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ORIGINAL PAPER

The fear-factor stress test: an ethical, non-invasive laboratory method that produces consistent and sustained cortisol responding in men and women

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Abstract We describe a method to administer a controlled, effective stressor to humans in the laboratory. The method combines the Trier Social Stress Test (TSST) and the Cold Pressor Test into a single, believable procedure called the Fear-Factor Stress Test (FFST). In the procedure, participants imagine auditioning for the reality television show Fear Factor. They stand before a video recorder and a panel of judges while (a) delivering a motivational speech, (b) performing a verbal arithmetic task, and (c) placing one hand into a bucket of ice water for up to 2 min. We measured subjective anxiety, heart rate, and salivary cortisol in three groups of young adults (n=30 each, equal numbers of men and women): FFST, TSST, and Control (a placebo version of the FFST). Although the FFST and TSST groups were not distinguishable at the cortisol measure taken 5 min post-manipulation, at 35 min postmanipulation average cortisol levels in the TSST group had returned to baseline, whereas those in the FFST group continued to rise. The proportion of individual cortisol responders (≥ 2 nmol/l increase over baseline) in the TSST and FFST groups did not differ at the 5-min measure, but at the 35-min measure the FFST group contained significantly more responders. The findings indicate that the FFST induces a more robust and sustained cortisol response (which we assume is a marker of an HPA-axis response) than the TSST, and that it does so without increasing participant discomfort or incurring appreciably greater resource and time costs.

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Exposure to external (environmental) or internal (psychological) stressors activates two primary stress response systems in the body. Both these systems are characterized by the release of stress hormones that facilitate adaptation to the threatening situation (McEwen 1998; de Kloet et al. 2005; Herbert et al. 2006). The first system is orchestrated primarily by the sympathetic nervous system (SNS) and sees the rapid release of catecholamines (e.g., epinephrine and norepinephrine) that produce increases in heart rate, respiration, and blood pressure. The second system is activated more slowly, and is initiated solely by the hypothalamic-pituitary-adrenal (HPA) axis (Roozendaal et al. 2006, 2009). This activation results in the release of glucocorticoids (corticosterone in non-human animals; cortisol in humans) from the adrenal cortex. Glucocorticoids cross the blood–brain barrier easily, and thereafter bind to receptors in various subcortical and cortical brain regions (Lupien et al. 2008; Arnsten 2009). Occupation of receptor sites (most notably the mineralocorticoid type) in these regions has marked effects on both cognitive and emotional processing (Erickson et al. 2003), while hyperactivity of the HPA axis is linked to stress-related illnesses (Kudielka et al. 2009; Foley and Kirschbaum 2010).

Empirical study of the ways in which stress affects human psychobiological, cognitive, and affective processes (and, therefore, the ways in which stress affects human health) demands that researchers have reliable methods of experimentally stimulating the HPA axis. The Trier Social Stress Test (TSST; Kirschbaum et al. 1993) and the Cold Pressor Test (CPT; Hines and Brown 1932) are two methods that neuroscientists use frequently to induce stress in the laboratory. The TSST involves participants undergoing a mock job interview;

it consists of a short preparation period, a 5-min speech and a 5-min verbal arithmetic task. The CPT, in contrast, requires participants to hold their hands and forearms in ice water for up to 3 min. These two methods, therefore, induce stress responses differently: Whereas the TSST manipulates psychological and social-evaluative elements, the CPT manipulates physiological elements.

Neither method, however, consistently activates the HPA axis in all participants. Typically, the CPT elicits strong SNS activation but only moderate HPA-axis responses (Duncko et al. 2007; Schwabe et al. 2008). Typically, the TSST elicits a stronger HPA-axis response than the CPT (McRae et al. 2006), but it does not elicit consistent HPA-axis responses across participants. For example, some researchers (e.g., Buchanan and Tranel 2008; Schoofs and Wolf 2009) have reported low numbers of cortisol responders to the TSST, while others (e.g., Kuhlmann et al. 2005; Nater et al. 2007; Schoofs et al. 2008; Luethi et al. 2009) have chosen to use male-only samples because the TSST typically elicits a larger stress response in men than in women. These sex differences in TSST response appear related to HPA-axis (re)activity (Kudielka et al. 2009). Furthermore, potential explanations for the TSST's ability to induce a greater HPA-axis response than the CPT include the nature (psychosocial vs. physiological), duration (20 min vs. 3 min), and uncontrollability/unpredictability of the procedures (Dickerson and Kemeny 2004; Smeets et al. 2012).

Recently, at least two different laboratories, outside of ours, have investigated the question of whether combining elements of the TSST and CPT might lead to stronger, and more consistent, HPA-axis responses. The rationale for combining elements of the two stress-induction procedures is this: The CPT induces, because of the experience of physical pain, a rapid stress response via activation of the autonomic nervous system and HPA axis. Neural correlates believed to underlie this reflexive physical response are the brainstem and hypothalamus (Ulrich-Lai and Herman 2009). In contrast, the TSST utilises psychosocial stress elements that require cognitive appraisal. This cognitive evaluation of the stressor is associated with activity in the frontal lobes and thalamus, which sees resulting connections from prefrontal and limbic regions to the hypothalamus activating the HPA axis (Dickerson and Kemeny 2004; Ulrich-Lai and Herman 2009). Combining psychosocial and physical stressors can, therefore, be expected to strongly activate both autonomic and HPA-axis responses.

Consistent with this expectation, several new stress induction methods that combine psychological and physiological elements of the TSST and CPT have reported encouraging results. For example, Schwabe et al. (2008) demonstrated that adding a socio-evaluative component to the CPT (i.e., being watched by a confederate and being videotaped while dipping a hand into ice water) increased HPA-axis response over the standard CPT. Similarly, Smeets et al. (2012) demonstrated that the Maastricht Acute Stress Test (MAST), which features the addition of another component of the TSST (a socially evaluated mental arithmetic task) to Schwabe et al.'s Socially Evaluated CPT (SECPT), elicited even greater HPA-axis activation than the standard CPT and than the SECPT. However, cortisol elevations in response to the MAST were similar to those elicited by the TSST.

In the current study, we ask if combining *all* the components of the TSST with those of the CPT will elicit a greater cortisol response than the standard TSST. Hence, we describe an ecologically valid procedure that contains the *entirety* of both the CPT and the TSST, and that maintains, to a large degree, the fiction set forth in the latter. The method, which involves participants undergoing a mock audition for the reality television show *Fear Factor*, combines the psychological aspects of the TSST and the physiological aspects of the CPT into a single, believable, and ethical procedure. We compared changes in subjective anxiety, heart rate, and cortisol levels produced by the *Fear-Factor Stress Test* (FFST), the TSST, and a control procedure with similar mental and physical demands to the FFST but devoid of its stress-inducing features.

Methods

Participants

Ninety healthy university students (45 men, 45 women) between the ages of 18 and 27 years (M=19.76; SD=1.82) met the eligibility criteria for participation. Exclusion criteria included smoking tobacco, using any prescription medication (including oral contraceptives), and scoring≥29 on the Beck Depression Inventory - Second Edition (BDI-II; Beck et al. 1996). Participants were asked to refrain from eating, drinking, or doing physical exercise for at least 2 h before testing.

All participants provided written informed consent. These records of consent are archived by the first author (CdP). The consent form described the study procedures clearly, assured the confidentiality of participation, outlined what would be expected of participants, stated they could end their participation at any time without penalty or prejudice, and confirmed they would receive course credit as compensation. No participant took the option to withdraw, and none reported remaining in a subjectively distressed state at the end of the study. Had any been in such a state, a clinical psychologist was on stand-by, and contact details of other counselling services would have been provided. The research described here followed the ethical guidelines for research subjects outlined by the Health Professions Council of South Africa and the University of Cape Town (UCT) Codes for Research. The Research Ethics Committee of the UCT Department of Psychology approved all study procedures.

Materials and procedure

Figure 1 illustrates the timeline of events in the experimental procedure.

Test sessions started at either 14 h00, 16 h00, or 18 h00. The last session each day ended at 19 h30. Participants rated their current level of anxiety three times using the State form of the State-Trait Anxiety Inventory (STAI; Spielberger et al. 1983): the first, a baseline, shortly after entering the research laboratory ($STAI_B$), the second immediately following their assigned test condition ($STAI_1$), and the third 35 min after the end of the manipulation ($STAI_2$).

To measure heart rate, a research assistant (RA) fitted a VrijeUniversiteit Ambulatory Monitoring System (VU-AMS, Version 5 fs; de Geus and van Doornen 1996) immediately upon the participant's arrival in the laboratory. The VU-AMS is a portable device that allows recording of the electrocardiogram (ECG) and impedance cardiogram (ICG). The device measured heart rate (HR) continuously until the RA removed it immediately before the participant departed. HR decreases in response to parasympathetic nervous system activation, and increases in response to sympathetic nervous system activation.

After the RA attached the equipment, we waited 5 min and then sampled HR for 2 min. The average HR during these 2 min represented a baseline (HR_B) for each participant. We sampled throughout the final 12 min (10 min for the TSST group) of the stress or control manipulation. The average during this period, which encompassed the speaking and mental arithmetic tasks, as well as the water immersion task for the FFST and Control groups, represented heart rate during the manipulation (HR_1). Finally, we sampled heart rate for 2 min starting at 35 min after the manipulation ended, taking the average of those 2 min as the final representation of heart rate (HR_2). Using the VU-DAMS software suite, we extracted indicators of HR (i.e., number of heartbeats per unit of time) from the VU-AMS ECG and ICG signal recordings.

The RA collected saliva samples, using Sarstedt Salivettes (Sarstedt, Nümbrecht, Germany), three times: the first, a baseline, shortly after entering the research laboratory ($CORT_B$), the second 5 min after the stress or control manipulation ended ($CORT_1$), and the third 35 min after the end of the manipulation ($CORT_2$). At each collection, the RA instructed participants to chew gently on the cotton swab for a full minute. Thereafter, the RA immediately placed the swab into a storage tube and placed the tube in a freezer where it remained until transported to a laboratory for salivary cortisol analyses. The assay used the Roche E170 platform; further details are provided by Pillay et al. (2008).

Immediately following data collection, the RA debriefed the participants completely, and the study concluded.

Experimental manipulations

We pseudo-randomly assigned participants to one of three experimental groups: Fear-Factor Stress Test (FFST; n=30); Trier Social Stress Test (TSST; n=30); and Control (n=30). Each group contained equal numbers of men and women because previous studies in this field have reported sex differences in the magnitude of HPA-axis activation following stress induction in the laboratory (e.g., Kirschbaum et al. 1992; Kudielka and Kirschbaum 2005). We did not include

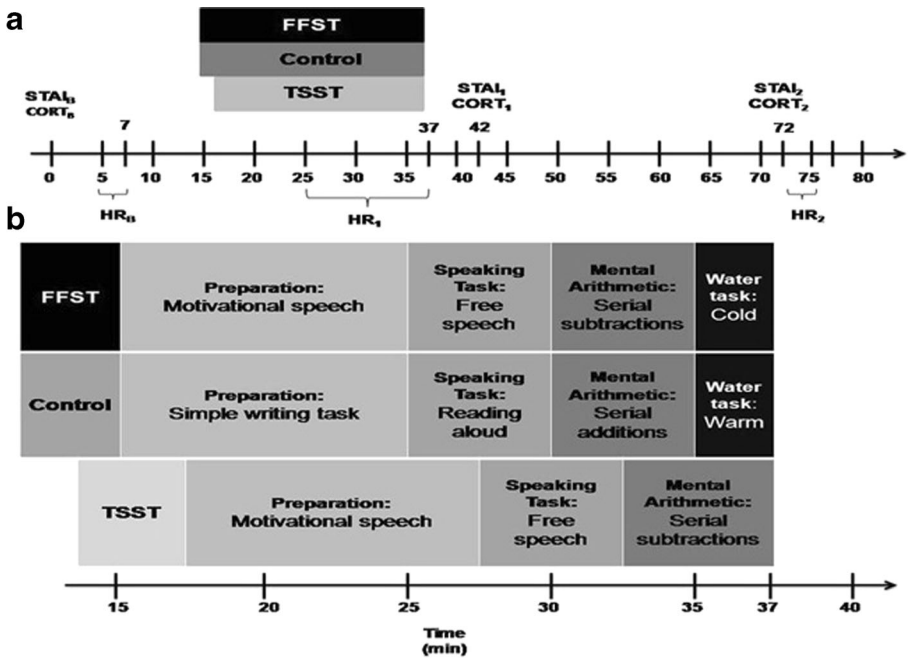

Fig. 1 Panel **a** shows the timeline of events, from 0 min to 80 min, during the experimental procedures. *FFST* Fear-Factor Stress Test group; *TSST* Trier Social Stress Test group; *STAI* Spielberger State-Trait Anxiety Inventory; *HR* heart rate (measured in beats per minute); *CORT* salivary cortisol (measured in nmol/l). *Subscripts* represent measurement point (e.g., $STAI_B$ is the first STAI measurement point, or baseline). Panel **b** focuses in on minutes 15 to 37 of the experimental procedures, showing the sequence of tasks for the FFST, TSST and Control procedures

a CPT group because previous studies demonstrate that stress-induction methods that include social evaluative components (e.g., TSST, SECPT) elicit greater HPA-axis responses than the standard CPT (McRae et al. 2006; Schwabe et al. 2008; Smeets et al. 2012).

In the FFST group, the RA instructed participants to imagine auditioning for the reality television show *Fear Factor*, and then read a set of standardized instructions detailing the process of the audition. Participants were informed that they would complete three tasks: (1) a 5-min free motivational speech as to why they should appear on *Fear Factor*; (2) a 5-min mental arithmetic task to test thinking under pressure; and (3) a test of pain resilience that measured the ability to withstand the physical demands of the show. The RA told participants they would complete the three tasks in front of a panel two of judges, who would decide on their suitability for the show.

Participants received a blank sheet of paper and were given 10 min to prepare the speech. After preparation, the RA took them to a room illuminated by a halogen lamp; this room contained a video camera and a panel of judges. Two undergraduates (one man, one woman) served as judges. They were smartly dressed and seated behind a desk. The participants were given 5 min to present their speech extemporaneously; if they stopped speaking before 5 min elapsed, the judge of the opposite sex to the participant asked a set of standard prompting questions (e.g., "You still have time left, please continue" or "What is your ultimate fear and how do you think you will be able to overcome it in front of the camera?"). Following the speech, participants performed the mental arithmetic task (subtractions of 17 starting from 2043). If the participant answered incorrectly, the same judge asked him/her to restart at 2043. Finally, the judge of the same sex as the participant asked him/her to submerge the dominant arm, up to the elbow, in cold water (between 0° and 4 °C) for as long as possible, up to a maximum of 2 min. Participants remained standing for all three tasks, with the judges watching throughout.

In the TSST group, participants underwent a standard TSST that differed only slightly from the original (Kirschbaum et al. 1993). First, the RA instructed participants to write and present a speech detailing their suitability for a job of their choice. The participants prepared the speech for 10 min and were then taken to an interview room where the two judges were seated behind a desk. The interview room was identical to that described for the FFST group. The remaining protocol, including the extemporaneous speech and the arithmetic task, proceeded precisely as Kirschbaum et al. (1993) described. Participants remained standing for both tasks.

In the Control group, the RA provided participants a blank sheet of paper and instructed them to write a summary of everything they had done on that day. The participants wrote for 10 min and were then taken to a well-lit room, where they were told to stand and read aloud from a general-interest magazine. The RA left the room and permitted the participants to read aloud and alone for 5 min. The RA then re-entered the room and instructed the participant to count upwards in multiples of five, starting from zero. The RA then left the room and permitted the participants to perform this task aloud and alone for 5 min. The RA then re-entered and instructed the participant to submerge his/her dominant arm into warm water (34–38 °C) for as long as possible, up to a maximum of 2 min. The RA remained in the room but did not directly watch the participant, who remained standing.

Statistical analyses

We tested the prediction that the Fear-Factor Stress Test produces greater increases in subjective anxiety, heart rate, and cortisol than the Trier Social Stress Test or a no-stress Control condition. Hence, we expected this pattern of data for all outcome variables: FFST>TSST>Control.

To test this set of predictions, we used two broad analytic strategies. First, we used three separate repeated-measures ANOVAs (one for each class of outcome variable). Between-subject variables were Group (FFST versus TSST 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 twice post-manipulation (5 and 35 min after the end of the manipulation). Second, due to significant between-group differences in baseline levels of cortisol, we used six separate 3 (Group: FFST versus TSST versus Control)×2 (Sex: male versus female) factorial ANOVAs to examine between-group differences on the outcome variables of interest. For the latter analyses, we derived outcome variables by subtracting the baseline measure from those at the second and third measurement points, as follows:

$$STAI_{\Delta 1} = STAI_1 - STAI_B$$

$$STAI_{\Delta 2} = STAI_2 - STAI_B$$

$$HR_{\Delta 1} = HR_1 - HR_B$$

$$HR_{\Delta 2} = HR_2 - HR_B$$

$$CORT_{\Delta 1} = CORT_1 - CORT_B$$

$$CORT_{\Delta 2} = CORT_2 - CORT_B$$

For the factorial ANOVAs, we used Scheffé post-hoc tests to analyze significant main effects further.

To analyze the CORT data further, we split the groups, on a post-hoc basis, into cortisol responders and cortisol non-responders. Following Fehm-Wolfsdorf et al. (1993), we classified participants as *cortisol responders* if their $CORT_1$ or $CORT_2$ values represented a 2 nmol/l or more increase over baseline ($CORT_B$). In the current sample, an increase of 2 nmol/l relative to baseline was equivalent to a 49 % cortisol increase. We analyzed between-group differences in number of cortisol responders using Pearson's χ^2 tests of independence.

We conducted all statistical analyses using SPSS 21. We set the threshold level of statistical significance (α) at .05, and calculated the appropriate effect size estimate for each analysis. In most cases, data distributions met the required assumptions for the relevant inferential statistical analyses; we made necessary adjustments where assumptions were violated (e.g., the use of Greenhouse-Geisser degrees of freedom corrections).

Results

Subjective anxiety

Repeated-measures ANOVA detected significant main effects of Time, $F(1.58, 132.42)=75.83$, $p<.001$, $\eta_p^2 = .47$, and Group, $F(2, 84)=7.40$, $p=.001$, $\eta_p^2 = .15$, in the absence of significant main effect of Sex, $p=.431$, $\eta_p^2 = .01$. Additionally, the analysis detected a significant Group×Time interaction, $F(3.15, 132.42)=23.80$, $p<.001$, $\eta_p^2 = .36$, in the absence of a significant Time×Sex interaction, $p=.896$, $\eta_p^2 < .01$, Group×Sex interaction, $p=1.00$, $\eta_p^2 < .01$, or Time×Group×Sex interaction, $p=.114$, $\eta_p^2 = .05$.

Post-hoc within-group analyses across Time showed that the FFST group displayed a significant increase in self-reported anxiety from $STAI_B$ to $STAI_1$, $p<.001$, but returned to near baseline levels by $STAI_2$, $p=1.00$ (see Table 1). The TSST group showed a similar significant increase from $STAI_B$ to $STAI_1$, $p<.001$, and also returned to near baseline levels by $STAI_2$, $p=1.00$. The Control group, in contrast, displayed a non-significant decrease in self-reported anxiety from $STAI_B$ to $STAI_1$, $p=.25$, and reported significantly decreased anxiety levels by from $STAI_B$ to $STAI_2$, $p=.02$.

Factorial ANOVA of mean $STAI_{\Delta 1}$ scores detected a significant main effect of Group, $F(2, 83)=26.03$, $p<.001$, $\eta_p^2 = .39$. That analysis detected no main effect of Sex, $p=.84$, $\eta_p^2 < .001$, and no significant Group×Sex interaction, $p=.19$, $\eta_p^2 = .04$. Post-hoc pairwise comparisons detected a significant difference for the mean of the FFST group versus that of Control group, $p<.001$, but not for the mean of the FFST and Control groups taken together versus that of the TSST group, $p=.10$. This set of decisions implies the following order of true means: *FFST*>*TSST*>*Control*, a pattern that matches the sample data displayed in Table 1.

Table 1 Subjective anxiety, heart rate, and cortisol data ($N=90$)

Variable	Group		
	FFST ($n=30$)	TSST ($n=30$)	Control ($n=30$)
Subjective anxiety			
$STAI_B$	29.13 (7.42)	34.07 (6.81)	31.70 (6.50)
$STAI_1$	45.30 (12.89)	44.10 (11.78)	29.33 (6.41)
$STAI_2$	28.53 (6.31)	31.57 (6.92)	28.57 (6.35)
Heart rate			
HR_B	74.78 (11.72)	74.76 (12.82)[b]	73.27 (18.22)[a]
HR_1	94.13 (15.06)	103.93 (19.61)[b]	85.48 (21.69)[a]
HR_2	71.78 (11.54)	71.74 (9.92)[b]	70.03 (17.33)[a]
Salivary cortisol			
$CORT_B$	3.71 (1.43)[a]	1.96 (1.94)	4.55 (5.42)
$CORT_1$	6.01 (2.83)[a]	5.61 (4.18)	3.41 (2.87)
$CORT_2$	7.90 (7.20)[a]	2.23 (2.19)	2.46 (2.00)

Means are presented, with standard deviations in parentheses

FFST Fear-Factor Stress Test group; *TSST* Trier Social Stress Test group; *STAI* Sp/ielberger State-Trait Anxiety Inventory; *HR* heart rate (measured in beats per minute); *CORT* salivary cortisol (measured in nmol/l)

Subscripts represent measurement point (e.g., $STAI_B$ is the first STAI measurement point, or baseline)

[a] $n=29$

[b] $n=21$

Factorial ANOVA of mean $STAI_{\Delta 2}$ scores detected no significant main effects of Group or of Sex, and no significant Group×Sex interaction effect, $p=.29$, $\eta_p^2 = .03$, $p=.76$, $\eta_p^2 < .01$, and $p=.78$, $\eta_p^2 = .01$, respectively. These analyses confirm the pattern of data shown in Table 1, where it appears that, at $STAI_2$, self-reported anxiety levels returned to, and dipped below, baseline in all groups.

Heart rate

We visually inspected the recorded interbeat interval time series for implausible (> 3 *SD* from the mean) readings. These implausible readings can result from hardware failures, or from electrodes not making full contact with a participant's skin. If more than 10 % of the data over a critical period consisted of artefacts, we excluded the variable for that participant. We excluded 10 participants (9 in the TSST group and 1 in the Control group) following this reasoning.

Repeated-measures ANOVA detected significant main effects of Time, $F(1.57, 115.85)=351.11$, $p<.001$, $\eta_p^2 = .83$, and Sex, $F(1, 74)=16.08$, $p<.001$, $\eta_p^2 = .18$ (Men: $M=75.75$, $SD=12.48$; Women: $M=85.44$, $SD=12.52$),in the absence of a significant main effect of Group, $p=.28$, $\eta_p^2 = .03$.

Additionally, the analysis detected significant interactions between Group and Time, $F(3.13, 115.85)=15.56$, $p<.001$, $\eta_p^2=.30$, and between Time and Sex, $F(1.57, 115.85)=8.23$, $p=.001$, $\eta_p^2=.10$, in the absence of a significant Time×Group×Sex interaction, $p=.08$, $\eta_p^2=.06$.

Post-hoc within-group analyses across Time showed that, on average, participants in all three groups showed a significant increase in heart rate levels from HR_B to HR_1, all p's<.001, and returned to below baseline levels by HR_2, all p's≤.001 (see Table 1).

Factorial ANOVA of mean $HR_{\Delta 1}$ values detected a significant main effect of Group, $F(2, 74)=17.41$, $p<.001$, $\eta_p^2=.32$. That analysis detected no significant main effect of Sex, $p=.14$, $\eta_p^2=.03$, and no significant Group×Sex interaction, $p=.85$, $\eta_p^2<.01$. Post-hoc pairwise comparisons detected a significant difference for the mean of the FFST group versus that of the Control group, $p=.02$, but not for the mean of the FFST and Control groups taken together versus that of the TSST group, $p=.23$. This set of decisions implies the following order of true $HR_{\Delta 1}$ means: *FFST>TSST>Control*, a pattern that matches the sample data displayed in Table 1.

Factorial ANOVA of mean $HR_{\Delta 2}$ values detected no significant main effects of Group or of Sex, and no significant Group×Sex interaction effect, $p=.73$, $\eta_p^2<.01$, $p=.54$, $\eta_p^2<.01$, and $p=.11$, $\eta_p^2=.05$, respectively. These analyses confirm the pattern of data shown in Table 1, where it appears that, at HR_2, heart rate returned to, and dipped below, baseline in all groups.

Cortisol responses

Due to experimenter error, cortisol data for one man in the FFST group were lost. Repeated-measures ANOVA detected significant main effects of Time, $F(1.75, 145.34)=6.39$, $p=.003$, $\eta_p^2=.07$, and of Group, $F(2, 83)=7.35$, $p=.001$, $\eta_p^2=.15$, in the absence of a significant main effect of Sex, $p=.36$, $\eta_p^2=.01$. Additionally, the analysis detected a significant Group×Time interaction, $F(3.50, 145.34)=12.66$, $p<.001$, $\eta_p^2=.23$, in the absence of a significant Time×Sex interaction, $p=0.18$, $\eta_p^2=.02$, Group×Sex interaction, $p=0.63$, $\eta_p^2=.01$, or Time×Group×Sex interaction, $p=0.89$, $\eta_p^2<.01$.

Post-hoc within-group analyses across Time showed that the FFST group displayed a significant increase in cortisol levels from $CORT_B$ to $CORT_1$, $p<.001$ (see Table 1). FFST participants' cortisol levels continued to show a sustained increase at $CORT_2$, when they were significantly higher than at baseline, $p=.010$. The TSST group also showed a significant increase in cortisol levels from $CORT_B$ to $CORT_1$, $p<.001$. By $CORT_2$, however, average levels in the TSST group had returned to near baseline levels, and were not significantly different from $CORT_B$, $p=1.00$. The Control group, in contrast, displayed a non-significant decrease from $CORT_B$ to $CORT_1$, $p=.26$. By $CORT_2$, average levels in the Control group were significantly lower than at $CORT_B$, $p=.01$.

Between-group analysis at $CORT_B$ showed a significant main effect of Group, $F(2, 86)=4.42$, $p=0.02$, $\eta_p^2=0.09$. Bonferroni post-hoc comparisons confirmed a significant difference between cortisol levels for the TSST and Control groups, $p=0.01$, but not between the FFST and TSST groups, $p=0.16$. It is largely due to these pre-existing between-group differences at $CORT_B$ that further between-group comparisons at $CORT_1$ and $CORT_2$ were performed using change scores.

Figure 2 illustrates these change scores, and shows there were noteworthy differences in cortisol responding among the groups across time. A visual impression of the figure suggests that 5 min after the end of the manipulation (i.e., at $CORT_1$), both stress manipulations resulted in cortisol levels greater than those in the Control group. Thirty minutes later, however, cortisol *declined* in the TSST group but *continued to increase* in the FFST group.

Statistical analyses confirmed this impression. Factorial ANOVA of mean $CORT_{\Delta 1}$ values detected a significant main effect of Group, $F(2, 83)=9.80$, $p<.001$, $\eta_p^2=.19$. That analysis detected no significant main effect of Sex, $p=.17$, $\eta_p^2=.02$, and no significant Group×Sex interaction, $p=.08$, $\eta_p^2=.06$. Post-hoc pairwise comparisons detected no significant difference for the mean $CORT_{\Delta 1}$ value obtained from the FFST group versus that obtained from the TSST group, $p=.13$, but a significant difference for the mean of the $CORT_{\Delta 1}$ values obtained from the FFST and TSST groups taken together versus that of the Control group, $p<.001$. This set of decisions implies the following order of true $CORT_{\Delta 1}$ means: *FFST=TSST>Control*, a pattern that matches the sample data displayed in Table 1.

Factorial ANOVA of mean $CORT_{\Delta 2}$ values also detected a significant main effect of Group, $F(2, 83)=11.30$, $p<.001$, $\eta_p^2=.21$. That analysis detected no significant main effect of Sex, $p=.58$, $\eta_p^2<.01$, and no significant Group×Sex interaction, $p=.37$, $\eta_p^2=.02$. Post-hoc pairwise comparisons detected a significant difference for the mean $CORT_{\Delta 2}$ value obtained from the FFST versus that obtained from the TSST group, $p<.001$, and a significant difference for the mean of the $CORT_{\Delta 2}$ values obtained from the FFST and TSST groups taken together versus that of the Control group, $p<.001$. This set of decisions implies the following order of true means: *FFST>TSST>Control*, a pattern that matches the sample data displayed in Table 1.

Cortisol responders

At $CORT_1$, 14 of the 29 (48 %) participants in the FFST group (8 men, 6 women) were cortisol responders. Similarly, 17 of the 30 (57 %) participants in the TSST group (11 men and 6 women) were responders. Only 1 of the 30 (3 %) participants

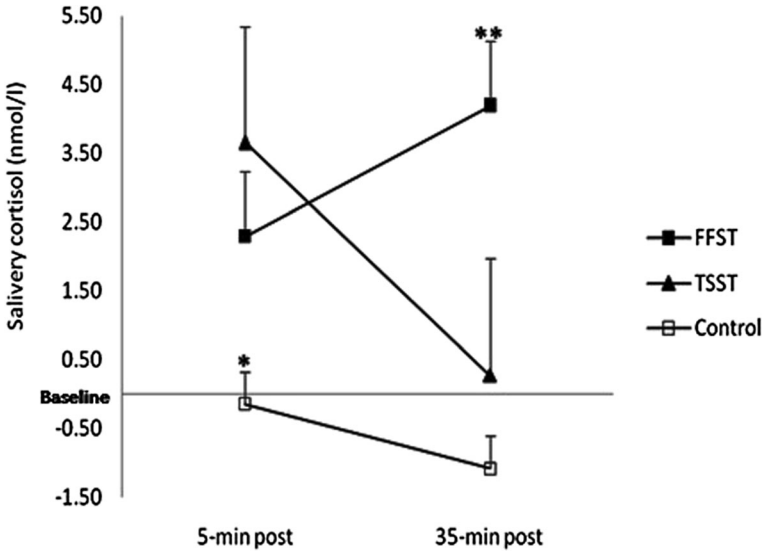

Fig. 2 Salivary cortisol in nanomoles per liter (mean + standard error) at two measurement points in the study.*Significant difference between the Control group and the other two groups, p=.004. **Significant difference between FFST group and other two groups, p<.001

in the Control group (1 man) was a responder. A chi-squared test of independence confirmed that the overall proportion of responders between groups differed significantly, $\chi^2(2)$= 18.38, p<.001, Cramer's V=.45. The proportion of responders in the FFST and TSST groups did not differ significantly, $\chi^2(1)$=0.42, p=.52, Cramer's V=.08.

In contrast, at $CORT_2$, 15 of the 29 (52 %) participants in the FFST group (9 men, 6 women) were cortisol responders. Only 4 of the 30 (13 %) participants in the TSST group (4 men) were responders. A chi-squared test of independence confirmed that the overall proportion of responders between groups differed significantly, $\chi^2(2)$=21.99, p<.001, Cramer's V=.50. The proportion of responders in the FFST group differed significantly from that in TSST group, $\chi^2(1)$=9.95, p=.002, Cramer's V=.41. Importantly, there was no significant difference between the proportion of men and women responders within the FFST group, $\chi^2(1)$=1.71, p=.19, Cramer's V=.24.

We observed individual differences in maintenance of cortisol response over time. In the FFST group, 10 of the 14 participants who responded initially maintained or increased that response from $CORT_1$ to $CORT_2$, whereas 4 returned to baseline. In contrast, 4 of the 17 participants in the TSST group sustained responding from $CORT_1$ to $CORT_2$, whereas the remaining 13 returned to baseline. The solitary cortisol responder in the Control group also did not sustain responding from $CORT_1$ to $CORT_2$; he returned to baseline. Naturally, this indicates that 6 participants in the FFST group were new responders at $CORT_2$ (i.e., they had been classed as non-responders at $CORT_1$), whereas there were no new responders in the TSST or Control groups. A chi-squared test of independence detected a significant between-group difference in terms of sustained responders, $\chi^2(2)=10.61$, p=.005, Cramer's V=.24.

Discussion

We have described the Fear-Factor Stress Test (FFST), a method of stress induction that combines a commonly used physiological stressor (the Cold Pressor Test) and a commonly used psychosocial stressor (the Trier Social Stress Test). The FFST draws on both the uncontrollable and social evaluative elements present in the TSST and features tasks (public speaking and mental arithmetic) known to increase cortisol levels reliably (Biondi and Picardi 1999; Dickerson and Kemeny 2004). We sought to determine if the FFST produces a more robust stress response than the TSST. We also compared the FFST and the TSST to a control version of the FFST procedure, similar in physical and mental demands but without negative stress-inducing components.

Participants in the FFST group did not show increased sympathetic activation (as measured by heart rate) relative to those in the TSST group, and did not rate the combined stressor experience as more anxiety-inducing than did those in the TSST group. In contrast, participants in the FFST group showed increased cortisol responding, which we assume is a marker of increased HPA-axis activity, relative to those in the TSST group. Specifically, although the FFST and TSST groups were not distinguishable in terms of magnitude of cortisol response from baseline to 5 min post-manipulation, they were statistically distinct in terms of change from baseline to 35 min post-manipulation. On average, participants in the FFST group sustained a relatively high level of cortisol responding, whereas those in the TSST group returned to baseline. Similarly, the proportion of cortisol responders (defined as those with 2 nmol/l increase over baseline) in the TSST and FFST groups did not differ significantly at the 5-min measure, but did differ significantly at the 35-min measure. Taken together, the absence of a sustained heart rate

response, the decline of subjective anxiety, and the sustained cortisol response, permits the inference that the FFST increases HPA-axis activation without additional psychological discomfort.

The present data indicate that psychosocial stressors and physiological stressors are distinct, and not merely alternative, methods of activating the HPA axis, and that there is increased HPA-axis response when activated in conjunction. This conclusion is consistent with those reached by Schwabe et al. (2008) and Smeets et al. (2012). The FFST, however, appears to deliver a more sustained cortisol response than that achieved by the Maastricht Acute Stress Test or the TSST (Smeets et al. 2012, Study 2). Direct comparisons of the three procedures remain to be described, however.

Another question that remains open is whether the interaction between TSST and CPT components produces unique effects (on cortisol and/or on cognition). Others who have combined stress-induction procedures in this way (Schwabe et al. 2008; Smeets et al. 2012) have made no mention of such unique interactive effects.

Consistent with our finding and with those of Schwabe et al. (2008) and Smeets et al. (2012), cognitive appraisal of a stressful event appears to have a large effect on the physiological response of an individual to that event (Dickerson et al. 2008). The current data indicate that the inclusion of psychosocial component to a physiological stressor results in a more robust stressor. Thus, it appears that if an individual perceives a stressor as a physical, intellectual, and social threat, then the physiological response to that stressor is greater than when the stressor incorporates merely a physical component.

Typically, research in this area reports means, or describes patterns of mean group differences, treating individual differences in cortisol response as unsystematic variance or error. We suspect, however, that the reported means hide important individual differences in cortisol responding. We found that most individuals in the FFST group who were responders at the 5-min measure continued to respond at the 35-min measure. In contrast, most of those in the TSST group who responded at the 5-min measure returned to baseline at the 35-min measure. In light of the statistical results for those in the FFST group, we consider it unlikely these individual differences represent unsystematic variance. Possible sources of systematic variance include traits (temperament, personality), recent or remote life experiences, phase of the menstrual cycle, or other pre-existing differences distributed unevenly across groups (Kudielka et al. 2009). Programmatic investigation of these possibilities may be a rewarding area of inquiry, as might be studies that determine why about 50 % of individuals exposed to laboratory-based stress tests do not show a significant cortisol response (Kudielka and Kirschbaum 2005; Kudielka and Wüst 2010).

Studies using the TSST frequently report sex differences in cortisol responses. Moreover, women are less likely than men to show an HPA-axis response to the TSST (Kirschbaum et al. 1992, 1999), leading many investigators to include only men in their studies (e.g., Kuhlmann et al. 2005; Schoofs et al. 2008; Smeets et al. 2012). Our analyses detected no significant sex differences in response to either the FFST or the TSST, however. Of note, though, is that we observed a statistically significant increase in the number of female sustained responders in the FFST group over the TSST group, despite the fact that we did not control for phase of the menstrual cycle (Kirschbaum et al. 1999). These data suggest that, by including a combination of stressors, the FFST holds promise as a stress-induction method that might attenuate the sex differences often seen in the TSST.

Interestingly, the women in the current study, irrespective of the experimental group, showed higher heart rate levels than the men. This finding is consistent with previous reports that women have higher resting heart rates than men (Pham 2003). The higher resting rates in women did not, however, seem to influence autonomic activation in response to the experimental manipulation.

A secondary aim of this study was to create and describe an effective control (placebo) version of the FFST. Any adequate control is identical to the intended treatment and differs only in psychological and/or physical effective characteristics (Mill 1843; Shapiro and Morris 1978). In the present case, the uncontrollable, social evaluative, and pain-inducing components were effective characteristics of the FFST. Participants in the Control group did not, on the average, show an increase in cortisol levels, and they reported significantly lower state anxiety levels than those in the FFST and TSST groups after the manipulation. Those in the Control group did, however, show an increase in heart rate during the manipulation, although the increase was significantly less than that observed in the FFST group. The cardiovascular nature of the control task may produce this increase, and the observed increase is consistent with results from the control version of the TSST (Het et al. 2009). Hence, the absence of uncontrollability, social evaluative components, and pain stimuli in the control task produced moderate sympathetic activation without concomitant HPA-axis activation.

We acknowledge six factors that may limit the generalizability of the present findings. First, we did not control for participants' body mass index (BMI). Although it appears traditional to control for BMI in this literature (e.g., Schwabe et al. 2008; Smeets et al. 2012), Wirtz and colleagues (2008) established that BMI and salivary cortisol (either in reaction to stress or in circadian cortisol secretion) are not related. Hence, it is not completely clear that such a control is necessary.

Second, we did not control for phase of the menstrual cycle in the women who participated in this study. Previous studies show that responses to a stressor change as a function of phase of the menstrual cycle (Kirschbaum et al. 1999). Hence, some

of the variability we describe in these data may be due to this factor, which is, obviously, interesting in its own right.

Third, we collected our subjective measure of stress at the end of the stress manipulation. Subjective measures of stress are greater when measured *during* the manipulation than when measured *following* it (Hellhammer and Schubert 2012). Hence, we may have missed differences in participant levels of stress *during* stress-induction.

Fourth, the FFST manipulation tested in the current study was slightly longer (by about 2 mins) than the TSST. Although there is no suggestion in the literature of an optimal length for a stress-induction procedure, there is a possibility that the length of the manipulation is positively correlated with HPA-axis response. Future studies might serve to tease apart the effects of length of the stressor exposure versus characteristics of the stressor.

Fifth, there were large differences in baseline cortisol levels between the three groups in the current study. Although we are unable to account precisely for the source of these differences, we suggest that they might be associated with (a) variations in phase of menstrual cycle amongst female participants, and (b) variations in time of day at which participants were exposed to the experimental manipulations. Although we are unaware of literature suggesting that such differences at baseline are associated with the magnitude of cortisol response, there nevertheless exists a possibility of such an association. However, given that *magnitude* of response was the critical outcome variable, we are confident that the reported results are sound and valuable. Furthermore, if one argues that higher baseline cortisol values leave less room for a large cortisol response to the stressor (i.e., that individuals with lower baseline cortisol values are farther away from the physiological limits of circulating cortisol, and so can show larger magnitude of cortisol responses relative to individuals with lower baseline values), then our argument for the value of the FFST over the TSST is even stronger: In this study, the TSST group's baseline cortisol values were significantly lower than those of the FFST group.

Finally, cortisol levels of FFST participants did not return to baseline by the end of the study. Hence, the duration of this response remains to be determined.

The current study demonstrates that the FFST, in tandem with its control comparison, is a promising research tool. Similar cognitive and physical demands characterize the experimental and control conditions; in so doing, they increase internal validity by eliminating confounding variables (Krauth 2000). Differences between the procedural demands and response burden of the FFST and the TSST are trivial (e.g., it takes about 2 min longer to administer than the TSST). The use of the FFST offers an advantage to the working scientist and to those who participate in our studies in that it elicits a more robust and sustained HPA-axis response than the TSST or the CPT without (a) increasing participant discomfort or (b) requiring increased resources and costs. Such a research tool could prove valuable in helping disentangle the actions of stress and HPA axis-related hormones on human cognitive, affective, and behavioural functioning.

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Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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