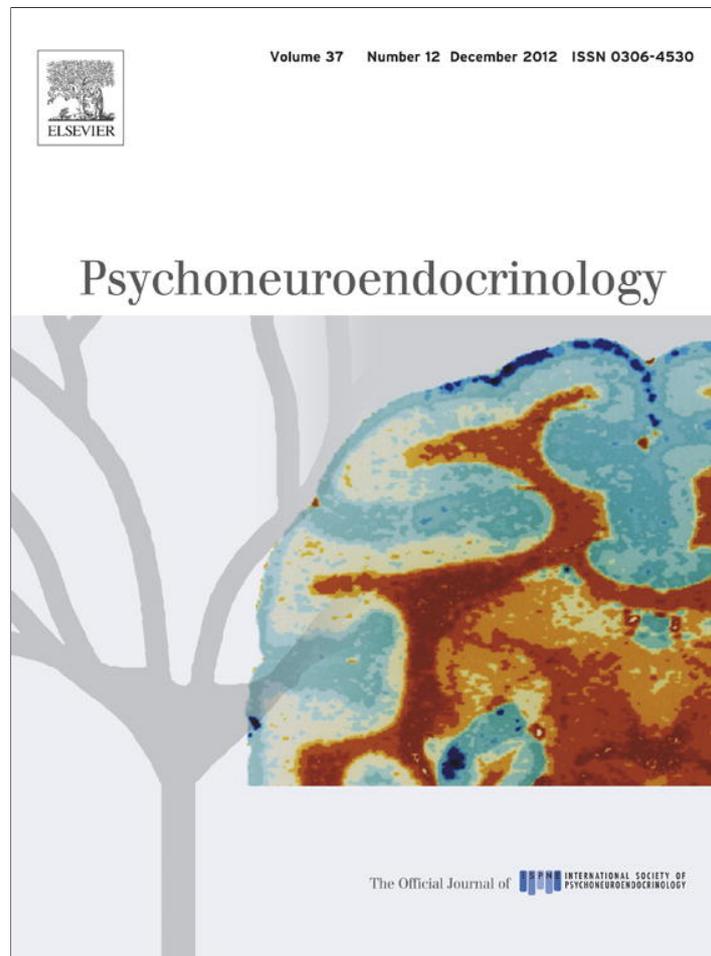


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Introducing the Maastricht Acute Stress Test (MAST): A quick and non-invasive approach to elicit robust autonomic and glucocorticoid stress responses

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Summary Stress-related research has employed several procedures to activate the human stress system. Two of the most commonly used laboratory paradigms are the Trier Social Stress Test (TSST) and the Cold Pressor Test (CPT). We combined their most stressful features to create a simple laboratory stress test capable of eliciting strong autonomic and glucocorticoid stress responses. In comparison with the CPT and its variations, our stress tool (labeled the Maastricht Acute Stress Test; MAST) was found to yield superior salivary cortisol responses, while being equally effective in eliciting subjective stress reactions and (systolic and diastolic) blood pressure increases (study 1; $N = 80$). In study 2 ($N = 20$), we directly compared the effectiveness of the MAST and TSST and found that both methods elicited similar subjective, salivary alpha-amylase, and salivary cortisol stress responses. Finally, we developed and evaluated an appropriate no-stress control version of the MAST that was similar to the stress version, although it did not comprise stressful components (study 3; $N = 40$). Collectively, our results confirm the effectiveness of the MAST in terms of subjective, autonomic, and – most importantly – glucocorticoid stress responses. Thus, as a brief and simple stress protocol, the MAST holds considerable promise for future research.

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1. Introduction

Exposure to stressful events increases the activity of the stress-responsive sympatho-adrenal-medullary (SAM) and hypothalamus-pituitary-adrenal (HPA) axes. Specifically,

stress suppresses the parasympathetic part of the nervous system (SNS), while simultaneously activating the sympathetic branch, causing the secretion of catecholamines (e.g., adrenalin and noradrenalin) that in turn produce increases in heart rate, blood pressure, and respiration frequency. The second major stress response relates to the activation of the HPA axis and commences with the hypothalamus releasing corticotropin releasing hormone, which then triggers the excretion of adrenocorticotrophic hormone by the pituitary gland. Ultimately, this causes the release of

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glucocorticoids (GCs; i.e., cortisol in humans and monkeys; corticosterone in many other species) by the adrenal cortex into the bloodstream (Ulrich-Lai and Herman, 2009). The stress-induced increase in activity of these stress systems leads to physiological and cognitive-behavioral alterations that collectively serve an adaptive purpose (i.e., increasing the chances of survival; McEwen, 1998, 2008; de Kloet et al., 2005; Schwabe et al., 2010). Neuroendocrine stress responses are also conceptualized as biomarkers reflecting individual differences in stress resilience and susceptibility to psychopathology and disease (Derijk and de Kloet, 2008; Feder et al., 2009; Smeets, 2010; Joëls, 2011).

Research regarding stress reactivity and the impact of stress responses on physiology, cognition, emotion, and behavior has employed several laboratory stress tasks that are capable of activating the human stress system. However, the degree to which they stimulate the SAM and HPA axes differs significantly between tasks. For example, the Cold Pressor Test (CPT; e.g., Lovallo, 1975; Mitchell et al., 2004), in which participants are instructed to immerse a hand up to the wrist in ice-cold (typically 0–5 °C) water for as long as possible with a maximum of 3 min, results in a robust and reliable activation of the SAM axis (e.g., blood pressure, skin conductance), but elicits only minor HPA axis reactivity in terms of cortisol responses. Alternatively, the Trier Social Stress Test (Kirschbaum et al., 1993; for review see Kudielka et al., 2007; Foley and Kirschbaum, 2010) is considered to be the gold standard among the various stress protocols. The TSST is a psychosocial challenge test that consists of a short preparation period, a 5 min speech (e.g., simulated job interview), and a 5 min mental arithmetic task, all performed in front of an audience while being audio- and videotaped. The TSST can be used with children and adults (e.g., Kirschbaum et al., 1992; Kudielka et al., 2004a,b; see also Yim et al., 2010) and is a procedure that reliably elicits strong neuroendocrine stress responses such as a 2- to 3-fold increase in salivary cortisol concentrations, rendering it the paramount stress protocol to stimulate the HPA axis (Dickerson and Kemeny, 2004).

Potential causes for the differential ability of the CPT and TSST to effectively stimulate the HPA axis likely include the nature of the challenge test (CPT: physical pain sensation; TSST: psychosocial evaluative threat), the duration of the stress protocol (CPT: max. 3 min; TSST: 15 min), and the uncontrollability and unpredictability of the procedure (e.g., Dickerson and Kemeny, 2004). Indeed, physical stressors (e.g., pain) require an immediate bodily reaction via reflexive mechanisms implicating the brainstem and hypothalamus (Ulrich-Lai and Herman, 2009), and thus generate a rapid activation of the autonomic nervous system and HPA axis. Psychosocial stressors are primarily evaluated and processed in the frontal lobes and thalamus, and the resulting cognitive appraisals trigger stress responses via connections of the prefrontal cortex with the limbic structures, which in turn through projections to the hypothalamus serve as the principal pathway to activating the HPA axis (Dickerson and Kemeny, 2004; Ulrich-Lai and Herman, 2009). Combining a physical stressor with social-evaluative components can therefore be expected to yield strong autonomic and HPA axis responses. In fact, the traditional CPT has been modified to produce stronger HPA (cortisol) stress responses by adding to it social-evaluative elements (i.e., the Socially Evaluated

Cold Pressor Test or SECPT; Schwabe et al., 2008). Specifically, in the SECPT participants perform the hand immersion task while being watched by an experimenter of the opposite sex and being videotaped. It was found that the SECPT resulted in significantly higher cortisol stress responses than the standard CPT or the control tasks (Schwabe et al., 2008).

Even though the SECPT has proven to be successful in reliably eliciting cortisol stress responses (see also Schwabe and Wolf, 2009a,b, 2010a,b, 2011; Schwabe et al., 2009a,b; Smeets, 2011), they remain smaller than those typically observed when employing the TSST. Thus, one could argue that the scientifically sound TSST should preferably be used in studies in which substantial glucocorticoid stress responses are deemed crucially important. However, one drawback of the various TSST procedures (e.g., Smeets et al., 2007; Yim et al., 2010; for review see Foley and Kirschbaum, 2010) is that they require a panel of judges to evaluate individual participants' performance on their speech and mathematical abilities, making the TSST less cost-effective than the (SE)CPT (requiring only a single experimenter). A related point is that TSST procedures may be jeopardized by practical problems such as scheduling conflicts between panel members, experimenter, and participants.

With this in mind, the aim of the current studies was to develop and evaluate an easy-to-administer stress protocol effective in eliciting robust cortisol responses. To this end, we combined the perceived most stressful features of the TSST (i.e., psychosocial evaluative threat, uncontrollability, and unpredictability) and the CPT (i.e., the painful aspect) so as to create a physically and psychologically challenging laboratory stress test, labeled the Maastricht Acute Stress Test (MAST). Basically, after a short instruction and preparation phase (5 min), participants are asked to perform 5 socially evaluated cold pressor trials that vary in duration (ranging from 60 s to 90 s) over a 10 min time span, with the water temperature held constant at 2 °C. During the inter-trial intervals, which also vary in duration, participants are instructed to – analogous to the TSST – perform mental arithmetic as fast and accurate as possible and receive negative feedback on their performance when mistakes are made. In study 1, the MAST was compared with the traditional CPT, the SECPT, and a prolonged (i.e., 15 min) SECPT in terms of subjective stress, systolic and diastolic blood pressure, and cortisol stress responses. Study 2 employed a within-subject crossover design to directly compare subjective stress, salivary alpha-amylase, and cortisol responses between the MAST and TSST. Finally, we designed and validated an appropriate no-stress control condition for the MAST (study 3).

2. Study 1

2.1. Study 1 method

2.1.1. Participants

Eighty healthy undergraduates with a mean age of 21.91 years ($SD = 2.72$) and a normal body mass index (BMI; means = 22.65; $SD = 2.22$) participated in the current study. They were recruited by means of advertisements that requested volunteers for a study examining individuals' resilience to physical and mental challenges. To rule out that

gender differences could play a confounding role in the cortisol reactions to the various stressors (e.g., Kudielka and Kirschbaum, 2005), only men were included in the present study. Eligibility was assessed using a semi-structured interview, with cardiovascular diseases, severe physical illnesses (e.g., fibromyalgia), hypertension, endocrine disorders, current or lifetime psychopathology, substance abuse, heavy smoking (>10 cigarettes/day) or being on any kind of medication known to affect the HPA axis serving as exclusion criteria. Test protocols were approved by the standing ethics committee of the Faculty of Psychology and Neuroscience, Maastricht University. All participants provided informed consent and received a small financial reward or partial course credit in return for their participation.

2.1.2. Stress induction equipment and procedures

Equipment. All stress induction procedures were carried out using a plexiglas box (JULABO Labortechnik GmbH, Seelbach, Germany) as the water bath. An electrical immersion cooler (JULABO type FT200) and a circulation pump (JULABO type ED-19) were used to cool the water and subsequently keep it constant (i.e., ± 0.03 °C) at a fixed temperature level (i.e., 2 °C).

2.1.2.1. Cold Pressor Test (CPT; $n = 20$). Participants in the CPT condition underwent the standard CPT procedure (e.g., Lovallo, 1975; Mitchell et al., 2004; Smeets et al., 2008). That is, they were instructed to immerse their hand up to and including the wrist in ice-cold (2 °C) water for as long as possible, with a maximum of 3 min. They were explicitly told that the procedure could be very uncomfortable and that they could remove their hand from the ice-cold water at their own discretion without consequences. Throughout the CPT, the experimenter remained in the test room to covertly monitor (i.e., without explicitly watching) participants' compliance with the test instructions from the corner of the test room.

2.1.2.2. Socially Evaluated Cold Pressor Test (SECPT; $n = 20$). In the SECPT (Schwabe et al., 2008) condition, participants had to immerse their hand up to and including the wrist in ice-cold (2 °C) water for as long as possible with a maximum of 3 min while being videotaped and closely monitored by an experimenter that displayed a lack of empathy. They were told that the videotapes would be analyzed for facial expressions of pain and had to provide written consent to the videotaping. It was also made clear that the procedure could be very uncomfortable and that they could remove their hand from the ice-cold water at their own discretion without consequences.

2.1.2.3. Prolonged Socially Evaluated Cold Pressor Test (P-SECPT; $n = 20$). The P-SECPT was designed as an extended version of the SECPT to more closely match the duration of the TSST (Kirschbaum et al., 1993). Specifically, during a 5 min preparation period, participants were seated in front of a computer screen and given instructions via a PowerPoint presentation. Similar to the SECPT condition, participants were informed about the hand immersion task, that they would be monitored by the experimenter and videotaped so as to later analyze their facial expressions, that they had to provide written consent to the videotaping, and that they

had the right to withdraw their hand at any time during the task. Instructions specific to the P-SECPT were that they had to immerse their hand in ice-cold (2 °C) water multiple times alternated with short resting periods during which they could rest their arm on a towel that was placed alongside the water bath. Also specific was the bogus instruction that the computer would randomly decide how long they had to immerse their hand in the water, but that trials would never exceed 90 s. Similarly, they were misleadingly told that the computer randomly decided the duration of the rest periods between the SECPT trials and that they would last a minimum of 45 s each. These bogus instructions served to increase participants' feelings of uncontrollability and unpredictability, which are known to increase the activity of the HPA axis (e.g., Dickerson and Kemeny, 2004). In fact, the order and duration of the hand immersion trials was fixed and identical to the MAST (see below).

2.1.2.4. Maastricht Acute Stress Test (MAST; $n = 20$). The MAST consists of a 5 min preparation phase and a 10 min acute stress phase that includes the physical aspects of the (SE)CPT and the unpredictability, uncontrollability, social-evaluative nature (i.e., negative feedback), and mental arithmetic elements of the TSST. The 5 min preparation period serves to seat participants in front of a computer screen and instruct them about the upcoming task via a PowerPoint presentation.¹ Similar to the SECPT condition, participants were informed about the hand immersion task, that they would be monitored by the experimenter as well as videotaped so as to later analyze their facial expressions, that they had to provide written consent to the videotaping, and that they had the right to withdraw at any time during the task. They were informed that there would be multiple trials in which they had to immerse their hand in ice-cold (2 °C) water, and that the duration of these trials would be randomly chosen by the computer yet never would exceed 90 s. In between the hand immersion trials, they were instructed to put their arm on a towel alongside the water bath and immediately engage in the mental arithmetic test, which consisted of counting backwards starting at 2043 in steps of 17 as fast and accurate as possible. Each time they made a mistake, they were given negative feedback and had to start over at 2043. They were told to continue with the mental arithmetic until the computer would signal the start of the next hand immersion trial, which would take at least 45 s. In reality, the duration of the various hand immersion trials alternated with the mental arithmetic was set in a fixed order and duration for all participants. The fixed order and duration is displayed in Fig. 1 (Panel b).

2.1.3. Subjective, cardiovascular, and neuroendocrine stress responses

2.1.3.1. Subjective stress. Immediately after the stress induction protocol, participants were asked to rate how stressful, how painful, and how unpleasant the stress induction procedure had been by appropriately marking 0–100 Visual Analog Scales (VASs; anchors: 0 = “not at all”; 100 = “extremely”).

¹ The PowerPoint presentation with specific instructions for the MAST is available upon request from the corresponding author.

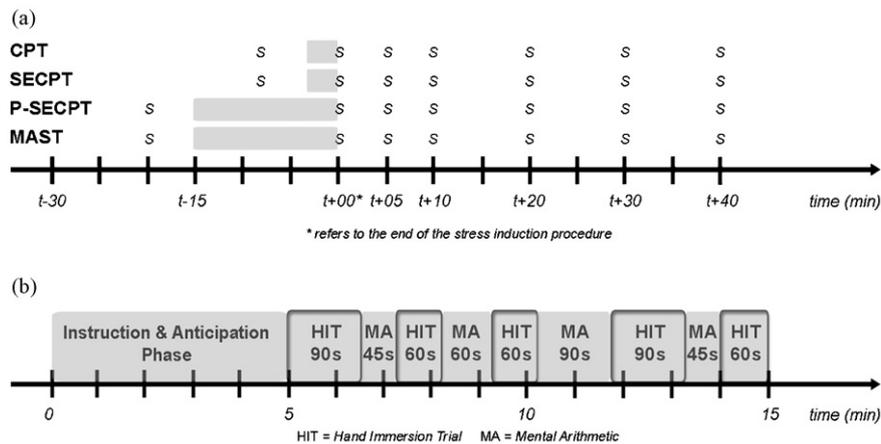


Figure 1 (Panel a) Sequence of the experimental events of study 1, with t_{+00} referring to end of the stress induction procedures and Ss denoting times when saliva was sampled. Gray areas refer to the stress induction procedures. (Panel b) Order of hand immersion trials (HIT) and mental arithmetic (MA) of the MAST and the duration (in s) of the various trials.

2.1.3.2. Systolic and diastolic blood pressure (SBP; DBP). SBP and DBP were measured using an Omron 705IT (HEM-759-E; Omron Healthcare Europe BV, Hoofddorp, the Netherlands), a fully automated upper-arm oscillometric blood pressure monitoring device clinically validated by the Association for the Advancement of Medical Instruments, the European Society of Hypertension, and the British Hypertension Society (e.g., Coleman et al., 2006; Stergiou et al., 2006). SBP and DBP were assessed 5 min before the start of the stress induction protocol as well as immediately and 5 min after the end of the stress induction protocol.

2.1.3.3. Cortisol. Salivary cortisol was measured in response to the stress induction procedures as a measure of activity of the stress-responsive HPA axis. Cortisol data were obtained with synthetic Salivette (Sarstedt®), Etten-Leur, the Netherlands) devices 5 min before (i.e., $t_{pre-stress}$) and 6 times afterwards (i.e., t_{+00} , t_{+05} , t_{+10} , t_{+20} , t_{+30} and t_{+40} min with reference to the end of the stressor; see Fig. 1 (Panel a)). Samples were stored at -20°C immediately on collection. Cortisol levels were determined by a commercially available luminescence immuno assay (IBL, Hamburg, Germany). Mean intra- and inter-assay coefficients of variation are typically less than 8% and 12%, respectively, and the lower and upper detection limits were $0.015\ \mu\text{g}/\text{dl}$ ($0.41\ \text{nmol}/\text{l}$) and $4.0\ \mu\text{g}/\text{dl}$ ($110.4\ \text{nmol}/\text{l}$), respectively.

2.2. Study 1 procedure

Participants were tested in individual sessions run between 13 h and 18 h. To allow for controlled saliva collection participants were asked not to brush their teeth and to refrain from food, drinks, and intense physical exercise at least 1 h prior to the test phase. None of the participants reported to have violated these directives. After arrival in the laboratory, participants received information about the study and the measurements that would be taken, and provided written informed consent. Participants were randomly assigned to one of the four stress induction procedures (i.e., CPT, SECPT, P-SECPT, or MAST), and following completion of the stress task were asked to rate the stressfulness of the procedure using the VASs. During the remainder of the session participants were

asked to relax and engaged in non-stressful filler tasks (e.g., reading a neutral text).

2.3. Study 1 statistical analyses

Data were checked for non-normality using Q–Q plots and Shapiro–Wilk tests of normality. Subjective stress ratings (stressfulness, painfulness, pleasantness) were analyzed using univariate analyses of variance (ANOVAs). Blood pressure was analyzed using a $4(\text{Group: CPT, SECPT, P-SECPT, MAST}) \times 3(\text{Time: } t_{pre-stress}, t_{+00}, t_{+05})$ repeated measures ANOVA. As the cortisol data were highly skewed, a log-transformation was performed before these data were used in subsequent analyses. Cortisol data were analyzed with a $4(\text{Group: CPT, SECPT, P-SECPT, MAST}) \times 7(\text{Time: } t_{pre-stress}, t_{+00}, t_{+05}, t_{+10}, t_{+20}, t_{+30}, t_{+40})$ ANOVA, with the latter factor being a repeated measure. We also computed the Area Under the Curve with respect to increase (AUCi) as a single measure of the total hormone concentration in response to the various stressors (Pruessner et al., 2003) and analyzed them with a univariate ANOVA. For descriptive purposes, a responder rate of participants showing a cortisol increase equal to or larger than $2.5\ \text{nmol}/\text{l}$ (see, for example, Kirschbaum et al., 1993; Smeets et al., 2006a), which is thought to reflect a cortisol secretory episode (Van Cauter and Refetoff, 1985), was calculated. When sphericity assumptions were violated, Greenhouse–Geisser corrected p -values are reported. Alpha was set at 0.05 and adjusted (Bonferroni) for multiple comparisons where necessary. In case of significant results, ANOVAs are supplemented with Partial Eta Squared (η_p^2) values as a measure of effect size, which represent the proportion of total variation attributable to the independent variable after partialling out the contribution of the other variables under investigation. η_p^2 values of 0.01 indicate small effects, 0.06 represent medium effects, and 0.14 constitute large effects (Fritz et al., 2012).

2.4. Study 1 results and discussion

Table 1 shows subjective stress ratings and SBP/DBP for each of the 4 groups. Groups did not differ in their ratings of subjective stressfulness, painfulness, or unpleasantness [All

Table 1 Means (\pm SE) of subjective stress and systolic and diastolic blood pressure responses for the 4 stress induction procedures.

	CPT	SECPT	P-SECPT	MAST
Subjective stress (0–100)				
Stress	39.65 (4.32)	41.38 (4.43)	39.45 (4.10)	35.98 (4.44)
Pain	46.05 (3.77)	44.50 (3.22)	48.10 (4.69)	45.48 (3.85)
Unpleasantness	61.70 (4.37)	57.65 (3.86)	53.70 (4.41)	61.10 (4.39)
Systolic BP (mm/HG)				
Pre	122.00 (1.40)	121.95 (2.35)	124.90 (2.88)	123.50 (2.43)
Stress	139.20 (2.13)	137.70 (3.16)	142.35 (2.19)	143.45 (3.21)
Post	<i>124.30 (1.76)</i>	<i>126.15 (1.86)</i>	<i>131.05 (1.94)</i>	<i>132.50 (2.92)</i>
Diastolic BP (mm/HG)				
Pre	69.45 (0.93)	70.35 (1.54)	71.00 (1.64)	68.95 (2.60)
Stress	77.80 (1.95)	79.35 (1.47)	81.60 (1.70)	79.85 (2.68)
Post	<i>69.20 (2.36)</i>	<i>72.00 (2.10)</i>	<i>75.40 (1.83)</i>	<i>73.30 (2.49)</i>

Note: Values printed in bold denote significant ($p < 0.001$) within-group differences from pre-stress to immediately following stress induction; values printed in italics denote significant ($p < 0.001$) within-group differences from immediately following stress induction to post-stress.

$F_s(3,76) < 1$; all $p_s > 0.52$], indicating that participants perceived the CPT, SECPT, P-SECPT, and MAST as equally distressing.

For SBP, ANOVA showed a main effect of Time [$F(2,152) = 124.01$; $p < 0.001$; $\eta_p^2 = 0.62$], but no main effect of Group [$F(3,76) = 1.59$; $p = 0.20$] or a Group \times Time interaction [$F(6,152) < 1$; $p = 0.47$]. SBP increased significantly ($p < 0.001$) from $t_{\text{pre-stress}}$ to immediately following stress induction (t_{+00}), and decreased from t_{+00} to t_{+05} ($p < 0.001$). Similarly, analyses of DBP yielded a main effect of Time [$F(2,152) = 55.96$; $p < 0.001$; $\eta_p^2 = 0.42$] in the absence of a main Group [$F(3,76) < 1$; $p = 0.46$] or Group \times Time interaction [$F(6,152) < 1$; $p = 0.64$] effect. DBP also increased significantly from $t_{\text{pre-stress}}$ to t_{+00} ($p < 0.001$) and decreased from t_{+00} to t_{+05} ($p < 0.001$). Thus, the newly developed P-SECPT and MAST procedures reliably induced systolic and diastolic blood pressure responses that were highly similar to the cardiovascular responses elicited by the CPT and SECPT procedures.

Cortisol responses to the CPT, SECPT, P-SECPT, and MAST are displayed in Fig. 2. As the ANOVA showed a significant Group \times Time [$F(18,456) = 4.49$; $p < 0.001$; $\eta_p^2 = 0.15$] interaction, simple effects were computed for each time point. Groups differed significantly in cortisol concentrations at t_{+05} [$F(1,76) = 4.20$; $p = 0.008$; $\eta_p^2 = 0.14$] and t_{+10} [$F(1,76) = 4.30$; $p = 0.007$; $\eta_p^2 = 0.15$], but not at any other sampling point (all $F_s(1,76) < 1.83$; all $p_s > 0.15$). Follow-up tests showed that the differences at t_{+05} were qualified by higher cortisol concentrations for the MAST group relative to the CPT ($p = 0.010$) and SECPT ($p = 0.045$) groups, while the MAST group differed only marginally from the P-SECPT group ($p = 0.091$). The CPT, SECPT, and P-SECPT did not differ from each other at t_{+05} (all $p_s > 0.99$). Similarly, at t_{+10} the MAST group displayed higher cortisol concentrations than the CPT ($p = 0.009$) and SECPT ($p = 0.030$) groups, but the difference with the P-SECPT group fell short of significance ($p = 0.43$). The CPT, SECPT, and P-SECPT again did not differ from each other (all $p_s > 0.90$). ANOVA on the AUCi values showed a main effect of Group [$F(3,76) = 3.25$; $p = 0.026$; $\eta_p^2 = 0.11$], with follow-up tests showing that the MAST differed from the CPT ($p = 0.017$) while all other comparisons remained non-significant (all $p_s > 0.35$). The percentage of participants who could be

classified as cortisol responders (i.e., cortisol increase ≥ 2.5 nmol/l; cf. supra) was 40% (8/20) for the standard CPT, 65% (13/20) for the SECPT, 70% (14/20) for the P-SECPT, and 85% (17/20) for the MAST. Collectively, these results suggest that the MAST yields stronger cortisol responses than the CPT and SECPT.

3. Study 2

Study 1 indicated that the CPT, SECPT, P-SECPT, and MAST were equally effective in eliciting subjective and

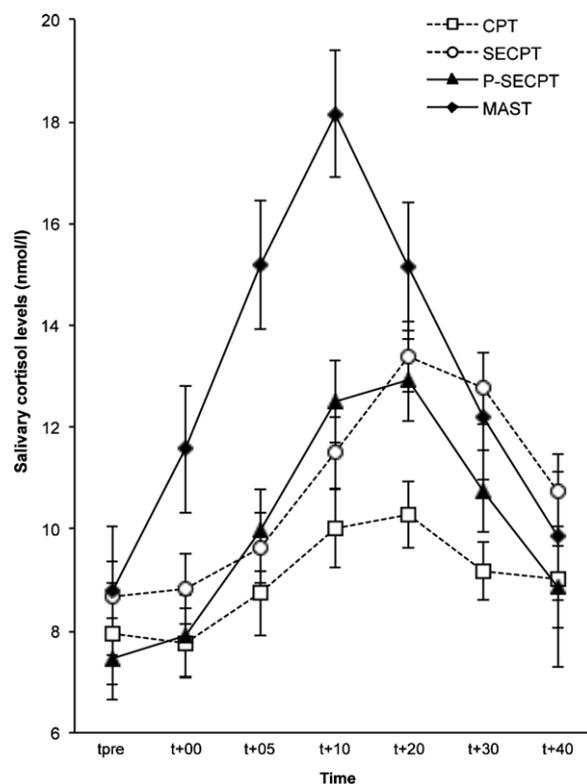


Figure 2 Cortisol responses to the stress induction procedures in study 1. Graphs show means \pm SE.

cardiovascular (SBP and DBP) stress responses, and that the MAST appears to be most effective in terms of cortisol reactivity. Study 2 was designed to directly contrast the MAST and the gold standard in laboratory stress research, i.e., the TSST, using a within-subject crossover design. In addition to assessing subjective stress and cortisol responses, study 2 gauged the reactivity of salivary alpha-amylase (sAA), which has served as a measure of adrenergic activity of the stress-responsive SAM axis in recent research (for an overview see Nater and Rohleder, 2009).

3.1. Study 2 method

3.1.1. Participants

Twenty healthy male undergraduates with a mean age of 22.00 years ($SD = 3.87$) and a mean BMI of 22.05 ($SD = 2.44$) participated in the current study. Eligibility was assessed using the same semi-structured interview and exclusion criteria as in study 1. Test protocols were approved by the standing ethics committee of the Faculty of Psychology and Neuroscience, Maastricht University. All participants provided informed consent and received a small financial reward or partial course credit in return for their participation.

3.1.2. Stress induction

Study 2 involved two sessions, with a 1-week period in between, in which participants were exposed in a counter-balanced fashion to both the MAST and the TSST. The TSST (Kirschbaum et al., 1993) is a psychosocial challenge test that reliably induces psychological and neuroendocrine stress responses, consisting of a preparation period, a 5 min mental arithmetic task, and a 5 min speech in front of an audience. In keeping with our previous work (Smeets et al., 2006b, 2007, 2009, 2012), participants were asked to critically describe their own personality characteristics in English (i.e., a non-native language) while standing in front of a live audience and being audio- and videotaped.

3.1.3. Subjective and neuroendocrine stress responses

3.1.3.1. Subjective stress. Subjective stress prior to and immediately following the MAST/TSST was assessed using the Negative Affect subscale of the Positive and Negative Affect Schedule, state version (PANAS; Watson et al., 1988). The PANAS is a sound psychometric tool consisting of two subscales that quantify positive affect (PA) and negative affect (NA). The NA subscale comprises 10 items for which respondents indicate on 5-point scales (anchors: 1 = *very slightly or not at all*; 5 = *extremely*) the extent to which certain feelings and emotions apply to them. Higher scores are indicative of higher levels of experienced negative affect.

3.1.3.2. Salivary alpha-amylase (sAA) and cortisol. sAA and cortisol were measured in response to the MAST/TSST as measures of activity of the stress-responsive SAM and HPA axis, respectively. sAA and cortisol data were obtained with synthetic Salivette devices 5 min before (i.e., $t_{\text{pre-stress}}$) and 6 times after the stress induction protocol (i.e., t_{+00} , t_{+05} , t_{+10} , t_{+20} , t_{+30} and t_{+40} min with reference to the end of the stressor). Saliva samples were stored at -20°C immediately

on collection. sAA levels were determined from the saliva samples using a commercially available kinetic reaction assay (Salimetrics, Penn State, PA). Mean intra- and inter-assay coefficients of variation of the sAA analyses are typically less than 8% and 6%, respectively. As in study 1, cortisol levels were determined by a commercially available luminescence immuno assay (IBL, Hamburg, Germany).

3.2. Study 2 procedure

Participants were tested individually between 09 h and 12 h. All participants reported not to have brushed their teeth, consumed foods or drinks, or engaged in intense physical exercise at least 1 h prior to the test phase to allow controlled saliva sampling. Upon arrival in the laboratory, participants received information about the study and provided written informed consent. Participants were randomly assigned to one of two groups, with one group first being exposed to the MAST and subsequently after a 1-week interval to the TSST, and the other group receiving the reverse order.

3.3. Study 2 statistical analyses

Non-normality and violations of sphericity were treated analogous to study 1. As there were no order (i.e., MAST–TSST vs. TSST–MAST) effects in any of the analyses, order is not further considered in the analyses reported below. Subjective stress (i.e., negative affect) was analyzed with a 2(Group: MAST, TSST) \times 2(Time: pre, post) ANOVA with both factors being repeated measures. sAA and cortisol data were analyzed with a 2(Group: MAST, TSST) \times 7(Time: $t_{\text{pre-stress}}$, t_{+00} , t_{+05} , t_{+10} , t_{+20} , t_{+30} , t_{+40}) repeated measures ANOVA. AUCi was also calculated for sAA and cortisol and analyzed using paired samples t -tests. The criterion to define cortisol responding was the same as that employed in study 1.

3.4. Study 2 results and discussion

Fig. 3 shows PANAS NA scores and sAA and cortisol responses to the MAST and TSST, respectively. For PANAS NA, a main effect of Time [$F(1,19) = 4.69$; $p = 0.043$; $\eta_p^2 = 0.20$], but no main [$F(1,19) = 1.57$; $p = 0.23$] or interaction [$F(1,19) < 1$; $p = 0.67$] effect involving Group was found. As can be seen in Fig. 3, there was an increase in negative affect in response to both stressors.

With regard to sAA, ANOVA yielded a main effect of Time [$F(6,114) = 4.85$; $p = 0.003$; $\eta_p^2 = 0.20$], but no main effect of Group [$F(1,19) < 1$; $p = 0.78$] or a Group \times Time interaction [$F(6,114) < 1$; $p = 0.59$]. Follow-up tests regarding the main effect of Time showed significant increases in sAA between $t_{\text{pre-stress}}$ and t_{+00} ($p < 0.001$), followed by declines between t_{+00} to t_{+05} ($p = 0.020$), only to remain stable afterwards (i.e., between t_{+05} , t_{+10} , t_{+20} , t_{+30} , and t_{+40} ; all $ps > 0.99$). AUCi values with respect to sAA did not differ between the MAST and TSST [$t(19) < 1$; $p = 0.65$].

Cortisol responses to the MAST and TSST were also of a similar magnitude, as evidenced by a non-significant Group \times Time interaction [$F(6,114) = 1.15$; $p = 0.33$] and a non-significant main effect of Group [$F(1,19) < 1$; $p = 0.57$]. As was expected, a main effect of Time [$F(6,114) = 34.79$;

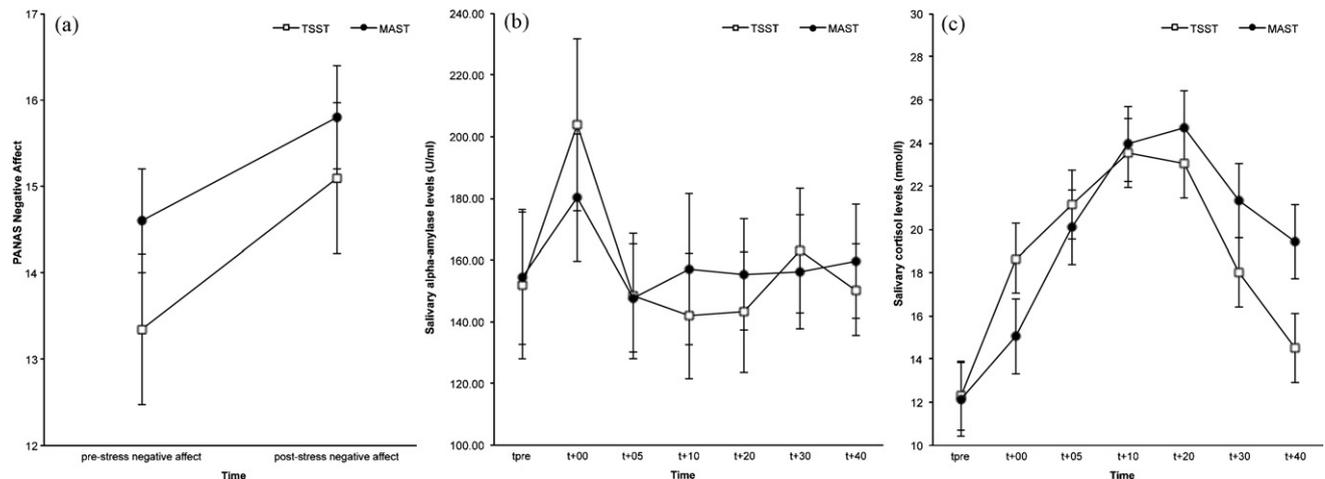


Figure 3 PANAS NA (panel a), salivary alpha-amylase (panel b), and cortisol (panel c) responses to the Maastricht Acute Stress Test (MAST) and Trier Social Stress Test (TSST) in study 2. Graphs show means \pm SE.

$p < 0.001$; $\eta_p^2 = 0.65$] emerged. Follow-up tests showed significant increases in cortisol between $t_{\text{pre-stress}}$ and t_{+00} ($p < 0.001$), t_{+00} and t_{+05} ($p < 0.001$), and t_{+05} and t_{+10} ($p = 0.001$), non-significant changes between t_{+10} and t_{+20} ($p > 0.99$), followed by significant declines between t_{+20} and t_{+30} ($p < 0.001$) and between t_{+30} and t_{+40} ($p < 0.001$). There were no differences between the MAST and TSST for cortisol AUCi values [$t(19) < 1$; $p = 0.82$]. Cortisol responder percentages for the TSST and MAST were 95% (19/20) and 90% (18/20), respectively.

In sum, using a within-subject crossover design, study 2 showed that the MAST yields subjective and neuroendocrine stress responses that are similar to those of the TSST.

4. Study 3

The aim of study 3 was to develop and evaluate a no-stress control version of the MAST that is comparable in terms of duration and physical and cognitive load, but does not include the MAST's stressful components. Thus, a 15 min hand immersion task with lukewarm water and a simplified counting task was developed and contrasted with the MAST.

4.1. Study 3 method

4.1.1. Participants

Forty healthy male undergraduates with a mean age of 20.55 years ($SD = 1.99$) and a mean BMI of 21.99 ($SD = 2.12$) participated in the current study. Eligibility was assessed using the same semi-structured interview and exclusion criteria as in studies 1 and 2. Test protocols were approved by the standing ethics committee of the Faculty of Psychology and Neuroscience, Maastricht University. All participants provided informed consent and received a small financial reward or partial course credit in return for their participation.

4.1.2. Stress induction versus no-stress control

Equivalent to the MAST, its placebo (i.e., no-stress control) version consists of a 5 min preparation phase and a 10 min hand immersion phase alternated with a simple counting

test. The 5 min preparation phase was identical to that of the MAST, except that no mention of audio- or videotaping was made and that participants were told that the water would be lukewarm (35–37 °C). In between the hand immersion trials, participants rested their arm on a towel alongside the water bath and immediately started counting consecutively from 1 to 25 at their own pace, and had to start anew at 1 when 25 was reached. The experimenter remained in the room so as to check participants' compliance with the instructions, but they were not given any feedback on their performance. The duration of the various trials and their order paralleled that of the MAST (see Fig. 1 (Panel b)).

4.1.3. Neuroendocrine stress responses

sAA and cortisol measurements were taken at 4 time points: a pre-stress measure obtained 5 min prior to the onset of the MAST or placebo MAST ($t_{\text{pre-stress}}$) and 3 post-stress measures (i.e., t_{+00} , t_{+10} , and t_{+30} with reference to the end of the (placebo) MAST). Collection and handling of the samples was identical to study 2.

4.2. Study 3 procedure

Participants were tested individually between 13 h and 16 h. Participants refrained from brushing their teeth, consuming foods or drinks, or engaging in intense physical exercise at least 1 h prior to the test phase. Information about the study was provided upon arrival and participants provided written informed consent. Participants were then randomly assigned to either the MAST ($n = 20$) or the placebo MAST ($n = 20$) group. Five min prior to the beginning of the (placebo) MAST, a first saliva sample was taken. Immediately following the (placebo) MAST, a second saliva sample was taken. While awaiting the third and fourth saliva sampling procedure, participants were asked to relax and perform non-stressful filler tasks.

4.3. Study 3 statistical analyses

Analyses of sAA and cortisol data were similar to those of studies 1 and 2.

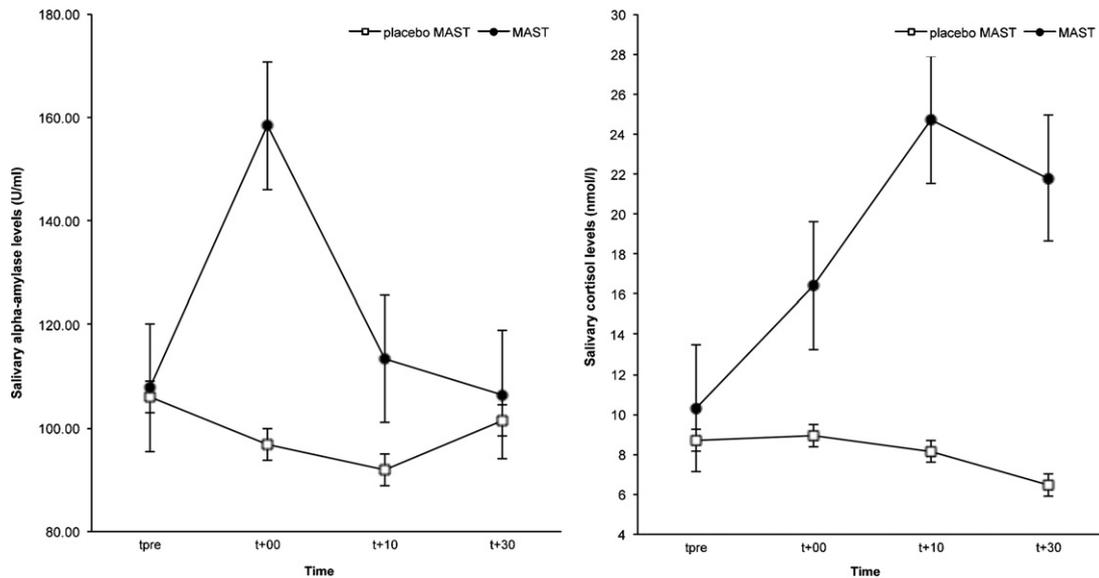


Figure 4 Salivary alpha-amylase (panel a) and cortisol (panel b) responses to the Maastricht Acute Stress Test (MAST) and its no-stress control counterpart (placebo MAST) in study 3. Graphs show means \pm SE.

4.4. Study 3 results and discussion

Fig. 4 shows sAA and cortisol responses to the MAST and placebo MAST. As expected, ANOVA regarding sAA data yielded a significant Group \times Time interaction [$F(3,114) = 6.27$; $p = 0.001$; $\eta_p^2 = 0.14$]. Simple effects showed that groups differed significantly in sAA levels at t_{+00} [$F(1,38) = 4.13$; $p = 0.049$; $\eta_p^2 = 0.10$], with higher sAA levels in the MAST relative to the placebo MAST group. Both groups did not differ in sAA at $t_{\text{pre-stress}}$, t_{+10} , or t_{+30} (all $F_s(1,38) < 1$; all $p_s > 0.35$). Importantly, no significant sAA increases were apparent in the placebo MAST condition (see Fig. 4). ANOVA on the AUCi values for sAA resulted in the expected main effect of Group [$F(1,38) = 11.98$; $p = 0.001$; $\eta_p^2 = 0.24$], with the MAST yielding higher sAA AUCi values than the placebo MAST.

The MAST and its placebo version also showed the anticipated differential cortisol reactivity, as evidenced by a significant Group \times Time interaction [$F(3,114) = 17.42$; $p < 0.001$; $\eta_p^2 = 0.31$]. Simple effects showed differences in cortisol concentrations between groups at t_{+00} [$F(1,38) = 17.10$; $p < 0.001$; $\eta_p^2 = 0.31$], t_{+10} [$F(1,38) = 32.89$; $p < 0.001$; $\eta_p^2 = 0.46$], and t_{+30} [$F(1,38) = 25.44$; $p < 0.001$; $\eta_p^2 = 0.40$] but not at $t_{\text{pre-stress}}$ [$F(1,38) = 2.08$; $p = 0.16$], supporting the differential cortisol reactivity of the MAST and its placebo counterpart. Notably, there were no significant cortisol responses in the placebo MAST condition (also see Fig. 4). Again, AUCi values for cortisol showed higher AUCi values for the MAST group compared to the placebo MAST [$F(1,38) = 21.02$; $p < 0.001$; $\eta_p^2 = 0.36$]. The percentage of cortisol responders for the MAST group was 80% (16/20).

5. Discussion

The current studies investigated the effectiveness of the MAST as a straightforward laboratory stress test capable of eliciting subjective, autonomic, and HPA axis stress responses. The main results can be summarized as follows.

Study 1 indicated that the MAST elicited the strongest salivary cortisol responses compared to the traditional CPT, the SECPT, and a prolonged version of the SECPT (i.e., P-SECPT). All stressors were, however, equally effective in terms of subjective stress ratings and blood pressure responses. In study 2, the MAST was found to yield similar subjective, sAA, and cortisol stress responses as the TSST, underscoring the outstanding ability of the MAST to stimulate the HPA axis. Finally, study 3 once more provided evidence for the effectiveness of the MAST in eliciting sAA and cortisol stress reactions, and presented an essential placebo procedure similar to the MAST regarding physical and cognitive load, but without stimulating the stress-responsive systems (i.e., without producing significant sAA and cortisol reactions; cf. Het et al., 2009).

Notably, study 1 showed that the MAST was the most powerful stress induction procedure for eliciting cortisol responses. Further attesting to the effectiveness of the MAST in arousing the HPA axis, we found that 85% of the total sample (51/60 for studies 1–3 combined) displayed cortisol increases larger than 2.5 nmol/l that are indicative of cortisol secretory episodes (Van Cauter and Refetoff, 1985). At the same time, however, the MAST yielded subjective stress ratings (i.e., stressfulness, painfulness, and unpleasantness) and systolic and diastolic blood pressure responses that were comparable to those of the CPT and its various versions. This points to a selective amplification of the HPA axis stimulation and suggests that not only social evaluation (e.g., Dickerson and Kemeny, 2004), but also the extended duration and combination of a physical challenge with a demanding cognitive test (i.e., mental arithmetic) promotes robust cortisol responses. This latter explanation is supported by the idea that physical stressors instantaneously trigger the activation of the autonomic nervous system and HPA axis through mechanisms involving the hypothalamus and the brainstem, while psychosocial stressors elicit responses via the frontal lobes and limbic structures that connect to the hypothalamus (e.g., Ulrich-Lai and Herman, 2009). Also important to note is

that Dickerson and Kemeny (2004) showed that uncontrollability boosted cortisol reactivity only in the context of a motivated performance task, which suggests that the mental arithmetic task is essential to the ability of the MAST to generate strong cortisol responses.

It is well known that laboratory stressors, including but not limited to the CPT and TSST, generate sex differences in HPA axis stress responses (e.g., Kirschbaum et al., 1992; for review, see Kudielka and Kirschbaum, 2005; Kajantie and Phillips, 2006). For example, men usually display greater HPA reactivity than women, which to a large extent seems dependent on hormonal activity related to the female menstrual cycle (e.g., Kirschbaum et al., 1999) and an associated differential activation of stress response circuitry (e.g., Goldstein et al., 2010). As in the current studies only men were included, no specific predictions can be made regarding the effectiveness of the MAST to elicit HPA axis responses in women and whether – in line with research employing the TSST – cortisol responses to the MAST are comparable to those of men when naturally cycling women are tested in the late luteal phase (e.g., Kirschbaum et al., 1999). In addition, sex differences in subjective stress ratings to the MAST are expected to vary as a function of changes in pain perception across the menstrual cycle phase (e.g., Riley III et al., 1999). It also remains to be determined whether the MAST shows the typical rapid habituation of cortisol reactivity as is found when repeatedly subjecting participants to the TSST (e.g., Kirschbaum et al., 1995; Pruessner et al., 1997). Varying the order of the hand immersion and mental arithmetic trials when repeatedly exposing participants to the MAST could diminish the potential for such habituation effects to occur. Correspondingly, one could vary the number one has to start at in the mental arithmetic challenge so as to avoid practice effects over sessions. An advantage of the TSST relative to the MAST and the recently developed SECPT (Schwabe et al., 2008) is the vast body of research supporting its effectiveness in generating autonomic and HPA axis responses and also in producing other neuroendocrine (e.g., adrenocorticotrophic hormone, prolactin, testosterone, dehydroepiandrosterone) as well as immunological (e.g., interleukin-6, lymphocytes) responses (for reviews, see Kudielka et al., 2007; Foley and Kirschbaum, 2010; Kirschbaum, 2010). Whether these other neuroendocrine, immunological, and neural responses to the MAST are comparable to those typical for the TSST remains open to further empirical testing.

The fact that across 3 studies the MAST succeeded in generating compelling cortisol responses as well as subjective and autonomic stress reactions, suggests that the MAST can be a powerful tool in laboratory stress research in which meaningful cortisol responses are deemed important. Moreover, the MAST may prove to be a worthy alternative to the CPT, SECPT, and also the TSST in research that seeks a combination of a physical and psychosocial component rather than either alone. Also important to note is that the MAST is an economical and practical laboratory stressor. For example, as only one experimenter is needed the personnel costs involved in running the MAST are considerably lower than those of the TSST. Recently, however, von Dawans and colleagues introduced a group version of the TSST in which up to 6 participants can be simultaneously stressed (von Dawans et al., 2011). While this group version presents researchers with new opportunities (e.g., for studies in

social neuroscience on the role of social support in stress reactivity or behavioral economics research involving group decision making under stress), while simultaneously decreasing the cost per participant, it increases the potential for scheduling conflicts to arise between experimenters and participants. Such scheduling conflicts are less likely to occur in research employing a single experimenter, as is the case in the CPT, SECPT, and MAST.

Another potential advantage of the MAST over the TSST relates to its no-stress placebo version. That is, the placebo version of the TSST (Het et al., 2009) requires participants to talk aloud for 5 min about a movie, a novel, or a recent holiday trip, after which they are asked to add up the number 15 starting at 0. Het et al. suggested asking participants for the number that was reached while performing the serial addition task in order to check whether participants complied with the instructions. There is, however, no way to verify the accuracy of participants' responses to this question as the speech and serial addition tasks of the TSST's placebo version are performed in an empty room in the absence of audio or video recordings. Thus, one might be concerned with the extent to which participants take the speech and serial addition task serious in the placebo TSST. This concern does not apply to the no-stress placebo version of the MAST, in which the lukewarm water trials and simple counting test are performed while the experimenter is present in the room to covertly check for compliance. On the other hand, there may also be drawbacks in reliance on the MAST. For one, the MAST may not be as well suited as the TSST when the psychosocial nature of a stress situation is considered crucial (e.g., research in social phobias; see Soravia et al., 2006). As well, the MAST's physical component might be too intense for certain samples (e.g., young children, clinical groups such as fibromyalgia patients). Moreover, one relative advantage of the 3 min SECPT over the 15 min MAST is that it could be more appropriate for studies in which a very brief stressor is needed. Finally, it should be noted that compared with the TSST, one also needs the proper equipment (e.g., water bath) to run a CPT, SECPT or MAST protocol.

A few limitations of the current studies need to be acknowledged. First, Hellhammer and Schubert (2012) recently showed that subjective stress ratings were significantly higher when obtained during stress exposure relative to those obtained after the stressor. As such, it is desirable to collect multiple subjective stress ratings instead of the single measure we obtained at the end of the stress induction procedure in study 1. Second, as cortisol concentrations had not yet returned to baseline forty min after cessation of the MAST, one could argue that the current studies would have benefited from a longer measurement period. Third, while the within-subject crossover design that we employed in study 2 typically has many advantages over a between subject design (e.g., reducing error variance due to inter-individual differences), it is also associated with the risk at carry-over effects. This is especially important given that the mental arithmetic task of the MAST was analogous to that of the TSST. Albeit no order effects were found on any of the parameters in study 2, a further evaluation of the similarities and differences between the TSST and MAST using a between-subject design seems appropriate.

Taken together, the current studies demonstrate the value of the MAST as a concise, straightforward, and economical

stress protocol for future research that is capable of reliably eliciting robust subjective, autonomic, and glucocorticoid stress responses.

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Conflict of interest

No conflicts of interest are declared.

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