RESEARCH ARTICLE

Stress reactivity during evaluation by the opposite sex: comparison of responses induced by different psychosocial stress tests

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Abstract

It is becoming increasingly difficult for researchers to continue their high rates of publication when funding budgets are running tighter than ever. It is therefore in a researcher's best interest to utilize more economical tests whenever possible. This project aims to compare various stress tests in order to determine whether the new, cost-efficient Maastricht Acute Stress Test (MAST) activates a physiological and subjective stress response with the same effectiveness as pre-existing, more resource-intensive tests. This study demonstrated that the MAST produces a response similar to that of the previously predominant Trier Social Stress Test (TSST). Meanwhile, database data shows that the purely physiological Cold Pressor Task (CPT) lags behind in terms of response elicited. These findings may allow for a more cost-efficient yet highly effective stress task to become available to researchers.

Introduction

Stress is a major factor in modern daily life; we encounter it at school, at work, in our social relationships, and even at home. A stressful stimulus activates several interacting physiological stress pathways, the two main ones being the Sympathetic-Adrenal-Medullary (SAM) and the Hypothalamic-Pituitary-Adrenal (HPA) axes. Stimulation of the HPA axis following exposure to a stressor results in the release of corticotropin-releasing hormone (CRH) from the hypothalamus, which triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH then stimulates the secretion of glucocorticoids from the adrenal cortex. Conversely, in SAM axis action, ACTH also acts upon the adrenal medulla, which releases epinephrine and norepinephrine. This triggers an acute stress response from the sympathetic nervous system (such as increased blood pressure and heart rate).

While the SAM axis is predominantly responsible for an immediate fight-or-flight response (7), HPA axis activation maintains homeostasis after a challenge through a cascade of hormonal responses, which take up to 30 minutes to fully unfold (1). Thus, the two axes differ in how quickly they respond and how long they remain active. Although acute HPA activation is necessary for maintaining homeostasis, chronic activation of the HPA axis has been associated with complications such as immunosuppression, depression, and increased risk of coronary heart disease (15).

In humans, the main glucocorticoid released by stimulation of the HPA axis is cortisol, which plays an important function in modulating the stress response towards the re-establishment of homeostasis. Freely circulating levels of cortisol (i.e. cortisol not bound to proteins in the blood) serve as indicators of HPA axis activation and can be easily measured in blood and saliva (10, 11).

Furthermore, the HPA axis is especially sensitive to non-physical stressors involving a social context. Its activation is therefore considered a strong indicator of exposure to psychosocial stress. One of the most frequently used standard protocols for inducing such stress is the Trier Social Stress Test (TSST). The TSST involves a period of anticipation, then requires the participant to present a free speech in front of a panel of "experts" (personnel in lab coats), and afterwards to perform a mental arithmetic challenge (9). Other stress tests such as the Cold Pressor Task (CPT), which involves submerging one's hand in ice-cold water (20), fail to achieve similar increases in ACTH and cortisol levels as the TSST (14). Thus, it appears that a challenging task with a social-evaluative component that is outside of the individual's control and threatens their social status causes greater HPA activation than one with a physically stressful component alone (2, 18).

One limitation of the TSST is that it is time-and-resource intensive. The design of a simpler and more rapid test would be advantageous and economical. Recently, Schwabe *et al.* (2008) introduced a modified

version of the CPT that included a social evaluative component: the Socially Evaluated Cold Pressor Task (SECPT) (18). This test induces higher levels of salivary cortisol than the CPT alone, reinforcing the idea that social evaluation significantly increases HPA axis activity.

Most recently, Smeets *et al.* (2012) introduced the Maastricht Acute Stress Test (MAST) consisting of fixed SECPT intervals (CPT with social evaluation) separated by mental arithmetic challenge periods. The authors compared the responses achieved with the MAST with those from other stress paradigms, in this case CPT and SECPT, or TSST. They concluded that subjects' cortisol levels after MAST appeared higher than in both CPT and SECPT. A comparison of the MAST and TSST suggested that cortisol response curves were comparable in magnitude, and followed a similar progression (19). Carrying out all four tests within the framework of the same study allowed variables such as collection techniques, exclusions, protocol, and group size to be controlled. These results therefore suggest that the MAST may be a valid alternative to the TSST. The effectiveness of the MAST may be result of the fact that its CPT component provides a physical stressor that predominantly activates the SAM axis (indicated by elevated heart rate and blood pressure), while the social evaluation and mental arithmetic components of the TSST activate the HPA axis (indicated by higher levels of salivary free cortisol) (19).

The effect of subjects' sex is another potentially significant factor. Numerous studies have shown that the two groups who display greatest cortisol responses to a standard TSST are women in the luteal phase of their menstrual cycle, and men (10). However, a recent study by Duchesne *et al.* (2011) has shown that women in the follicular phase of their menstrual cycle and men show higher cortisol levels compared to women in the luteal phase and controls when the social evaluation during the TSST was carried out by an experimenter of the opposite sex (4). This is possibly a result of the fact that women in the follicular phase of their menstrual cycle display increased responsiveness and physiological arousal towards men and masculine stimuli (e.g. facial and vocal cues of men with high testosterone levels) (4, 6, 17).

However, it is unclear whether any such gender differences exist in the MAST. The work by Smeets *et al.* (2012), introducing the MAST, tested it on twenty men and no women. The results were subsequently compared to men-only TSST and CPT versions. As of yet, no project has directly compared all three stress tests at one time while including women. Such a study would be able to determine whether the MAST affects women in the follicular phase of the menstrual cycle differently from men when they are evaluated by experimenters of the opposite sex.

We hypothesized that the results of the current project would demonstrate that the administration of a psychosocial stress test by an experimenter of the opposite sex would lead to a comparable stress response in women in the follicular phase of their menstrual cycle and men. Further, we hypothesized that the magnitude of the stress response would differ between different psychosocial stress tests (TSST, CPT and MAST), in that the MAST and the TSST would induce a greater cortisol (HPA axis) and autonomic (SAM axis) stress response than the CPT.

Materials & Methods

Subjects

For the MAST, we recruited 9 men (ages = 20-27 years, mean = 23.33 years, sd = 2.45 years) and 8 women (ages = 18-21 years, mean = 19.50 years, sd = 1.20 years) via advertisements posted in a variety of Montréal electronic classifieds. Interested individuals filled out a screening questionnaire, and we contacted those eligible for the study in order to conduct a follow-up interview. In order to avoid any influence of hormonal changes associated with age (such as puberty and menopause) the participants recruited were all between 18 and 35 years of age. We excluded applicants who smoked more than 7 cigarettes a day, had a past history of psychiatric illness, had a body mass index (BMI, in kg/m^2) outside the range of 18-27, or were currently using steroid hormone medications and/or recreational drugs, as these criteria have been known to affect baseline hormone levels (4, 10). We scheduled female applicants who reported not using any oral contraceptives to undergo the MAST when they were in the follicular phase of their menstrual cycle (by testing them 2-13 days following the onset of menstruation; assessed via self-report and monitoring through phone calls). Women reporting themselves as pregnant were not allowed to participate. The Douglas Research Ethics Board approved this project

For the TSST, we included a total of 23 subjects (n_{men} = 12, n_{women} = 11; age mean = 23.09 years), and for the CPT, a total of 17 subjects (n_{men} = 11, n_{women} = 6, age mean = 23.35). Although different experimenters carried out each test, laboratory standards in all stress tests ensured that the same exclusion criteria were used, that participants were within the same age brackets and chosen from the same (predominantly undergraduate) student population, and that the same cortisol analysis methods and measurement instruments were used. Furthermore, only participants tested by an opposite-sex experimenter were included in our analysis so as to match our MAST sample. Only opposite-sex testing was employed due to time and resource constraints on carrying both same-sex and opposite-sex testing. Across all stress tests, the only outstanding difference that emerged during our analysis was that the CPT study involved "high" and "low" self-esteem groups (as measured by the Rosenberg Self-Esteem Scale (RSES)). Therefore, analyses were run with CPT divided into CPT-high and CPT-low groups.

General Procedure

Testing took place at the Douglas Research Institute between 1 and 5

pm to control for diurnal variations of cortisol, as cortisol levels are highest in the morning and decline throughout the day (10). Upon the participant's arrival in the laboratory, they were greeted by Experimenter #1 (the "coordinator") and taken to a waiting room for 40 minutes. This waiting period allowed hormonal levels and physiological measures to return to baseline should they have been increased due to previous spontaneous stimulation by random factors outside of the control of the experiment. During this time period, we gave the participants consent form to sign and the Visual Analogue Scale (VAS) of subjective stress to fill out. We took baseline saliva sample, heart rate (HR), and blood pressure (BP) measurements. Following the rest period, we moved the participant to the MAST testing room and introduced to Experimenter #2.

Experimenter #2 administered the MAST and was always of the opposite sex to the participant. We seated the participant with a pail of ice water (4 ± 0.5°C) placed next to their dominant hand and read them a set of instructions explaining the testing protocol. The experimenter stood in front of them next to a video camera. This camera was solely used to further induce social stress and was not operational; however, we told participants that it would be recording their facial expression for later inspection by behavioural analysts, and that they should therefore keep their gaze directed toward the camera for the duration of the procedure.
The MAST intervals were as follows: [90s-45s], [60s-60s], [60s-90s], [90s-45s] & [60s], where the first number in each bracket indicates the amount of time the hand must be submerged in ice cold water and the second number indicates the time spent doing the mental arithmetic challenge. The arithmetic challenge consisted of counting down as rapidly and accurately as possible from 2043 by steps of 17 (with their hand out of water). In order to increase the stressfulness of the situation, if a mistake in the counting was made, the experimenter stopped the participant and instructed them to begin again from 2043. If the participant was counting down with sufficient accuracy and speed, the experimenter would instruct them to speed up. After the completion of the test, the participant remained in the room for the following hour such that all samples and measurements could be obtained. Finally, we debriefed the participant about the procedure and hypothesis, and compensated them with $50 for their time.

We took saliva samples, heart rate, and blood pressure at ten-minute intervals during the entire length of the testing protocol: three times before the MAST and six times after it, for a total of ten saliva samples (to measure cortisol levels) and ten stress analogue scales (to measure the degree of subjective stress) (Fig.1). HR and BP served as indicators of SAM axis activity.

The protocol we used for the TSST data acquisition was based upon Kirschbaum's original task description (9) and the specific details follow those in Duchesne's study (4). The protocol used for the CPT was the same as that described by von Baeyer *et al.* (2005).

Physiological and Psychological Measurements

Each subject provided samples of salivary cortisol, measures of systolic and diastolic blood pressure, heart rate, and subjective stress ratings at 10-minute intervals throughout the procedure (producing a total of 10 measures per category) (Fig.1). We collected saliva using Salivettes (Sarstedt Inc., Quebec City, Quebec, Canada), which we then stored at -20°C until analysis by time-resolved fluorescence immunoassay (intra- and inter-assay variabilities of 10% and 12%, respectively) (3). We took blood pressure and heart-rate readings with a digital inflatable-wrist-cuff blood pressure monitor (Life Source, UB-512). Finally, we assessed subjective stress values using visual analogue scales (VAS), which involve asking participants to rate how stressed they feel at that time on a continuous scale from 1 to 10 (4).

Data Analysis

To determine the effectiveness of a stress test in inducing a stress response, physiological effects in response to a particular stress test (TSST, CPT or MAST) must be measured over a period of time. Thus, the dependent variables were cortisol levels (in nmol/L), heart rate (in beats per minute), systolic and diastolic blood pressure (in mmHg), and subjective stress (in centimetres, as measured on VAS),

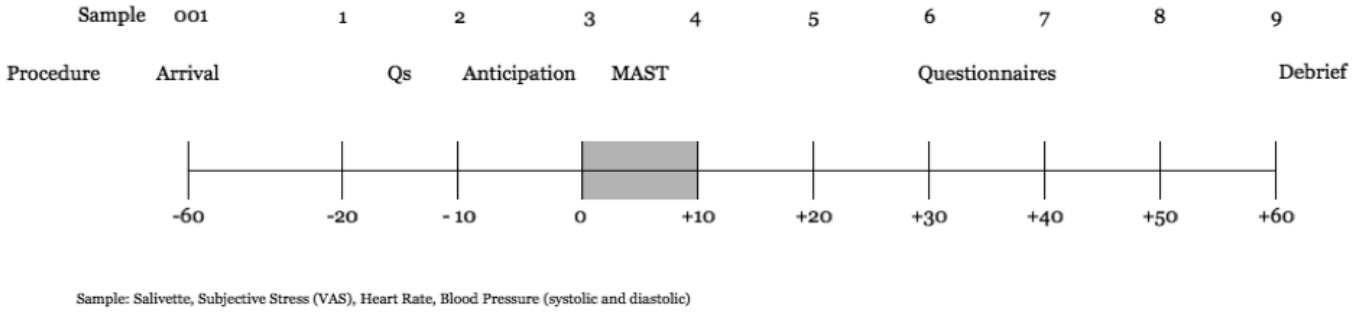

Fig. 1
Timeline of the MAST protocol. Qs= Questionnaires.

while our independent variable was time (measured in minutes).

We compared MAST with TSST and CPT data from the lab's database by using a mixed design analysis of variance (ANOVA). The ANOVA was of time by group by test (group = women in the follicular phase of their menstrual cycle or men, test = TSST, CPT or MAST) with repeated measures of cortisol, HR, BP (systolic and diastolic), and subjective stress. We determined significant main effects through pairwise comparisons corrected with Bonferroni's confidence interval adjustment. We set ninety-five-percent confidence intervals and a significance level of α=0.05 for all variables.

We conducted univariate ANOVAs on area-under-the-curve increase (AUCi), area-under-the-curve with respect to ground (AUCg) and Delta Peak (ΔPk) measures for cortisol, systolic and diastolic blood pressure, heart rate, and subjective stress. AUCi represents the increase in the area under the curve from baseline measures, AUCg represents the increase from zero, and ΔPk represents the difference between the height of peak and baseline values.

Results

Descriptive Statistics

We compared the results from the MAST (n = 17; n_{men} = 9, n_{women} = 8) with those of the TSST (n = 25; n_{men} = 13, n_{women} = 12) and the CPT (n = 18; n_{men} = 12, n_{women} = 6). Across all groups, four subjects' data had to be removed from the analysis since their scores were more than 3 SD away from the mean. We applied logarithmic transformations to non-normally distributed variables; this was the case for all measures except heart rate. Participants' age did not significantly differ between test groups or between sexes.

Effect of Sex and Stress Type on Salivary Cortisol Levels

No sex differences in cortisol levels existed in any of the stress tests. A mixed-design ANOVA with repeated measures of log-transformed cortisol levels showed that neither the main nor the interaction effects of sex were significant ($F(1,50) = 2.634$, $p = 0.111$; $F(2,50) = 1.435$, $p = 0.248$). In contrast, the same ANOVA showed that TSST and MAST produced comparable cortisol responses, which were higher than those of the CPT. All three tests produced a significant increase in cortisol levels over time within subjects (main effect of time ($F(2.818, 140.909) = 14.003$, $p = 0.000$) and time by test ($F(5.636, 140.909) = 4.104$, $p = 0.001$). Pair-wise comparisons carried out on time showed that cortisol levels at time points +10, +20, and +30 were significantly greater than cortisol levels at the -10min baseline.

The cortisol response did differ significantly between-tests (main effect of test between-subjects ($F(2,50) = 3.927$, $p = 0.026$)). Pair-wise comparisons revealed that the TSST and MAST were significantly higher than the CPT ($p<.05$; Fig.2-4).

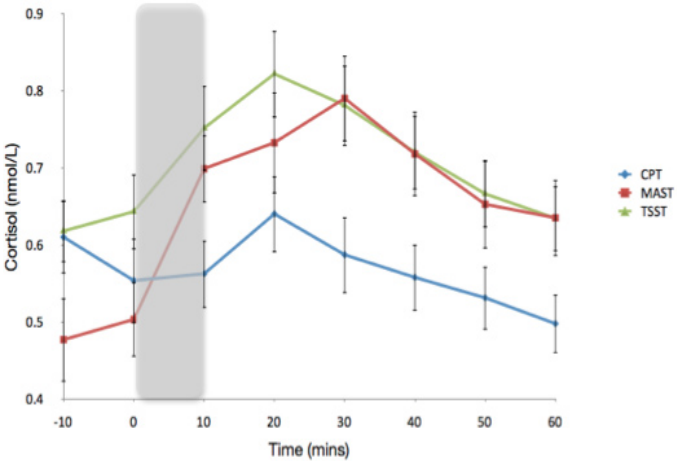

Fig. 2
Plots of estimated marginal means of log-transformed cortisol (nmol/L) levels in all three tests over time. There exists a significant effect of test between-subjects ($F(2,50)=3.927$, $p=0.026$) but only TSST and CPT are significantly different from each other ($p=0.022$). The error bars represent SEM.

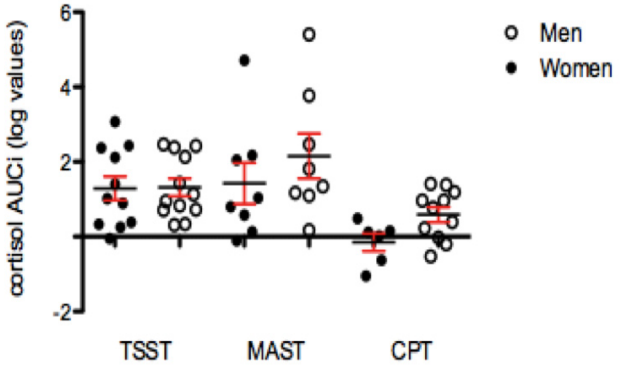

Fig. 3
Impact of three different stress tests on cortisol. Log-transformed cortisol data was used to calculate the AUCis. A significant effect of test was found in cort AUCi ($F(2,50)=8.304$, $p=0.001$). Means are displayed by full lines, SEM in red.

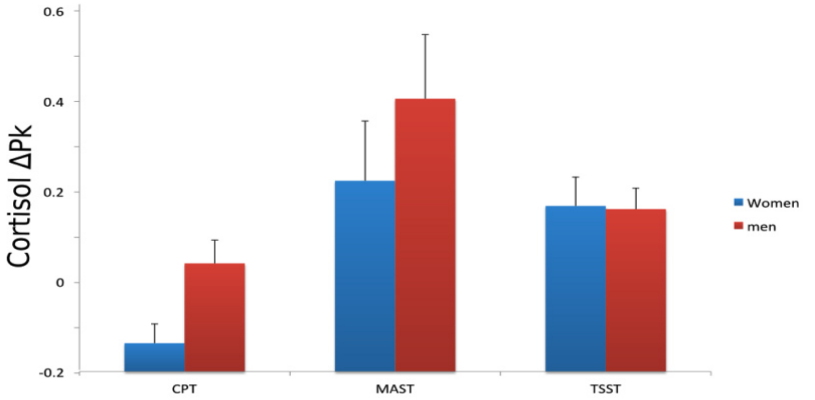

Fig. 4
Plots of estimated marginal means of log transformed cortisol ΔPk of both sexes over time in all three tests. Men's peak in cortisol is significantly greater than that of women (p=0.011). ΔPk is significantly different between TSST and CPT (p=0.024) and between MAST and CPT (p=0.006). The error bars represent SEM.

Effect of Sex and Stress Type on Blood Pressure and Heart Rate Measures

We carried out a logarithmic transformation on all blood pressure data because it was not normally distributed. HR data was normally distributed and therefore needed no transformation.

When looking at MAST data alone, the systolic BP values showed a significant between-subject effect of sex (p = 0.008) such that men's were greater.

We then ran a two-way repeated-measure ANOVA on both log systolic and log diastolic data. Both BP and HR significantly increased over time during all stress tests (lgsys F(1.823, 87.526) = 5.899, p = 0.005; lgdia F(2.852, 136.906) = 5.267, p = 0.002; HR F(4.750, 227.99) = 4.249, p = 0.001), however only HR ΔPk data showed a significant difference between tests supporting cortisol results. Responses of both TSST and MAST were comparable (p=1.000) and both were significantly greater than CPT (pTSST = 0.002; pMAST = 0.020) (Fig.5).

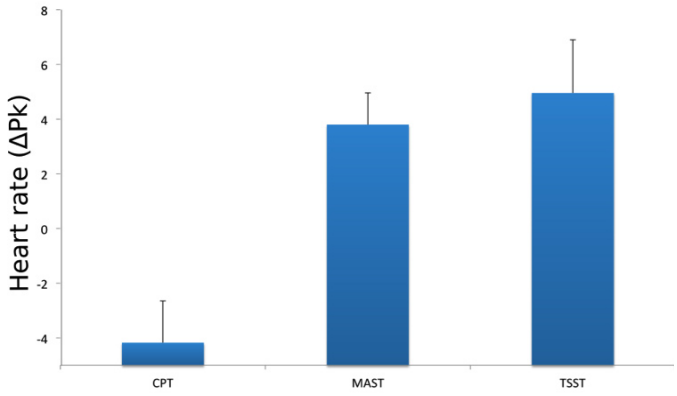

Fig. 5
Plot of estimated marginal means of heart rate ΔPk. A significant between-subjects effect of test was revealed (F(2,48)=7.021, p=0.002) where there is a significant difference between TSST and CPT (p=0.002) and between MAST and CPT (p=0.020). The error bars represent SEM.

Effect of Sex and Stress Type on Subjective Stress Ratings

Only TSST data showed a significant difference between sexes in their subjective stress responses over time (F(2.637, 55.382)=3.568, p=0.024), where women's subjective stress ratings were higher before and during the TSST.

A mixed-design repeated-measures ANOVA of subjective stress score showed that all tests produced a significant change in subjective stress ratings within-subjects over time (main effect of time (p=0.000) and time by test (p=0.031)).

Although self-esteem groups made no significant difference across all physiological measures, subjective stress did differ significantly between tests (p=0.000). Pair-wise comparisons demonstrated that subjective stress was strongly affected by whether CPT participants had high or low self-esteem; subjective stress scores of the CPT high self-esteem group were significantly lower than TSST (p=0.022), MAST (p=0.000), and CPT low self-esteem (p=0.002) (Fig.6).

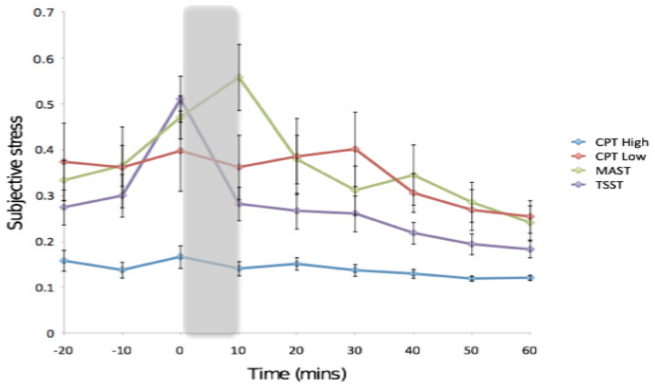

Fig. 6
Plot of estimated marginal means of log transformed subjective stress with CPT divided into high and low self-esteem groups. There exists a significant between-subjects main effect of test (F=7.420, p=0.000). CPT high-self-esteem group was significantly different from TSST (p=0.022), MAST (p=0.000) and CPT low-self-esteem (p=0.002).

Discussions

Opposite Sex Effects

Our main objective in this project was to determine whether opposite-sex testing significantly affected the stress responses of participants in a differential manner depending on their sex. Overall, opposite-sex testing did not seem to affect men and women in a differential manner. Apart from the subjective stress difference in the TSST (women rated higher in subjective stress) and the systolic BP difference in the MAST (men showed a greater systolic BP response), participants did not significantly differ in overall stress response. It is possible that the TSST produced a greater subjective stress response, being more sensitive to opposite sex evaluation due to its "job interview"-like, public speech context, period of preparation, and long speech time in front of an opposite-sex panel. Furthermore, it is likely that women in the follicular phase of their menstrual cycle are more susceptible to feeling stressed in this situation due to their heightened sensitivity to male stimuli (6).

Tests

In this project, we compared physiological and subjective responses to the TSST, the CPT, and the MAST stress tests when the tests were administered by experimenters of the opposite sex. Since the MAST is a newly developed stress task, this project was the first to both include women and directly compare its efficacy to that of the TSST and the CPT when administered by opposite-sex experimenters. In all tests, exposure produced a main effect of time in all repeated measures (cortisol, systolic and diastolic blood pressure, heart rate and subjective stress) indicating that all three stress tests were successful in producing significant responses in participants.

Our main hypothesis for this project was that the MAST administered by experimenters of the opposite sex would induce a comparable cortisol response to the TSST, and that both would generate higher responses than the CPT. Analyses of overall cortisol levels suggest that the CPT produced a significantly lower cortisol response than the TSST. The cortisol levels induced by the MAST were not significantly different from those induced by the TSST. In addition, a significant cortisol time by test effect was observed within subjects, where TSST and MAST cortisol values closely mirrored each other and CPT values were significantly lower. Subjects' cortisol responses over time were increasing following TSST, MAST and CPT. Therefore, at least in terms of HPA axis activation as indicated by salivary cortisol levels (and thus the effectiveness of a stress test to induce a stress at a psychosocial level), TSST and MAST seem to be equally effective.

Although blood pressure did not differ as a result of exposure to any of the tests, each test elicited different heart rate variations in participants over time. This suggests that both the MAST and the TSST are effective in producing an autonomic response in heart rate and that they are also both better at eliciting this response than the CPT. Therefore, it seems that stress induction tests with a psychosocial quality are not only better at activating the HPA axis, but also at producing an increased autonomic heart rate response.

When the participants of the CPT were divided into "high" and "low" self-esteem groups, there was a significant change in participants' subjective stress ratings over time. This change varied between tests. Each test thus elicited a significantly different subjective stress response within each participant throughout the testing period. Significant test effects between subjects also showed that the TSST, the MAST, and the CPT low-self-esteem groups all produced significantly greater subjective stress ratings than the CPT high-self-esteem group. This could perhaps be because individuals with very high self-esteem tend to be markedly more confident in their ability to cope with stressors and therefore perceive less stress throughout the procedure. The opposite would then be true for those with low self-esteem (i.e. less confidence) (5). In other words, self-esteem seems to buffer the subjective perception of stress during stress exposure in high-self-esteem individuals (5).

The results obtained from the MAST alone demonstrate that it causes a significant change in activation of both the HPA and SAM axes as well as in subjective stress over time in each participant. This is shown by a significant time effect within participants in cortisol levels, systolic and diastolic blood pressure, and heart rate measures as well as subjective stress ratings, in that all increase subsequently to task onset. These results further confirmed those of Smeets *et al.* (2012) as well as our hypothesis that the MAST would be an effective stress induction test that could significantly activate both the HPA and the SAM axes. Its ability to do so is possibly related to the fact that the MAST contains aspects of both psychosocial and physical stressors.

When compared to the other tests, our findings clearly demonstrate that the MAST is comparable to the effectiveness of the TSST. Furthermore, both the TSST and the MAST seem to be more effective stress induction tests than the CPT, especially when it comes to HPA axis activation and heart rate. These conclusions supported our initial statements that MAST is as effective a stress test as TSST, and that both are superior to CPT. However, our findings do not support our hypothesis since in no case did the stress response (as measured by indicators of HPA and SAM axis activation) induced by MAST exceed the one induced by TSST.

Limitations

Due to the restricted time available for this project, the major limitations were the small group sizes and the dependence on database data from previous TSST and CPT studies for analyses. Data from the TSST and the CPT were collected by different experimenters, with different protocols and different variables and covariates than the

MAST data collected specifically for this project. This was particularly true in the case of the CPT, where the added factor of self-esteem and the lack of emphasis on opposite-sex testing may have affected our results in sex differences.

Small sample sizes producing greater inter-individual variation may also have affected our results. Therefore, further studies with larger sample sizes would be required to confirm our conclusions. Finally, our main hypothesis tested for a similarity in stress responses between the sexes, thus interpreting the beta-error. As a consequence, we should have included computations for this error type in our statistical analysis, to show that if differences existed between men and women in the entire population, we possessed sufficient statistical power to demonstrate such an effect in our sample. However, because of financial and economic constraints in our testing regimen, we were unable to increase our sample size for this project, thus any power calculations would have been without consequence.

Concluding Statements

Based on the findings of this project, the MAST performed equally well in inducing a physiological stress response as the TSST in women in the follicular phase as well as men, and performed better in all cases than the CPT. Future directions of this line of research should investigate the effect of opposite versus same-sex testing, and the effect in women across all phases of the menstrual cycle or with the use of oral contraceptives.

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