maheu, Collicut, Kornik, Moszkowski, & Lupien, 2005).

We ran the study in two venues in the Department of Psychology at the University of Cape Town (UCT). In the first venue, a computer laboratory, participants completed all self-report and physiological measures. In the second venue, a nearby smaller room, participants were exposed to the experimental manipulation.

Participants

Forty-four healthy university students (23 men, 21 women) met the inclusion criteria and provided largely complete data sets for analysis. A power analysis suggested that using a sample of this size leads to an achieved power of .94 when α is set at .05 and the assumed effect size is set at .25 (i.e., is of medium magnitude; Faul, Erdfelder, Lang, & Buchner, 2007). Cohen (1992) advises a statistical power greater than .80 in order to achieve an

acceptable effect size. Therefore, we argue that our design achieved an acceptable level of statistical power. High levels of statistical power are important as they demonstrate the ability of laboratory-based findings to find an effect in the data when the effect exists in the real world (Faul et al., 2007).

We recruited participants from undergraduate psychology classes at UCT using the Student Research and Participation Programme (SRPP); hence, we employed a self-selected sampling method. Potential participants were notified of the study's availability and of the exclusion criteria via the SRPP website. Male participants signed up for a test session using the site interface. Sign-up procedures for females are documented below.

Participants who survived the exclusion criteria listed below were pseudorandomly assigned to one of four groups: Stress-Female, Control-Female, Stress-Male, or Control-Male.

Exclusion criteria. We investigated participant eligibility using a sociodemographic questionnaire and the other screening instruments described below. Exclusion criteria included: (a) smoking, (b) a Beck Depression Inventory – Second Edition (BDI-II; Beck, Steer, & Brown, 1996) score of ≥ 29 (indicating current experience of severe depression), (c) the use of any prescription or steroid-based medication, including oral contraceptives, and (d) a body mass index (BMI) of more than 31 or less than 18. Research investigating the effects of psychosocial stress on cognitive performance has identified these factors as potentially confounding variables (Kirschbaum et al., 1999; Wirtz, Ehlert, Emini, & Suter, 2008).

To improve on a previous methodological study conducted by our laboratory (du Plooy et al., under review), we controlled for females' menstrual cycle phase and oral contraceptive use. Both of these have a significant effect on HPA-axis responsiveness to psychosocial stress (Kirschbaum et al., 1995, 1999). Hence, we required that female participants be in the luteal phase of their cycle (i.e., the 12 days preceding the start of their menses; Kirschbaum, Wüst & Hellhammer, 1992) when they experienced the experimental protocols; this phase is when the female HPA-axis response is most similar to that of men. Hence, to sign up for the study, we asked female participants to contact us directly, via e-mail, with their name, student number, and contact number. In the e-mail, they were asked to verify details regarding the regularity of their menstrual cycle, as well as the estimated starting date of their next period. Thereafter, we offered and confirmed a date for a test session on a day within the 12-day period of their luteal phase.

Materials and Manipulations

Participant self-report measurements. The following instruments collected information on participant eligibility, affective traits, and affective state before, during, and after the experimental manipulation.

Sociodemographic questionnaire. This questionnaire (see Appendix A) gathered information regarding age, sex, smoking habits, medication intake, and current and past psychological illness.

Beck Depression Inventory-II (BDI-II). This is a self-report questionnaire containing 21 items. Each item has four possible responses, indicating differing degrees of possible depressive symptomatology. Respondents are asked to choose the response that best approximates how they have been feeling for the 2 weeks prior to reporting. Higher scores indicate greater levels of depression; scores ≥ 29 (the cut-off in this study) indicate severe depression (Beck et al., 1996).

The BDI-II was developed to comply with the diagnostic criteria for major depression listed in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; American Psychiatric Association, 1994). It has high levels of internal consistency ($\alpha = .91$; Dozois, Dobson, & Ahnberg, 1998), and good test-retest reliability ($\alpha = .93$; Beck et al., 1996).

We used the BDI-II as a screening measure: Participants scoring ≥ 29 or above were excluded from the study. Those scoring less than 29 but scoring 2 or more on suicidal ideation items were also excluded because the experimental manipulation required that participants contemplate their death.

State-Trait Anxiety Inventory (STAI). The STAI (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) contains (1) the STAI-State (Form Y-1), which measures an individual's anxiety at a particular time point (state anxiety), and (2) the STAI-Trait (Form Y-2), which measures an individual's general level of anxiety (trait anxiety). Both forms consist of 20 items expressed as statements; each statement is rated using a 4-point Likert-type scale.

The STAI has been used widely with undergraduate student populations and has acceptable reliability levels ($\alpha = .92$). Additionally, it has a reliable factor structure, high levels of validity, and high levels of internal consistency (Spielberger & Vagg, 1984).

We used the STAI-Trait to measure general levels of anxiety in the participants and to ensure that, across groups, participants were experiencing comparable levels of anxiety in

their daily lives. The STAI-State measured changes in self-reported anxiety at five points during the study.

Physiological measurements. We used the apparatus described below to collect information on participant physiological states before, during, and after the experimental manipulation.

Salivary cortisol. We used SARSTEDT Salivette[®] Cortisol swabs (Sarstedt, Nümbrecht, Germany) to collect saliva samples from each participant. The swab is a simple and non-intrusive means of collecting cortisol samples without causing participants undue discomfort (Garde & Hansen, 2005). For each collection, we instructed the participant to chew on a cotton swab for 1 minute. Thereafter, we stored the samples in individual tubes and froze them until they were transported to the National Health Laboratory Services at Groote Schuur Hospital, where they underwent analysis.

Heart rate. We fitted participants with a Vrije Universiteit Ambulatory Monitoring System, version 5fs (VU-AMS; Vrije Universiteit, Amsterdam, Holland) at the beginning of each session. The VU-AMS is a non-invasive, portable device that allowed participants to move between the two study venues whilst wearing it. The device measured heart rate continuously until it was removed at the end of the study.

The experimental manipulation. Each participant experienced either the MSST or a control condition that mimicked the MSST but was devoid of its purported stress-inducing components.

Mortality Salience Stress Test (MSST). This is a laboratory-based psychosocial stressor that consists of three components: (a) a 5-minute free-form speech, (b) a 5-minute mental arithmetic task, and (c) a 2-minute physical challenge in which the participant is asked to submerge his/her hand in ice water. This stressor aims to improve on the FFST by modifying the protocol to include an MS manipulation: Instead of asking participants to write a speech that will convince the judges of their suitability for the television show, it asks them to write a speech wherein they speak about their own imminent death. In comparison with the content of the TSST and FFST speech components, the MSST speech component has no competitive element and thus seeks to reduce achievement challenges present in the other protocols.

In accordance with research utilising the FFST protocol (du Plooy et al., under review; Human et al., 2013), we informed participants that they would be auditioning for a place on the reality television show *Fear Factor*. We then gave them a set of standardised instructions that introduced them to the task. The instructions informed them they would be

auditioning for the role in front of a panel of behavioural health experts who would analyse their verbal and nonverbal behaviour with the aid of a video recording. We told participants that the audition comprised three tasks: (a) a speech about the circumstances of their death that would demonstrate their ability to withstand the psychological pressures associated with an appearance on *Fear Factor*; (b) a mental arithmetic task that would test their ability to perform under pressure; and (c) a test of pain tolerance that would test their ability to withstand the physical demands of the television show.

We then instructed the participant to prepare a 5-minute speech describing the circumstances of their death in detail, the emotions and the thoughts that death arouses in them, and what they thought would happen to them as they physically die and after they are physically dead (Landau et al., 2007). The participant received a blank piece of paper and 10 minutes to prepare the speech. Thereafter, we took him/her to a room where s/he completed the rest of the stress manipulation. This room was lit only by a bright spotlight, directed at the participant, and contained a video camera and a panel of two judges (one man and one woman). We instructed the participant to stand directly in front of the judges, who were seated behind a desk and who were dressed smartly to convey the appearance of behavioural experts. Just before the procedure commenced, we removed, without warning, the piece of paper containing the participant's prepared speech. The judge of the opposite sex to the participant then instructed him/her to present the speech. If the participant hesitated or stopped speaking before the time elapsed, the same judge followed a script specifying how to respond to the participant (see Appendix B).

Following the speech, the judge of the opposite sex to the participant asked him/her to perform a serial subtraction task (subtracting 17 from 2043 continuously). If the participant performed an incorrect subtraction, the same judge instructed him/her to start the task from the beginning. Finally, the judge of the same sex as the participant asked him/her to submerge his/her arm in a bucket of ice water (between 0 and 4°C) for as long as possible, up to a maximum of 2 minutes.

For the duration of the manipulation, judges maintained eye contact with the participant, but did not engage with him/her or show any signs of reinforcement or support. For the duration of the manipulation, the participant remained standing and the researcher remained in the room. The MSST took 22 minutes to complete.

Control. We instructed participants to write, on a single sheet of paper, a summary of everything they had done that day. After 10 minutes they were taken to a well-lit room where we instructed them to stand and read aloud from a general interest magazine for 5 minutes.

We left the room for the duration of this task. Thereafter, we re-entered the room and asked participants to count upwards in multiples of 5, starting from 0, for 5 minutes. Again, we left the room for the duration of this task. We then re-entered the room and instructed the participant to submerge his/her arm into warm water (34-38 °C) for as long as possible, up to a maximum of 2 minutes.

Procedure

Figure 1 outlines the experimental procedures.

Similar to previous studies conducted in our laboratory (e.g., Human et al., 2013), we reminded participants via e-mail the day before their session to refrain from eating, drinking (except water), or doing physical exercise for 2 hours before testing. Upon arrival, participants read and signed an informed consent document (Appendix C). Thereafter, we administered the sociodemographic questionnaire, the BDI-II, and the STAI-Trait. Because participants who met the depression exclusion criterion were not allowed to continue with the rest of the experiment, we scored the BDI-II while participants completed the STAI-Trait. Thereafter we measured the participants' height and weight in order to estimate BMI.

If these screening procedures established the participant's eligibility to continue, we fitted him/her with the VU-AMS and allowed a 5-minute rest period for the device to normalise to his/her heart rate. Then followed a 2-minute baseline heart rate reading (HR_B). We then administered the first STAI-State ($STAI_B$) and thereafter collected the first saliva sample ($CORT_B$). We then gave participants instructions for the speech-writing component of the experimental manipulation. After the allotted preparation time had elapsed, we escorted participants to the room in which the experimental manipulation took place (see Figure 1, Panel B). Upon completion of the experimental manipulation, participants returned to the research laboratory for a 5-minute relaxation period.

We then took physiological (HR_{2-4} ; $CORT_{1-4}$) and self-report ($STAI_{1-4}$) measurements, identical to baseline measures, at 5 minutes, 20 minutes, 40 minutes, and 55 minutes following the end of the experimental manipulation (see Figure 1, Panel A). However, as an exception, participants' average heart rate over the final 10 minutes of the stressor represented the second heart rate measurement (HR_1). Other post-manipulation measures, not relevant to this study, took place between the abovementioned measurement points.

Upon conclusion of these measurements, we removed the VU-AMS. We then debriefed participants with regard to the purpose of the study and asked them not to disclose information about the study to anyone else so that results would not be confounded.

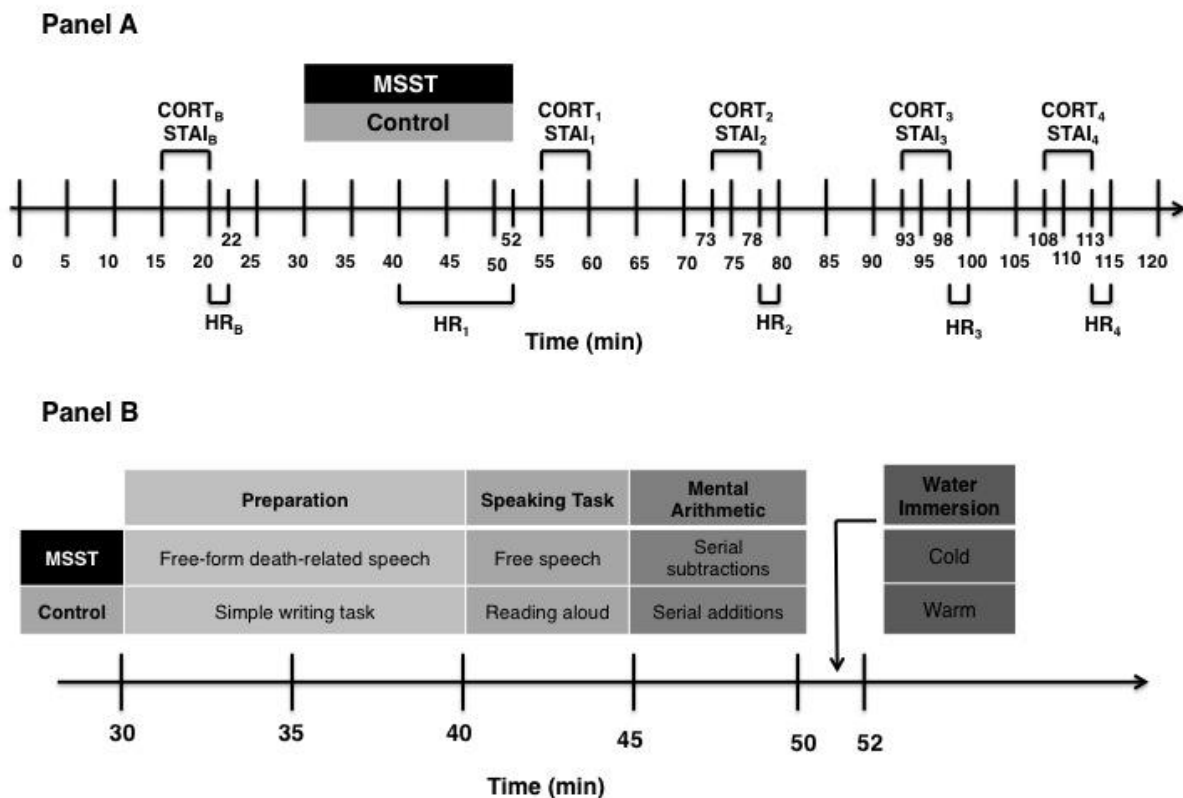


Figure 1. The procedure of the Mortality Salience Stress Test. (A) Timeline of events during the experimental procedures. (B) Sequence of tasks for the MSST. MSST = Mortality Salience Stress Test; CORT = salivary cortisol; STAI = State-Trait Anxiety Inventory, State form; HR = heart rate.

Ethical Considerations

The study adhered to the ethical guidelines for research with human subjects outlined by the Health Professions Council of South Africa and the University of Cape Town's Codes for Research. We received ethical approval from both the Human Research Ethics Committees of the UCT Department of Psychology and the UCT Faculty of Health Sciences (see Appendices D and E).

All individuals wishing to participate did so voluntarily. We issued potential participants with an informed consent document that outlined the purpose of the study, stated what would be expected of them should they agree to participate, and mentioned that participant confidentiality would be ensured and upheld. The consent form also notified

potential participants of their right to withdraw from the study at any point without penalties or negative consequences.

We debriefed all participants at the end of their participation. We also informed those who had been in the MSST condition that their performance in the ‘audition’ was not videotaped, and that their performance was not evaluated in any way by the judges. We explained that for the psychosocial stressor to have maximum effect, it was necessary to deceive them in this way.

The risks involved in participating included experiencing a mildly stressful situation. However, there were no other discomforts or risks associated with participation. If participants were excluded from the study based on their BDI-II scores/responses, or if they showed signs of subjective distress at the end of the study, we provided them with contact details for the UCT Student Wellness Centre so that they could seek counselling services if so desired. Although one participant (a 22-year-old female in the Stress condition) took the option to withdraw from the study following the experimental manipulation, no participants reported remaining in a subjectively distressed state at the end of the study.

Regarding benefits of participation, all participants received course credit via the SRPP system.

Data Management and Statistical Analyses

Details about specific analyses are provided before presentation of the results. We conducted all statistical analyses using IBM[®] SPSS[®] Statistics Version 21. We set the threshold level of statistical significance (α) at .05, and calculated the appropriate effect size estimate for each analysis.

Before beginning inferential analyses, we ensured that the data met the assumptions underlying each proposed parametric test. Unless otherwise stated, all of the required assumptions were upheld for each statistical analysis. In repeated-measures ANOVA analyses where Mauchley’s test indicated that the assumption of sphericity was violated, we used Greenhouse-Geisser estimates for corrected degrees of freedom. Furthermore, unless otherwise stated there were no significant differences in baseline measurements for physiological and self-report data, and therefore we did not make use of difference scores under these conditions.

Results

Final Sample

Reasons for participant exclusion. Figure 2 diagrams the sources of attrition during the data collection process. Three of the 50 participants who enrolled in the study did not complete it. Two were excluded after completing the screening measures. One, assigned to the Stress-Female group, did not meet the BDI-II eligibility criterion. The other, assigned to the Control-Male group, did not meet the BMI eligibility criterion. The third participant, assigned to the Stress-Female group, opted to withdraw before completing the MSST. Of note here is that this participant differed significantly, in terms of demographic characteristics, from participants who did complete the study (see Appendix F).

Post-experiment self-report verification revealed that 21 of the 24 female participants who completed the experimental procedures were tested either in the luteal phase of their menstrual cycles or ± 3 days outside of the desired phase (see Figure 3). The other three female participants were excluded because (a) one, assigned to the Stress-Female group, had not reported beginning her menses more than 2 months after testing, (b) another, also assigned to the Stress-Female group, was tested 7 days out of the luteal phase, and (c) the third, assigned to the Control-Female group, was tested 5 days out of the luteal phase. Hence, our final analyses excluded the data from these three participants.

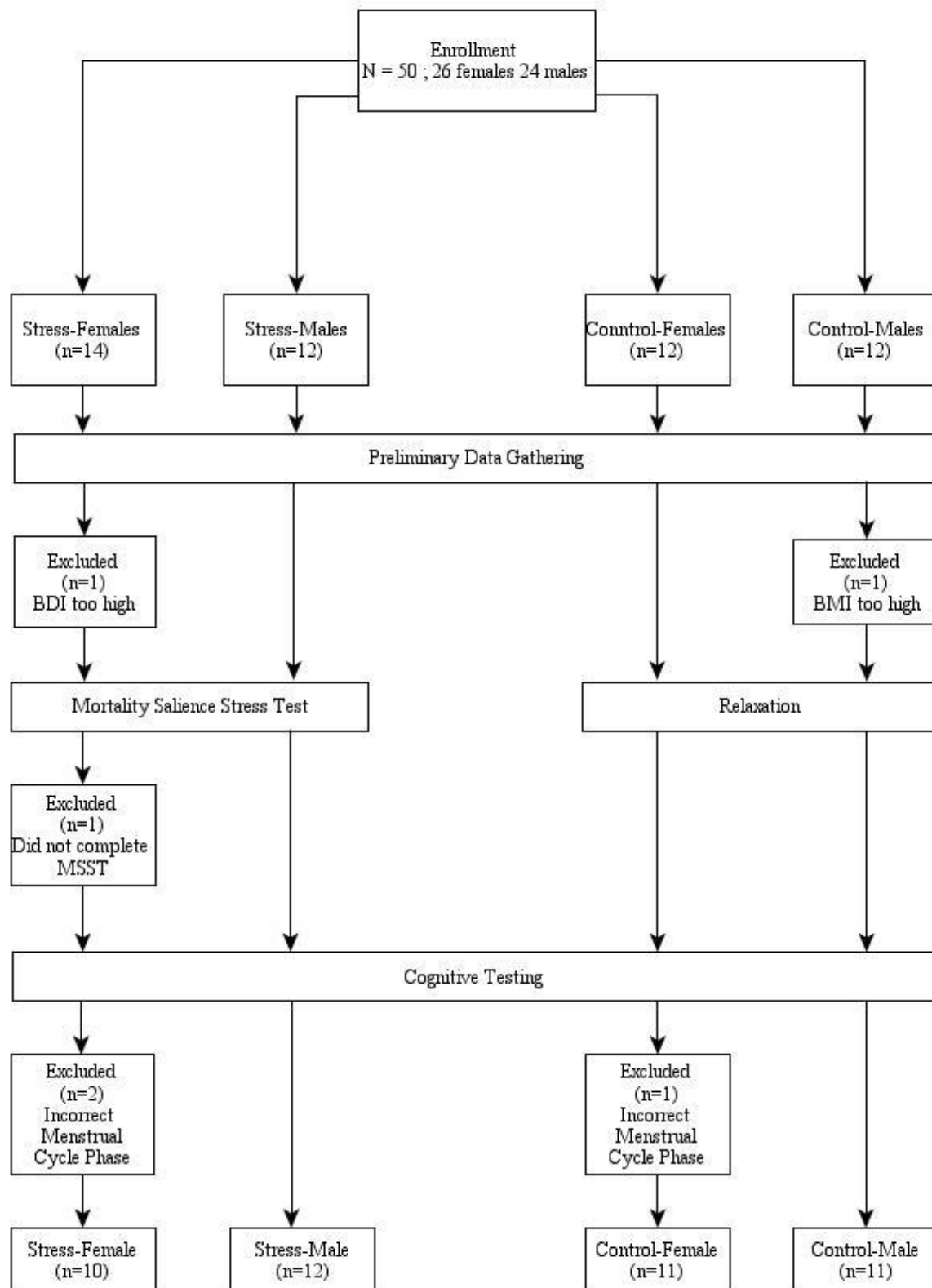


Figure 2. Diagram showing participant flow and attrition throughout the experimental procedures. Enrolled $N = 50$; final N for data analysis = 44.

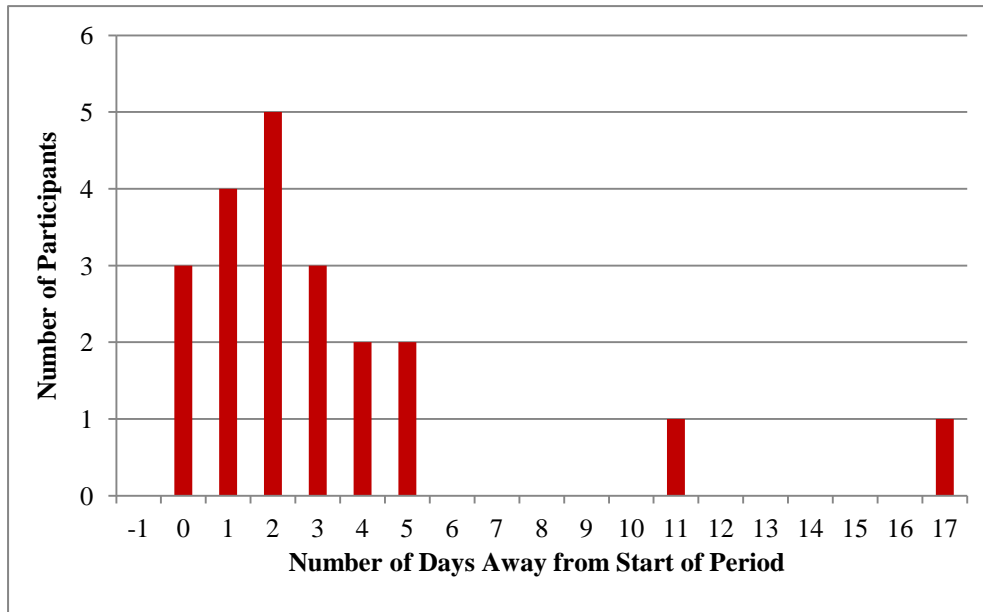


Figure 3. Female participants' proximity to menses phase.

Sample characteristics. Given the exclusions and attrition outlined above, data from 44 participants were included in the final analysis: Stress-Female $n = 10$; Stress-Male $n = 12$; Control-Female $n = 11$; Control-Male $n = 11$.

We conducted 2 x 2 (Experimental Condition x Sex) between-groups factorial ANOVAs to analyse participant data regarding age, BMI, BDI-II scores, and STAI-Trait scores (see Table 1). These analyses sought to ensure that the participants in the four groups were sampled from a similar population.

Table 1
Sample Demographic Characteristics ($N = 44$)

Variable	Group			
	Stress-Female ($n = 10$)	Stress-Male ($n = 12$)	Control-Female ($n = 11$)	Control-Male ($n = 11$)
Age	20.00 (2.31)	20.00 (0.85)	19.91 (1.22)	19.91 (1.30)
BMI	23.96 (3.54)	24.07 (2.60)	24.84 (3.80)	22.06 (1.85)
BDI-II	11.10 (6.38)	8.17 (6.52)	9.45 (7.34)	10.18 (9.22)
STAI-Trait	43.30 (9.18)	38.42 (10.51)	37.27 (8.73)	39.82 (12.29)

Note. Data presented are means, with standard deviations in parentheses. BMI = body mass index; BDI-II = Beck Depression Inventory-Second Edition; STAI = State-Trait Anxiety Inventory, Trait form.

Age. Overall, the participant age range was 18-26 years ($M = 19.95$, $SD = 1.43$). The factorial ANOVA did not detect significant any significant main or two-way interaction effects, $F_s < 0.05$, $p_s > .80$, η_p^2 's $< .01$.

BMI. Overall, the participant BMI range was 18-30 units ($M = 23.73$, $SD = 3.10$). The mean BMI for each group suggests that, on a group basis, all participants fell within the defined 'normal' range of 19-25. The factorial ANOVA did not detect any significant main or two-way interaction effects, $F_s < 2.50$, $p_s > .10$, η_p^2 s $< .07$.

BDI-II scores. Overall, the participant BDI range was 0-28 ($M = 9.66$, $SD = 7.27$). The mean BDI-II score for each group suggests that, on a group basis, the sample fell within the range described as 'minimal' depression, indicating low levels of depressive symptomatology (Beck et al., 1996).

The factorial ANOVA did not detect any significant main or two-way interaction effects, $F_s < 0.70$, $p_s > .40$, η_p^2 s $< .03$.

We conducted a single sample t -test to examine whether the average BDI-II score of the current sample differed from normative data for college students provided by the test manual ($M = 12.56$, $SD = 9.93$; Beck et al., 1996). The test detected a significant cross-sample difference, $t(43) = -2.65$, $p = .011$, $d = 0.07$. This result suggests that the current sample had significantly *lower* levels of depressive symptomatology than the general population of university students. This pattern of data does not present a problem for the interpretation of stress-related data.

STAI-Trait scores. Overall, the participant score range on this instrument was 23-58 ($M = 39.59$, $SD = 10.19$). The factorial ANOVA did not detect any significant main or two-way interaction effects, $F_s < 1.50$, $p_s > .20$, η_p^2 s $< .04$.

We conducted single-sample t -tests to examine whether male ($M = 39.09$, $SD = 11.16$) and female participants ($M = 40.14$, $SD = 9.25$) differed from normative data (men: $M = 38.30$, $SD = 9.18$; women: $M = 40.40$, $SD = 10.15$) for college students provided by the STAI manual (Spielberger et al., 1983). The analyses detected no significant differences: men, $t(22) = 0.338$, $p = .738$, $d = .08$; women, $t(20) = -.127$, $p = .900$, $d = -.03$. Hence, it appears that this sample is representative of the general population of college students.

Taken together, these results suggest there were no significant between-group differences in average age, BMI, BDI-II score, and STAI-Trait score. Therefore, the results presented below were not confounded by pre-existing differences in age, physique, depressive symptomatology, or trait anxiety.

Experimental Manipulation

To test the effectiveness of the experimental manipulation, we first conducted 2 (Experimental Condition) x 2 (Sex) x 5 (Time) repeated-measures ANOVAs for each major

outcome variable (salivary cortisol; heart rate; STAI-State scores). We followed up each ANOVA with a set of planned contrasts to test pre-existing hypotheses regarding between- and within-group differences.

Table 2 presents descriptive statistics for each of the relevant physiological and self-report outcome measures.

Table 2

Self-Report and Physiological Measures of Stress: Descriptive statistics (N = 44)

Measure	Group			
	Stress-Female (n = 10)	Stress-Male (n = 12)	Control-Female (n = 11)	Control-Male (n = 11)
Cortisol				
CORT _B	7.88 (2.65) ^a	15.84 (7.14)	10.31 (2.99)	11.77 (4.14)
CORT ₁	10.00 (5.25) ^a	22.84 (8.28)	8.72 (2.20)	8.58 (2.56)
CORT ₂	10.71 (5.04) ^a	23.26 (7.81)	8.96 (2.09)	8.93 (2.50)
CORT ₃	9.62 (4.41) ^a	18.07 (5.31)	8.21 (1.91)	9.18 (1.86)
CORT ₄	7.98 (3.00) ^a	15.00 (3.74)	8.08 (1.84)	8.68 (1.80)
CORT _{Δ1}	2.12 (3.06) ^a	7.00 (4.62)	-1.59 (1.48)	-3.20 (1.96)
CORT _{Δ2}	2.83 (4.01) ^a	7.42 (6.05)	-1.35 (1.82)	-2.84 (2.57)
CORT _{Δ3}	1.74 (3.07) ^a	2.23 (5.06)	-2.10 (2.10)	-2.59 (3.17)
CORT _{Δ4}	0.10 (1.88) ^a	-0.84 (5.48)	-2.23 (2.22)	-3.10 (3.32)
Heart Rate				
HR _B	73.13 (8.65)	77.24 (15.22) ^b	75.71 (8.80)	74.70 (12.23)
HR ₁	102.39 (11.91)	103.58 (24.05) ^b	88.00 (10.06)	88.40 (14.89)
HR ₂	76.89 (7.85)	79.98 (16.97) ^b	76.98 (10.23)	75.31 (12.22)
HR ₃	76.12 (7.11)	80.17 (16.48) ^b	75.68 (9.44)	74.69 (11.42)
HR ₄	78.33 (8.07)	79.88 (17.47) ^b	75.93 (9.06)	75.49 (13.28)
STAI-State				
STAI _B	35.56 (9.17) ^a	31.50 (5.93)	32.55 (8.81)	32.60 (7.44) ^b
STAI ₁	54.00 (13.90)	41.75 (9.57)	31.00 (4.65)	34.27 (9.64)
STAI ₂	38.80 (8.70)	31.25 (7.03)	29.82 (4.75)	31.55 (7.71)
STAI ₃	43.70 (9.50)	31.83 (10.43)	31.36 (5.99)	34.18 (9.66)
STAI ₄	38.00 (9.76)	30.67 (9.23)	29.18 (4.49)	35.82 (11.50)

Note. Means are presented with standard deviations in parentheses. Cortisol levels are measured in nanomoles per litre (nmol/l). Heart rate levels are measured in beats per minute (bpm). STAI = State-Trait Anxiety Inventory. Subscripts represent measurement point (e.g., HR_B is the first HR measurement point, or baseline; HR₁ is the second HR measurement point, or first beyond baseline; and so on). Δ represents a difference score, so that CORT_{Δ1} is CORT₁ - CORT_B, and so on.

^an = 9. ^bn = 10

Physiological measures of stress. Below, we present analyses of salivary cortisol and heart rate data separately.

Salivary cortisol. Data for a participant in the Stress-Female group were excluded from the analysis because her cortisol levels were much higher than the rest of the sample. Her baseline cortisol level (394.10 nmol/l) was 165 standard deviations above the mean of the group to which she had been assigned ($M = 7.87$, $SD = 2.38$), and was 59 standard deviations above the mean of all Stress-group participants ($M = 12.03$, $SD = 6.68$). Her final (CORT₄) sample (267.10 nmol/l) was 93 standard deviations above the mean of the Stress-Female group ($M = 7.68$, $SD = 2.86$) and was 56 standard deviations above the mean of all Stress-group participants ($M = 11.50$, $SD = 4.75$). Hence, it is possible that there were errors in data collection and/or cortisol analysis for this participant's saliva samples.

Informal inspection of group means suggested that men presented with considerably higher circulating levels of cortisol at baseline than women. Hence, we conducted a 2x2 factorial ANOVA on these baseline data. The analysis confirmed a significant main effect for Sex, $F(1, 39) = 10.46$, $p = .002$, $\eta_p^2 = .21$. Therefore, following du Plooy et al. (under review), we used difference scores as outcome data in our subsequent analyses of cortisol levels. To obtain these scores we subtracted the baseline measure from those at the second, third, fourth, and fifth measurement points as follows:

$$\text{CORT}_{\Delta 1} = \text{CORT}_1 - \text{CORT}_B$$

$$\text{CORT}_{\Delta 2} = \text{CORT}_2 - \text{CORT}_B$$

$$\text{CORT}_{\Delta 3} = \text{CORT}_3 - \text{CORT}_B$$

$$\text{CORT}_{\Delta 4} = \text{CORT}_4 - \text{CORT}_B$$

The repeated-measures ANOVA run on these difference-score data detected a significant main effect for Time, $F(1.91, 74.60) = 15.88$, $p < .001$, $\eta_p^2 = .29$, and a significant main effect for Experimental Condition, $F(1, 39) = 31.27$, $p < .001$, $\eta_p^2 = .45$. It did not, however, detect a significant main effect for Sex, $F(1, 39) = 0.38$, $p = .54$, $\eta_p^2 = .01$.

Regarding interaction effects, the analysis detected no significant Experimental Condition x Sex interaction, $F(1, 39) = 3.28$, $p = .08$, $\eta_p^2 = .08$. It did, however, detect the following significant interaction effects: Time x Experimental Condition, $F(1.91, 74.60) = 11.61$, $p < .001$, $\eta_p^2 = .23$, Time x Sex, $F(1.91, 74.60) = 3.26$, $p = .046$, $\eta_p^2 = .08$; and Time x Experimental Condition x Sex, $F(1.91, 74.60) = 6.14$, $p = .004$, $\eta_p^2 = .14$.

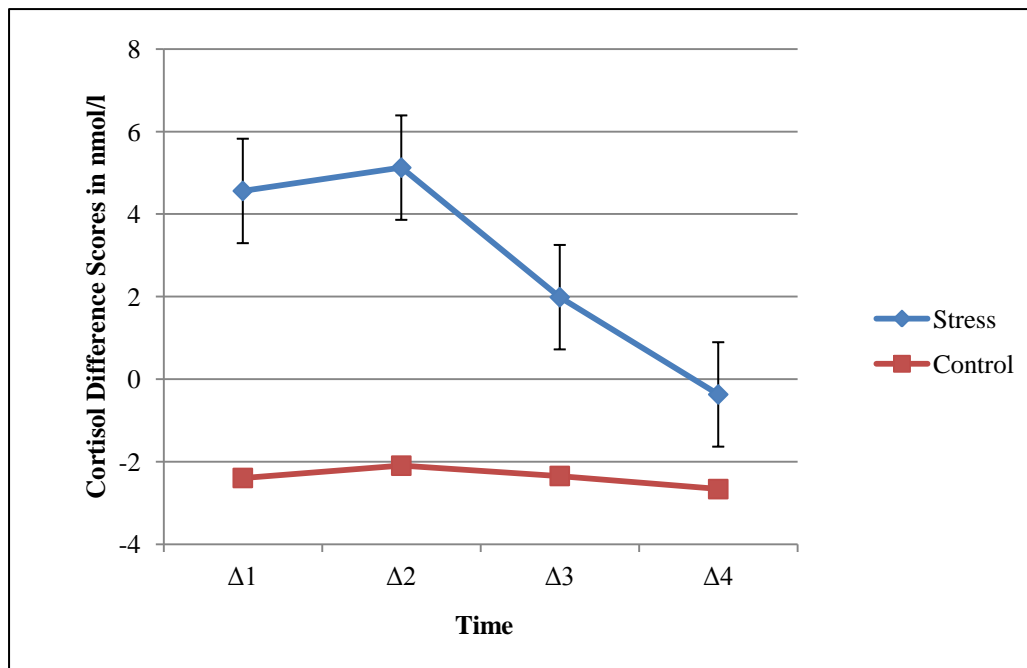


Figure 4. Changes in cortisol levels (measured in nmol/l) for the combined Stress and Control groups ($N = 43$). Error bars indicate standard error of means. Δ represents a difference score, so that $CORT_{\Delta 1}$ is $CORT_1 - CORT_B$, and so on.

We investigated the Time x Experimental Condition interaction further using a series of planned contrasts. These analyses revealed significant differences between the values of the combined Stress and Control groups at $CORT_{\Delta 1}$, $F(1, 164) = 40.60$, $p < .001$, $d = 1.98$; at $CORT_{\Delta 2}$, $F(1, 164) = 43.18$, $p < .001$, $d = 1.69$; at $CORT_{\Delta 3}$, $F(1, 164) = 14.84$, $p < .001$, $d = 1.24$; and at $CORT_{\Delta 4}$, $F(1, 164) = 4.11$, $p = .044$, $d = 0.64$. Figure 4 shows these differences.

To further investigate Hypothesis 1 (regarding elevated and sustained cortisol responses provoked by the MSST), we conducted a set of planned contrasts to examine, in the combined Stress and combined Control groups separately, differences in cortisol responses across time. In the Stress group, there was no significant difference between average values at $CORT_{\Delta 1}$ and $CORT_{\Delta 2}$, $F(1, 82) = 0.20$, $p = .65$, $d = 0.11$. There was, however, a significant difference between average values at $CORT_{\Delta 2}$ and $CORT_{\Delta 3}$, $F(1, 82) = 8.08$, $p = .006$, $d = 0.63$; values at $CORT_{\Delta 2}$ were significantly higher. Similarly, there was a significant difference between average values at $CORT_{\Delta 3}$ and $CORT_{\Delta 4}$, $F(1, 82) = 5.05$, $p = .027$, $d = 0.56$; values at $CORT_{\Delta 3}$ were significantly higher.

In contrast, in the combined Control group there were no significant differences between any of those consecutive pairs of values, $F_s < 0.10$, $p_s > .77$, $d_s < 0.19$.

Hence, as predicted, Control participants maintained low cortisol levels at all time points. Also as predicted, Stress-group participants showed elevated cortisol levels at the

immediate post-manipulation measure, and sustained this elevation for at least 20 minutes post-manipulation. Although there followed a decline in the response of the Stress group, they still demonstrated significantly higher responses than the Control group across all time points.

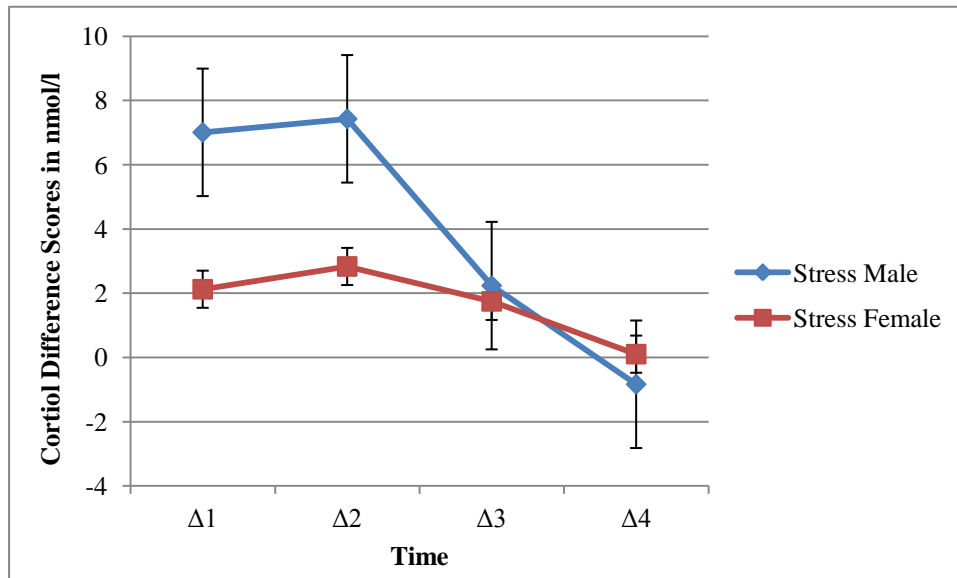


Figure 5. Changes in cortisol levels (measured in nmol/l) for the Stress-Male and Stress-Female groups ($N = 21$). Error bars indicate standard error of means. Δ represents a difference score, so that $CORT_{\Delta 1}$ is $CORT_1 - CORT_B$, and so on.

Figure 5 shows the differences in cortisol responses across time for the Stress-Male and Stress-Female groups. We conducted a set of planned contrasts on these data to investigate Hypothesis 4 regarding sex differences in cortisol responses. At $CORT_{\Delta 1}$ and $CORT_{\Delta 2}$, the Stress-Male group demonstrated significantly higher average values than the Stress-Female group, $F(1, 76) = 7.38, p = .008, d = 1.21$, and $F(1, 76) = 7.04, p = .01, d = 0.87$, respectively. There were no significant differences at either $CORT_{\Delta 3}$ or $CORT_{\Delta 4}$, however, $F_s < 0.90, p_s > .35, d_s < 0.23$.

The data suggest that men exposed to the MSST demonstrated, immediately following and at 20 minutes after the manipulation, significantly higher elevations in their cortisol responses than women exposed to the MSST. However, the men were not able to sustain this elevated response until the conclusion of the measurements at 55 minutes post-manipulation. Although the magnitude of female responses did not match that of male responses, they did demonstrate a sustained response until 40 minutes post-manipulation.

Cortisol responders and non-responders. Previous studies in this field (e.g., du Plooy et al., under review; Schwabe et al., 2008; Smeets et al., 2012) have presented analyses where

participants who have been exposed to an acute psychosocial stressor are classified, post-hoc, as either cortisol responders or non-responders. Such studies then go on to examine whether, for instance, effects on cognition are present only in the group of responders (see, e.g., Buchanan & Tranel, 2008; van den Bos, Harteveld, & Stoop, 2009).

Unfortunately, there is no standard criterion to classify a participant as a responder or non-responder. Hence, to compare our responder rates with those of previous studies, we created three different responder/non-responder sets. The first set followed Kirschbaum et al. (1993) and Smeets et al. (2012) by classifying cortisol responders as those showing at least a 2.5 nmol/l increase over baseline at any post-manipulation measurement point. The second set followed Fehm-Wolfsdorf et al. (1993) and du Plooy et al. (under review) by classifying cortisol responders as those showing at least a 2 nmol/l increase over baseline at any post-manipulation measurement point. The third set followed Buchanan and Tranel (2008) by classifying cortisol responders as those showing any increase over baseline at any post-manipulation measurement point. Table 3 shows the application of these criteria to the data.

Table 3

Cortisol Responders to the MSST Using Different Criteria (N = 21)

Criterion	Number of responders			
	CORT _{Δ1}	CORT _{Δ2}	CORT _{Δ3}	CORT _{Δ4}
+2.5 nmol/l	14 (67)	15 (71)	10 (48)	5 (24)
+2 nmol/l	15 (71)	15 (71)	10 (48)	7 (33)
Any increase	19 (90)	18 (86)	16 (76)	11 (52)

Note. Data presented are frequencies, with percentages in parentheses. Δ represents a difference score, so that CORT_{Δ1} is CORT₁ – CORT_B, and so on.

As the table shows, there were few differences between the +2.5 nmol/l and the +2 nmol/l criteria as they applied to our data: At the first two measurement points, both criteria classified most participants as responders; at the third measurement point, both classified almost half the participants as responders; and at the final measurement point, both classified the minority of participants as responders. In contrast, as one might expect, the “any increase” criterion classified almost all participants as responders at both the first and second measurement point; even at the final measurement point, this criterion classified more than half of the sample as responders.

We compared the response rates in our Stress group with those observed by du Plooy et al. (under review) in their comparison of the FFST and the TSST. Using the 2 nmol/l criterion, du Plooy et al. reported that, at CORT_{Δ1}, their TSST response rate was 57% (17 of

30 participants) and their FFST response rate was 48% (14 of 29 participants). In contrast, the MSST response rate observed here was 71% (15 of 21 participants). At $CORT_{\Delta 2}$, the TSST and the FFST produced response rates of 13% (4 of 30) and 52% (15 of 29), respectively, whereas the MSST produced a response rate of 48% (10 of 21) at the equivalent time ($CORT_{\Delta 3}$). The FFST's second measurement point occurred 35 minutes post-manipulation and the MSST's third measurement point occurred 40 minutes post-manipulation; this slight difference could account for the slightly lower response rate seen here.

Table 4 shows the application of the same three responder-classification criteria to our Stress-group data, this time split by sex. Again, the +2.5 nmol/l and +2 nmol/l criteria deliver almost identical results, with particular differences perhaps only at $CORT_{\Delta 4}$. Both of these criteria classify considerably more men than women as cortisol responders at each measurement point, however, with particularly stark sex differences at the first and second points. The most liberal ("Any increase") criterion delivered a different, and interesting, set of data. Under this criterion, sex differences are not as extreme; in fact, at $CORT_{\Delta 4}$ this criterion classified more female than male participants, and more than half of the female sample, as responders.

In their comparison of the CPT, Socially Evaluated CPT (SECPT; Schwabe et al., 2008), and MAST, Smeets et al. (2012) observed (using an all-male sample and a +2.5 nmol/l criterion) cortisol response rates of 40%, 65%, and 85%, respectively.¹ In the current Stress-Male group, there was a response rate of 92% using the +2.5 nmol/l criterion.

¹Smeets et al. (2012) did not report the time point used to determine these response rates. Therefore, we assume that these rates refer to the time at which the MAST attained its highest elevation in cortisol responses, and we applied this standard to the MSST in this comparison.

Table 4
Male and Female Cortisol Responders Using Different Criteria (N = 21)

Criterion	Number of responders	
	Men (<i>n</i> = 12)	Women (<i>n</i> = 10)
+2.5 nmol/l		
CORT _{Δ1}	11 (92)	3 (33)
CORT _{Δ2}	11 (92)	4 (44)
CORT _{Δ3}	7 (58)	3 (33)
CORT _{Δ4}	4 (33)	1 (11)
+2 nmol/l		
CORT _{Δ1}	11 (92)	4 (44)
CORT _{Δ2}	11 (92)	4 (44)
CORT _{Δ3}	7 (58)	3 (33)
CORT _{Δ4}	6 (42)	1 (22)
Any increase		
CORT _{Δ1}	11 (92)	8 (89)
CORT _{Δ2}	11 (92)	7 (78)
CORT _{Δ3}	9 (75)	7 (78)
CORT _{Δ4}	6 (50)	5 (56)

Note. Data presented are frequencies, with percentages in parentheses. Δ represents a difference score, so that CORT_{Δ1} is CORT₁ – CORT_B, and so on.

Area under the curve. In cortisol-related research (Buske-Kirschbaum et al., 2003; Het, Rohlerder, Schoofs, Kirschbaum & Wolf, 2009; Smeets et al., 2012), Area Under the Curve with respect to increase (AUC_I) has been calculated to illustrate that, under some conditions or in some populations, there is a blunted cortisol response in comparison with others. None of these studies, however, report raw data for their AUC_I values. Hence, we have no means of comparing our AUC_I value to theirs to determine whether cortisol response to the MSST was larger or smaller than other stress-induction protocols. We, however, report our value here to facilitate comparison in future studies. We used Pruessner, Kirschbaum, Meinlsmchmid and Hellhammer's (2003) formula for the calculation of AUC_I, and attained a value of 167.43 (see Appendix G).

Heart rate. Due to hardware malfunctions, heart rate data were not recorded for two participants in the Stress-Male group. Hence, the analyses reported below are based on the following sample sizes: Stress-Female *n* = 10; Stress-Male *n* = 10; Control-Female *n* = 11; Control-Male *n* = 11.

The repeated-measures ANOVA run on these data detected a significant (a) main effect for Time, $F(1.60, 60.87) = 133.45, p < .001, \eta_p^2 = .78$, and (b) interaction effect for Time x Experimental Condition, $F(1.60, 60.87) = 15.46, p < .001, \eta_p^2 = .29$. It did not detect

any other significant main, two-way, or three-way interaction effects, $F_s < 1.60$, $p_s > .20$, $\eta_p^2_s < .05$.

To further investigate Hypothesis 2 (regarding expected elevations in heart rate during the experimental manipulation and a decline immediately thereafter), we conducted a series of planned contrasts to compare data from the combined Stress and Control groups at all time points. Figure 6 shows those data. In the Stress-group, there was a significant increase in average heart rate from HR_B to HR₁, $F(1, 80) = 41.68$, $p < .001$, $d = 1.48$, and a significant decrease from HR₁ to HR₂, $F(1, 80) = 31.17$, $p < .001$, $d = 1.68$. Further planned contrasts analysing data from consecutive measurement points detected no other statistically significant differences. $F_s < 0.07$, $p_s > .79$, $d_s < 0.07$. Furthermore, there were no significant differences in average heart rate between HR_B and HR₄, $F(1, 80) = 1.11$, $p = .294$, $d = 0.31$.

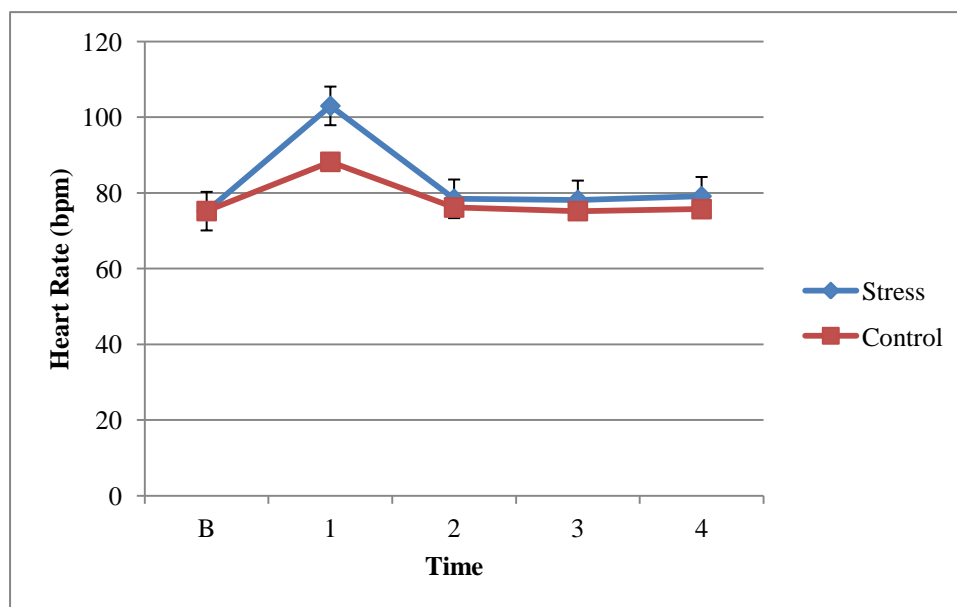


Figure 6. Changes in heart rate levels in the combined Stress and Control groups ($N = 42$). Error bars indicate standard error of means. Time points are represented on the x -axis (e.g. B is the first measurement point, or baseline; 1 is the first post-manipulation measurement point, and so on).

In the Control group, a similar pattern manifested: There was a significant increase in average heart rate from HR_B to HR₁, $F(1, 80) = 10.01$, $p = .002$, $d = 1.20$, and a significant decrease from HR₁ to HR₂, $F(1, 80) = 8.27$, $p = .005$, $d = 1.12$. Further planned contrasts analysing data from consecutive measurement points detected no other statistically significant differences, $F_s < 0.07$, $p_s > .79$, $d_s < 0.09$. Furthermore, there were no significant differences in average heart rate between HR_B and HR₄, $F(1, 80) = 0.02$, $p = .89$, $d = 0.05$.

Despite this similar pattern, a planned contrast comparing the combined Stress and Control groups revealed that, on average, Stress-group participants showed a significantly

higher heart rate than Control-group participants during the experimental manipulation (i.e., at HR₁), $F(1, 200) = 11.79, p = .001, d = 0.97$. There were no significant differences between the Stress and Control groups at any other time point, $F_s < 0.75, p_s > .39, d_s < 0.28$.

We conducted a series of planned contrasts to investigate Hypothesis 4 (regarding the attenuation of sex differences between Stress-Female and Stress-Male groups). As expected, this set of analyses revealed no significant between-group differences in terms of mean heart rate across all time points, $F(1, 90) = 0.03 - 0.41, p = .525 - .854, d = 0.04 - 0.13$.

A single sample *t*-test comparing the current heart rate data to those reported by du Plooy et al. (under review) indicated that, during the experimental manipulation (i.e., at HR₁), the MSST ($M = 102.98$ bpm, $SD = 18.47$) was able to elicit a significantly higher heart rate peak than the FFST ($M = 94.13, SD = 15.06$), $t(19) = 2.14, p = .02, d = 0.54$.

Self-report measurements. Below, we present analyses of STAI-State scores.

STAI-State scores. Data for two participants, one from the Control-Male group and one from the Stress-Female group, were excluded from this analysis because they did not respond to all questionnaire items at the Baseline measurement.

The repeated-measures ANOVA detected a significant (a) main effect for Time, $F(3.08, 117.35) = 14.55, p < .001, \eta_p^2 = .28$, (b) main effect for Experimental Condition, $F(1, 38) = 10.91, p = .002, \eta_p^2 = .22$, (c) interaction effects for Experimental Condition x Sex interaction, $F(1, 38) = 7.35, p = .01, \eta_p^2 = .16$, and (d) interaction effect for Time x Experimental Condition interaction, $F(3.08, 117.35) = 11.82, p < .001, \eta_p^2 = .24$. It did not detect any other significant main or interaction effects, $F_s < 4.05, p_s > .05, \eta_p^2_s < .10$.

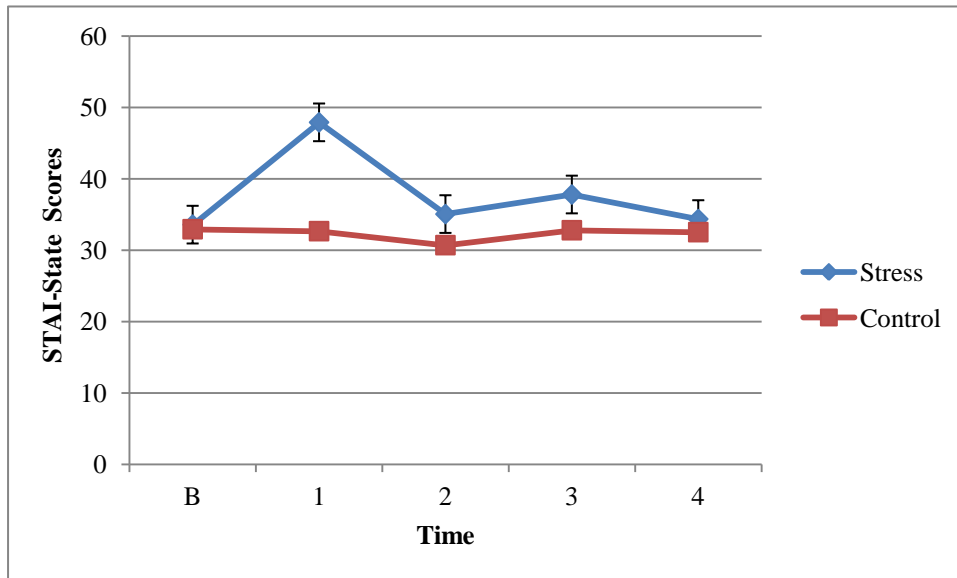


Figure 7. Changes in subjective anxiety levels in the combined Stress and Control groups ($N = 44$). Error bars indicate standard error of means. Measurement points are represented on the x -axis (e.g., B is the first measurement point, or baseline; 1 is the first post-manipulation measurement point, and so on).

To further investigate Hypothesis 3 (regarding elevations in subjective anxiety during the experimental manipulation and a decline immediately thereafter), we conducted a series of planned contrasts comparing data from the combined Stress and Control groups at all time points. Figure 7 shows changes in subjective anxiety levels. In the Stress group, there was a significant increase in subjective anxiety from $STAI_B$ to $STAI_1$, $F(1, 84) = 25.19$, $p < .001$, $d = 1.34$, and a significant decrease from $STAI_1$ to $STAI_2$, $F(1, 84) = 20.65$, $p < .001$, $d = 1.17$. As expected, further planned contrasts analysing data from consecutive measurement points detected no other statistically significant differences, $F_s < 1.21$, $p_s > .28$, $d_s < 0.31$. Furthermore, there were no significant differences in average levels of subjective anxiety between $STAI_B$ and $STAI_4$, $F(1, 84) = 0.06$, $p = .81$, $d = 0.09$.

In the Control group, the series of planned contrasts detected no significant differences in subjective anxiety between consecutive measurement points, and no significant differences between $STAI_B$ and $STAI_4$, $F_s < 0.62$, $p_s > .49$, $d_s < 0.01$.

Another planned contrast revealed that, on average, Stress-group participants reported significantly higher levels of subjective anxiety than the Control-group directly after the experimental manipulation (i.e., at $STAI_1$), $F(1, 210) = 28.48$, $p < .001$, $d = 1.43$. There were no other significant between-group differences at any other measurement points, $F_s < 2.62$, $p_s > .11$, $d_s < 0.58$.

We conducted a series of further planned contrasts comparing data from the Stress-Male and Stress-Female groups to investigate Hypothesis 4 (regarding the attenuation of sex differences in subjective anxiety levels). This set of analyses revealed no significant differences between the Stress-Female and Stress-Male at STAI_B, $F(1, 100) = 1.47, p = .23, d = 0.54$. However, women reported significantly higher levels of subjective anxiety than men at STAI₁, $F(1, 100) = 6.72, p = .011, d = 1.05$, at STAI₂, $F(1, 100) = 4.02, p = .048, d = 0.96$, and at STAI₃, $F(1, 100) = 8.74, p = .004, d = 1.18$, but not at STAI₄, $F(1, 100) = 3.92, p = .05, d = 0.77$.

Discussion

We described the Mortality Salience Stress Test (MSST), a stress-induction procedure that combines the format of an existing psychosocial stressor (the Fear Factor Stress Test) with a mortality salience manipulation. Several existing laboratory-based procedures utilise uncontrollability and social evaluative threat to produce consistent physiological stress responses through activation of the HPA axis; these include the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), the Maastricht Acute Stress Test (MAST; Smeets et al., 2012), and the Fear Factor Stress Test (FFST; du Plooy et al., under review). The laboratory convention, for decades, has been the TSST. However, this method is limited in that it fails to produce a consistent, robust, and sustained cortisol response in all participants. In particular, it does not elicit equivalent responses in men and women. Although the FFST and the MAST both address the limitations of the TSST, they both still fail to (a) provoke a robust and sustained cortisol response in *all* participants, (b) attenuate sex differences in cortisol responses, and (c) tackle issues of ecological validity. Hence, we sought to investigate whether the addition of a mortality salience (MS) manipulation to the FFST would result in a more efficient laboratory-based psychosocial stressor. Our results showed, overall, that the MSST was capable of eliciting HPA-axis, autonomic, and subjective responses to stress without causing additional psychological harm to participants.

The MSST produced elevated cortisol responses, which indicates that it provoked HPA-axis activity. Hypothesis 1 predicted elevated and sustained cortisol responses across all measurement points, during and post-manipulation. Cortisol responses were elevated above baseline for 40 minutes post-manipulation in both men and women. The elevated response observed in the Stress group directly after the experimental manipulation was sustained until at least 20 minutes post-manipulation.

Furthermore, comparisons of our cortisol responder rates to those of other studies indicated that the MSST was effective in producing more robust and sustained cortisol

responses than the FFST, the CPT, SECPT and MAST. Similar to other stress manipulations, cortisol response rates to the MSST declined significantly at longer intervals post-manipulation. However, the fact that a third of our participants were still classified as responders (using a criterion of 2 nmol/l over baseline) at 55 minutes post-manipulation shows promise for future research. Certainly, in comparison to its direct predecessor, the FFST, the MSST elicited a greater cortisol response rate.

Additionally, the MSST demonstrated its ability to produce significantly elevated responses in heart rate and subjective anxiety. Hypothesis 2 predicted elevated heart rate during the manipulation with a decline immediately post-manipulation. Results indicated that, although participants in both the Stress and Control groups experienced a peak in heart rate post-manipulation, as expected, the Stress group showed a significantly elevated heart rate at this time in comparison to Control participants. After this peak, heart rate declined rapidly until 20 minutes post-manipulation, and then stabilised for the remaining time points until the end of the study.

Hypothesis 3 predicted elevated subjective anxiety during the manipulation, with a decline immediately post-manipulation. Results indicated that Stress-group participants experienced elevated subjective anxiety post-manipulation and, as expected, showed significantly higher levels of subjective anxiety at this time in comparison to Control participants. After this initial increase, subjective anxiety declined until 20 minutes post-manipulation, and then stabilised for the remaining time points until the end of the study.

Hypothesis 4 predicted no sex differences on both physiological and self-report measures of stress. Although the MSST was successful in attenuating sex differences in autonomic responses to stress, it was unable to reduce the sex differences evident in cortisol responses and in subjective anxiety. Whereas men showed higher cortisol responses, women showed higher levels of subjective anxiety. Despite this failure, however, our protocol produced, relative to other stress manipulations, an increased proportion of cortisol responders among women.

We contend that the MSST achieved elevated hormonal and autonomic responses by, at least in part, adding an MS manipulation to the FFST protocol. We further contend that the success of the protocol demonstrates that requesting participants to contemplate emotions and thoughts surrounding death, as well as the physicality of death, assists in the creation of an unconscious and indiscriminate anxiety that results in elevated physiological responses to stress (Landau et al., 2007). This contention is consistent with MS literature asserting that, although death-related anxiety may be a somewhat unconscious phenomenon, it is

measurable through physiological assessment (Tritt et al., 2012). Furthermore, Landau et al. (2007) suggest that knowledge of one's death is the largest source of debilitating anxiety in psychological functioning. Consistent with this suggestion, the MSST provoked significantly increased elevations on measures of subjective state anxiety, suggesting that the inclusion of death-related stimuli may lead to increased anxiety. Biologically-based accounts of MS propose that death-related uncertainty is processed through brain-based anxiety systems, and that it results in raised levels of cortisol and other stress hormones. Similarly, previous research suggests that an appraisal of psychosocial stressors results in psychobiological responses, including the release of cortisol, via activation of the HPA axis (Kemeny, 2003). Taken together, these accounts suggest that elevated cortisol levels following MSST exposure can be explained by the combination of death-related subject matter and a psychosocial stressor that features elements of social evaluation and uncontrollability. Evidently, our improvements to the FFST, using theories from the MS literature, have resulted in an improved laboratory-based psychosocial stressor.

Stress researchers recognise there are clear sex differences in HPA-axis responses to stressors (Kirschbaum et al., 1993; Dickerson & Kemeny, 2004). In existing stress tests (e.g., TSST, CPT, and FFST; the developers of the MAST have not yet reported a study wherein that protocol is administered to women), men tend to display greater HPA reactivity (as indicated by larger cortisol responses) than women. These sex differences might be related to oral contraceptive use, hormonal activity as mediated by the female menstrual cycle, and differential reactions to achievement challenges. Regardless of why these sex differences arise, however, the fact that they are commonplace has led to many studies including only men in their samples. Through the addition of an MS manipulation, we sought to reduce the sex differences prevalent in existing stress-induction procedures. Specifically, we sought to reduce the achievement components evident in other stress manipulations by altering the content of the free-form speech.

Our findings were, however, consistent with previous research: The MSST provoked significantly larger cortisol responses in men than in women. This, despite the fact that we improved on existing protocols' controls for both oral contraceptive use and menstrual cycle phase. Furthermore, contrary to our hypothesis but consistent with most research in this field, men were not able to sustain this elevated response until the end of the study. In a divergent pattern, although women did not achieve cortisol elevations of the same magnitude as men, they were able to sustain their (smaller) responses over the duration of the study. Accordingly, with regard to the classification of participants as responders, the MSST

managed (relative to previously reported FFST data) to achieve higher and more sustained cortisol responder rates in women.

Previous research has shown that there are differences in brain activity between men and women in response to stressors (Goldstein, Jerram, Abbs, Whitfield-Gabrieli & Makris, 2010; Kudielka & Kirschbaum, 2005). These differences appear to be hormonally regulated by subcortical brain mechanisms, which enable women to attenuate their stress responses better than men. This regulation may explain the sex differences observed in the current study: Women may have been able to better mediate their responses to the psychosocial stressor because their brain circuitry supports such mediation. Another explanation for the observed sex differences considers the possibility of sex differences in psychological appraisals of stress-induction procedures. Previous research (e.g., Kudielka et al., 2009; Stroud et al., 2002) suggests that whereas women perceive social rejection challenges to be more stressful, men perceive achievement challenges as more stressful. Although the MSST attempted to alleviate these sex differences by reducing achievement components present in the protocol, our data suggest that this attempt may not have been altogether successful.

Limitations and Considerations for Future Research

Although the sex differences observed here were not a limitation in and of themselves, the way in which the study set out to attenuate potential sex differences (the presence of which we assumed, based on previous research in field) was. Reasons for the observed differences remain uncertain; certainly, they do not relate to oral contraceptive use and menstrual cycle phase, as we controlled for both of those. Above, we offered possible explanations for the observed sex differences. One of these considers sex differences in response to different stressor challenges (Stroud, et al., 2002). Regarding the latter, the MSST was still, largely, an achievement-based task rather than the type of social rejection challenge that has been shown to elicit greater cortisol responses in women (see, e.g., Stroud, Tanofsky-Kraff, Wilfley & Salovey, 2000). Future research may assist in the attenuation of sex differences by developing new laboratory-based stress induction procedures that feature social rejection more centrally, or by refining or modifying existing protocols to the same end. For instance, a task like the MSST might be modified by intensifying tasks in which performance is judged negatively by others in the female participant's social group.

A second limitation was that we observed significant sex differences in Baseline cortisol levels. It is possible that these differences might be related to participants experiencing anticipatory anxiety upon entering the laboratory: Previous research suggests that, in men only, the anticipation of an upcoming psychosocial stressor can result in

significant cortisol increases (Kudielka & Kirschbaum, 2005). Upon arrival at the laboratory, we informed all participants that they might have to give a 12-minute presentation; this instruction may have induced anxiety in male participants, and this anxiety might have been manifest in elevated cortisol levels at baseline. Although ethical considerations required us to inform participants they might be exposed to a mildly stressful situation, future research might either (a) rephrase this information in a less explicit and threatening (but still ethically sound) manner, or (b) take baseline measures of cortisol before relaying such information.

Finally, a clear direction for future research is to directly compare the MSST with the FFST, MAST, and TSST concurrently, with participants sampled from the same population. Although this kind of exhaustive comparison was beyond the scope of the current study, it is necessary in order to accurately determine the relative efficiency and efficacy of these stress-induction protocols.

Significance of Research

Despite these limitations, the incorporation of a mortality salience manipulation into an acute psychosocial stressor demonstrates clear steps towards creating a more ecologically valid stress-induction protocol: Death is an indiscriminate phenomenon that all humans experience, and anxiety surrounding one's death is natural. Ecological validity remains an important factor in laboratory-based research as it enables application of these findings to real-world settings (Orne & Holland, 1968). A continuing problem with existing laboratory-based stress-induction protocols is that they lack ecological validity, as they often require participants to perform tasks that do not necessarily approximate real-world events or settings. This, despite the fact that people often encounter acute stressors in everyday life and are expected to function effectively under these conditions. Currently, one purpose of stress-induction protocols is to aid in the investigation of the effects of stress on cognitive, behavioural, and affective functioning. The development of more ecologically valid protocols adds obvious value to such investigations. In turn, these investigations may assist in better equipping people to function effectively in stressful situations encountered in daily life.

Summary and Conclusion

We have shown that the MSST is a promising tool that offers improvements on existing laboratory-based psychosocial stressors: Compared to other stress-induction methods, it is more ecologically valid and it elicits more robust HPA-axis responses in men and ensures the maintenance of responses in women, all without increasing participant discomfort. Although this study demonstrated the value, feasibility, and potential uses of the MSST, it is clear that continued research into the improvement of laboratory-based stress-

induction procedures (e.g., around the issue of attenuating sex differences) is necessary. The growing prevalence of stress-related illnesses means that laboratory-based stress-induction procedures have an important role to play in uncovering neurobiological elements of those illnesses. Therefore, the development of ecologically valid stress-induction protocols may assist in increasing the efficacy with which these procedures are able to investigate potentially modifiable biological sources of stress-related illnesses.

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Appendix A
Sociodemographic Questionnaire

Name: _____

Surname: _____

Age: _____

Student Number: _____

Which Psychology course do you want these SRPP Points to go towards?

Do you smoke? (please tick the appropriate box)

Yes

No

Are you taking any form of chronic medication? (please tick the appropriate box)

Yes

No

Are you taking any form of steroid-based medication? (please tick the appropriate box)

Yes

No

Do you suffer from any chronic or psychological illness? (please tick the appropriate box)

Yes

No

Have you had anything to eat or drink (except water) in the last 2 hours?

(please tick the appropriate box)

Yes

No

Have you have you done any exercise in the last 2 hours? (please tick the appropriate box)

Yes

No

Appendix B

Script for Mortality Salience Stress Test

Remember not to be engaging with the participant (i.e., no positive or negative reinforcement / no sign of social support). The participant must not know how well or badly they are doing.

Part 1: Free-Form Speech (5 min)

Judge of the opposite sex to participant says, *“Good afternoon (Participant’s name). As (Experimenter’s name) has already explained to you this audition for Fear-Factor will comprise of 3 parts. Firstly, could you please describe the circumstances of your death, the emotions and the thoughts that death arouses in you, and what you think will happen as you physically die and after you are physically dead”*.

If the participant stops talking before 5 min is up, say:

- 1) *“You still have time left, please continue.”*
- 2) *“In your opinion, what is the worst way to die?”* If participant responds with a short answer, ask: *“Why?”*
- 3) *“What scares you the most about the process of dying?”*
- 4) *“How many people do you think would be at your funeral?”* If participant responds with a short answer, ask: *“Why?”*
- 5) *“When you die would you rather be cremated or buried?”* If participant responds with a short answer, ask: *“Why?”*
- 6) *“What do you think will happen to your body after your death?”*

After 5 minutes are up or the participant has nothing more to say (refuses to carry on talking), judge of the opposite sex to participant says, *“Thank you, that is fine. We are now going to proceed with the second part of the audition, which is a test of mental agility.”*

Part 2: Test of mental agility (5 min)

Judge of the opposite sex to participant says, *“We are now going to ask you to subtract 17 from 2043 continuously until we tell you to stop. If you make a mistake you will be asked to start from 2043 again. Please begin.”*

If the participant stops at a number say, *“Please carry on subtracting 17.”*

If an error is made say: *“That is incorrect, please start again from 2043.”*

Start: 2043 → 2026 → 2009 → 1992 → 1975 → 1958 → 1941 → 1924 → 1907 → 1890 → 1873 → 1856 → 1839 → 1822 → 1805 → 1788 → 1771 → 1754 → 1737 → 1720 → 1703 → 1686 → 1669 → 1652 → 1635 → 1618 → 1601 → 1584 → 1567 → 1550 → 1533 → 1516 → 1499 → 1482 → 1465 → 1448 → 1431 → 1414 → 1397 → 1380 → 1363 → 1346 → 1329 → 1312 → 1295 → 1278 → 1261 → 1244 → 1227 → 1210 → 1193 → 1176 → 1159 → 1142 → 1125 → 1108 → 1091 → 1074 → 1057 → 1040 → 1023 → 1006 → 989 → 972 → 955 → 938 → 921 → 904 → 887 → 870 → 853 → 836 → 819 → 802 → 785 → 768 → 751 → 734 → 717 → 700 → 683 → 666 → 649 → 632 → 615 → 598 → 581 → 564 → 547 → 530 → 513 → 496 → 479 → 462 → 445 → 428 → 411 → 394 → 377 → 360 → 343 → 326 → 309 → 292 → 275 → 258 → 241 → 224 → 207 → 190 → 173 → 156 → 139 → 122 → 105 → 88 → 71 → 54 → 37 → 20 → 3 → -14

After 5 minutes are up or a participant has completed the serial subtraction task say, “***Thank you, that is fine. We will now proceed onto the final part of the audition, which is a test of pain tolerance.***”

Part 3: Test of pain tolerance (2 min)

Judge of the same sex to participant says, “***We are now going to ask you to place your arm into the bucket of water. Please submerge your arm so that the palm of your hand is touching the bottom of the bucket. Please keep your arm submerged until we tell you to remove it or until you find it too painful to keep it there any longer.***”

After 2 minutes say, “***Thank you, that is fine. That concludes the final part of the audition. Thank you for your participation.***”

Appendix C

Informed Consent Document

*Informed Consent to Participate in Research
and Authorization for Collection, Use, and
Disclosure of Protected Health Information*

This form provides you with information about the study and seeks your authorisation for the collection, use and disclosure of your protected health information necessary for the study. The Principal Investigator (the person in charge of this research) or a representative of the Principal Investigator will also describe this study to you and answer all of your questions. Your participation is entirely voluntary. Before you decide whether or not to take part, read the information below and ask questions about anything you do not understand. By participating in this study you will not be penalised or lose any benefits to which you would otherwise be entitled.

1. Name of Participant ("Study Subject")

2. Title of Research Study

The Mortality Salience Stress Test: A laboratory-based acute psychosocial stressor.

3. Principal Investigators, Ethics Committee, and Telephone Numbers

Kevin G. F. Thomas, Ph.D.
Department of Psychology
University of Cape Town
021-650-4608
021-788-5536

Robyn Human, MA
PhD Candidate
Department of Psychology
University of Cape Town

Leanne Adams
Aimee Minnozzi
Honours students

Faculty of Health Sciences
Research Ethics Committee
Room E52-24, Groote Schuur Hospital, Old Main Building
Observatory 7925
Tel: 021-406-6338
Fax: 021-406-6411
Email: lamees.emjedi@uct.ac.za

4. What is the purpose of this research study?

The purpose of this research study is to better understand how exposure to acute psychological stress affects cognitive performance.

5. What will be done if you take part in this research study?

During this study, you will be required to complete two personality assessment tasks and may be required to complete a 20-minute presentation. Your levels of stress will be assessed through the collection of self-report data, heart rate measurements, skin conductance measurements and saliva samples with the aid of a cotton swab. These saliva samples will be used to analyse levels of cortisol, a stress hormone.

6. What are the possible discomforts and risks?

If you are one of the participants selected to complete the 20-minute presentation, you may be placed in a mildly stressful situation involving public speaking. Furthermore, you may be asked to place your hand in very cold water. There are no other discomforts and risks associated with participation in the study.

7. What are the possible benefits of this study?

One major benefit of this study is that scientists and society in general, will have better understanding of the effects of acute psychological stress on cognitive performance, and what variables moderate this relationship. This knowledge can then be applied to many different individuals and situations, including students who are taking exams, business managers who have to present to their boards, and so on.

8. Can you withdraw from this research study and if you withdraw, can information about you still be used and/or collected?

You may withdraw your consent and stop participation in this study at any time. Information already collected may be used.

9. Once personal information is collected, how will it be kept confidential in order to protect your privacy and what protected health information about you may be collected, used and shared with others?

Information collected will be stored in locked filing cabinets or in computers with security passwords. Only certain people - the researchers for this study and certain University of Cape Town officials - have the legal right to review these research records. Your research records will not be released without your permission unless required by law or a court order.

If you agree to be in this research study, it is possible that some of the information collected might be copied into a "limited data set" to be used for other research purposes. If so, the limited data set may only include information that does not directly identify you.

Signatures

As a representative of this study, I have explained to the participant the purpose, the procedures, the possible benefits, and the risks of this research study; the alternatives to being in the study; and how the participant's protected health information will be collected, used, and shared with others:

Signature of Person Obtaining Consent and Authorization

Date

You have been informed about this study's purpose, procedures, and risks; how your protected health information will be collected, used and shared with others. You have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time.

You voluntarily agree to participate in this study. You hereby authorize the collection, use and sharing of your protected health information. By signing this form, you are not waiving any of your legal rights.

Signature of Person Consenting and Authorizing

Date

Please indicate below if you would like to be notified of future research projects conducted by our research group:

_____ (initial) Yes, I would like to be added to your research participation pool and be notified of research projects in which I might participate in the future.

Method of contact:

Phone number: _____

E-mail address: _____

Appendix D

Ethical Approval from UCT Department of Psychology

1

**The Mortality Salience Stress Test:
A laboratory-based acute psychosocial stressor**

Leanne Adams and Airnee Minnozzi

ACSENT Laboratory
Department of Psychology
University of Cape Town

Ref. no PSY2013-012

*It has been approved
with small
amendments.
Glover
3/6/13*

Supervisor: Kevin G. F. Thomas
Co-supervisor: Robyn Human

Word Count: 4 994

Appendix E
Ethical Approval from UCT Faculty of Health Sciences Human Research Ethics Committee

UNIVERSITY OF CAPE TOWN



Faculty of Health Sciences
 Human Research Ethics Committee
 Room E52-24 Grootte Schuur Hospital Old Main Building
 Observatory 7925
 Telephone (021) 406 6338 • Facsimile (021) 406 6411
 e-mail: ghunetta.thomas@uct.ac.za
 Website: www.health.uct.ac.za/research/humanethics/forms

5 August 2013

HREC REF: 317/2013

Ms L Adams
 c/o Dr K Thomas
 Psychology
 PD Hahn Building
 Upper Campus

Dear Ms Adams

PROJECT TITLE: THE MORTALITY SALIENCE STRESS TEST: A LABORATORY-BASED ACUTE PSYCHOSOCIAL STRESSOR

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee dated 2nd August 2013.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year till the 15th August 2014

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
 (Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code of Federal Regulation Part 312.41, 312.56 and 312.62.

l.thomas@uct.ac.za

Appendix F

Comparison of Data from Participant who Withdrew From the MSST

Participant Data

Measure	Participant Data
Age	22
BMI	19.77
BDI-II	14
STAI _B	42
CORT _B	8.81

Comparison of Participant who Withdrew with Stress-Female and combined Female Group

Measure	<i>df</i>	Group				
		Stress-Females			Females	
	<i>df</i>	<i>t</i>	<i>p</i>	<i>df</i>	<i>t</i>	<i>p</i>
Age	9	-2.74	.01	20	-5.30	< .001
BMI	9	3.74	.005	20	5.89	< .001
BDI-II	9	-1.44	.09	20	-2.54	.01
STAI _B	8	-2.11	.03	19	-4.24	< .001
CORT _B	10	-1.31	.11	21	0.46	.33

Appendix G

The Calculation of Area Under the Curve

Pruessner, Kirschbaum, Meinlschmid, & Hellhammer (2003) offer two formulae for calculating area under the curve:

(1) AUC Ground (AUC_G) formula calculates the area under the curve using all time measurement points. This formula is presented below.

$$AUC_G = \frac{(m_2 + m_1) \cdot t_1}{2} + \frac{(m_3 + m_2) \cdot t_2}{2} + \frac{(m_4 + m_3) \cdot t_3}{2} \\ + \frac{(m_5 + m_4) \cdot t_4}{2} + \frac{(m_6 + m_5) \cdot t_5}{2}$$

(2) AUC with respect to Increase (AUC_I) refers to the area under the curve with respect to the first measurement; it ignores the distance from zero for all measurements. As we used difference scores for the cortisol measurement, whereby we also “ignore” the first measurement, we made use of the AUC_I formula to calculate area under the curve. The formula is illustrated below.

$$AUC_I = AUC_G - m_1 \cdot \sum_{i=1}^{n-1} t_i$$

Whereas the AUC_G formula takes into account both the change over time *and* the level at which changes occur, the AUC_I formula emphasizes changes observed over time.

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DATE: 24th October 2013