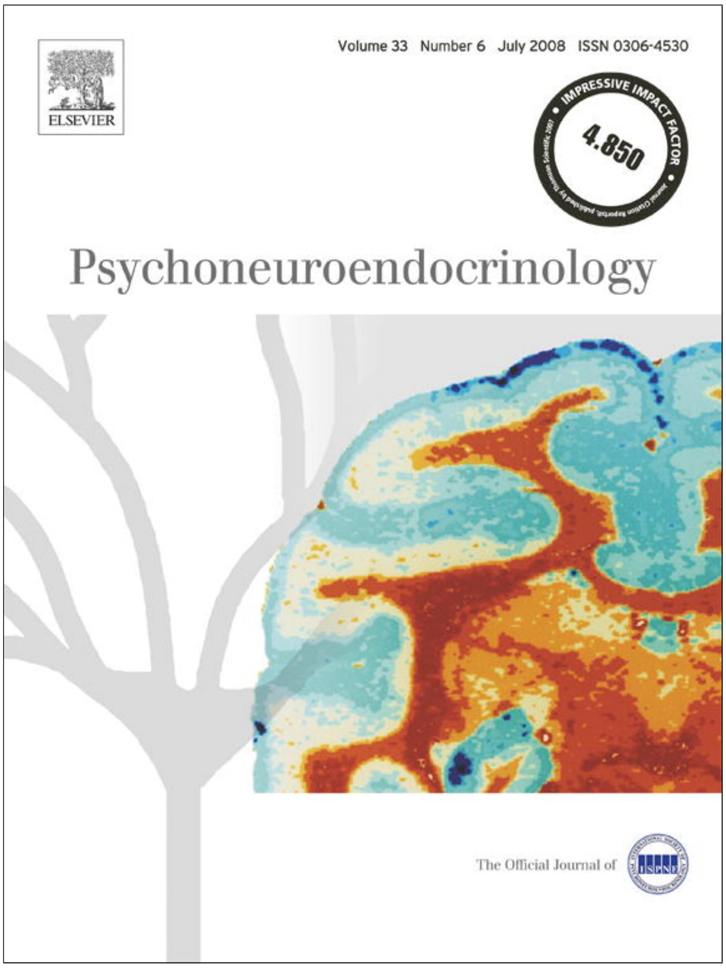
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SHORT COMMUNICATION

HPA axis activation by a socially evaluated cold-pressor test

Lars Schwabe*, Leila Haddad, Hartmut Schachinger

Department of Clinical Physiology, University of Trier, Johanniterufer 15, 54290 Trier, Germany

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Summary
The cold-pressor test (CPT) in which subjects immerse their hand in ice water is among the most commonly used laboratory stressors. While the CPT elicits strong sympathetic nervous system activation, cortisol elevations indicative for the reactivity of the hypothalamus–pituitary–adrenal (HPA) axis are moderate to low in response to the CPT. In the present study, we assessed whether cortisol responses to the CPT can be increased by adding social-evaluative elements. Therefore, 70 healthy young men immersed their hand in ice or warm water and were watched by a woman and videotaped during hand immersion or not. While the standard CPT and the socially evaluated cold-pressor test (SECPT) led to comparable increases in blood pressure and subjective stress ratings, saliva cortisol elevations and the proportion of subjects showing a saliva cortisol response (defined as increase >2 nmol/l) were significantly higher after the SECPT. Social evaluation during hand immersion in warm water did not affect saliva cortisol levels suggesting that both social evaluation and a challenge are required for HPA axis activation. These findings indicate that the incorporation of social-evaluative elements increases HPA axis responses to the CPT. The SECPT can serve as a tool for future stress research.

1. Introduction

Stress is an experience common to all of us. The perception or expectation of environmental or physical changes activates the sympathetic nervous system and the hypothalamus–pituitary–adrenal (HPA) axis, the two major stress systems of the body. For decades, research has employed challenge tests to study stress and its effects on health, cognition and emotion in a laboratory setting. One of the most frequently used stress protocols in humans is the cold-pressor test (CPT) in which participants immerse their hand for a few minutes into ice water (first described by Hines and Brown, 1932). The CPT elicits profound activation of the sympathetic nervous system expressed for example as increased skin conductance (Buchanan et al., 2006) and elevated blood pressure (al' Absi et al., 2002). However, the

*Corresponding author. Tel.: +49 234 3229324; fax: +49 234 3214308.
E-mail address: Lars.Schwabe@ruhr-uni-bochum.de (L. Schwabe).

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CPT is less capable to provoke the HPA axis. Several authors found only moderate increases in cortisol, the most important glucocorticoid in humans that indicates HPA axis activation, in response to the CPT (al' Absi et al., 2002; Gluck et al., 2004), others obtained no cortisol elevation at all (McRae et al., 2006; Duncko et al., 2007). Stress effects are multi-faceted, with numerous neuromodulator and hormonal effects on different neural and peripheral systems. However, cortisol has been identified as a key stress component mediating stress effects on cognitive functioning and emotional processing (Erickson et al., 2003). Thus, the lack of cortisol responses to the CPT reduces the value of the CPT as a tool in stress research.

According to a recent meta-analysis, profound activation of the HPA axis reflected in large cortisol elevations is associated with tasks that contain social-evaluative elements (Dickerson and Kemeny, 2004), such as the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) in which participants have to deliver a free speech and perform a mental arithmetic task in front of a camera and an audience. The present study aimed to examine whether cortisol responsiveness to the CPT could be increased by adding social-evaluative elements to the standard CPT procedure. Therefore, male subjects were watched by a woman (i.e. a member of the opposite sex) and videotaped during hand immersion into ice water. To exclude the possibility that the monitoring itself caused cortisol elevations independent of cold-pressor stress, a group of subjects was watched by a woman and videotaped while submerging their hand into warm water.

2. Materials and methods

2.1. Participants

Seventy male students (age: $M = 23.7$ yr; SD $= 2.9$ yr; range: 19–35 yr) were recruited by email announcements at the University of Trier. Only men were included to avoid gender and menstrual cycle effects on cortisol responses (Kirschbaum et al., 1999). Participation was limited to healthy non-smokers with normal BMI ($M = 22.8\,\mathrm{kg/m^2}$; SD $= 2.0\,\mathrm{kg/m^2}$; range: 19–27 $\mathrm{kg/m^2}$). Participants had to refrain from excessive exercise, alcohol, caffeine and meals within 3 h prior to the examination. All participants provided written informed consent. The study was approved by the local ethics committee.

2.2. Procedure

Experimental sessions were run between 1400 and 1700 h to control for diurnal cycle of cortisol. After arrival at the laboratory, blood pressure and ECG were recorded for 5 min (PRE measurement) and a baseline saliva sample was collected. At this point, participants were randomly assigned to one of four experimental conditions:

Warm water test ($n = 15$): Participants were asked to place their right hand up to and including the wrist for 3 min into warm water (35–37 °C). After 3 min they were instructed to remove their hand from the water. There was no camera; the (female) experimenter stayed in the room but did not watch the participants.

Socially evaluated warm water test ($n = 15$): Subjects were told that they would be video taped during the next part of the experiment and that the video recordings would be analyzed for facial expression. Participants were asked to provide written consent that these video recordings can be used for scientific purposes. Next, the camera was turned on and subjects were requested to look into the camera and place their right hand up to and including the wrist into warm water (35–37 °C). During the water immersion the (female) experimenter watched the subjects all the time. After 3 min, the experimenter told the subjects to take their hand out of the water.

Cold-pressor test (CPT; $n = 20$): This condition corresponded to the standard cold-pressor procedure. Participants were instructed to immerse their right hand up to and including the wrist into ice water (0–4 °C). Since this can be very uncomfortable, subjects were told to keep their hand as long as possible in the water, at maximum 3 min, and that they could remove their hand at their discretion. Those who kept their hand in the water for 3 min were instructed at that point to remove their hand. There was no camera present in this condition; the (female) experimenter was in the room but did not watch the participants. Participants in this condition kept their hand on average 166 s (SEM: 7.7 s) in the water.

Socially evaluated cold-pressor test (SECPT; $n = 20$): Subjects were informed that they will be videotaped and that these video recordings would be analyzed for facial expression. After participants provided written consent that the video recordings could be used for scientific purposes, they were asked to immerse their right hand up to and including the wrist into ice water (0–4 °C). Subjects were instructed to look into the camera and keep their hand as long as possible in the water. The (female) experimenter watched the participants all the time. Subjects kept their hand on average 170 s (SEM: 7.3 s) in the water. Those participants who kept their hand in the water for 3 min were instructed at that point to remove their hand.

Overall, the four groups were comparable in the time they kept their hands in the water ($F_{3,69} = 1.56$, $p > 0.20$). Blood pressure and ECG were measured during hand immersion in all conditions. Immediately after subjects took their hand out of the water, they rated on an 11-point scale ranging (in 10-point increments) from 0 (''not at all'') to 100 (''very much'') how unpleasant, stressful and painful the previous situation had been. Next, another saliva sample was collected and blood pressure was recorded for 5 min again (POST measurement). Thereafter, participants were guided to another room and asked to collect their saliva 10, 20, 30, 45 and 60 min after cessation of the stress manipulation. Subjects stayed alone in this room, but the experimenter checked repeatedly whether saliva samples were collected at the right time. Between collecting the saliva samples, subjects were allowed to read. At the end, participants were debriefed and paid a moderate monetary compensation for participation.

2.3. Cardiovascular data

Heart rate was derived from a single standard lead II ECG configuration employing telemetric HP 78100A transmitter and HP 78101A receiver system (Hewlett-Packard Corporation). ECG was sampled by 1 kHz with 12 bit resolution. Beat detection was performed offline by WinCPRS (Absolute Aliens Oy, Turku, Finland) as was artifact control.

Continuous blood pressure was recorded using the Finapres system (Ohmeda, Englewood, CO, USA); a cuff was placed on the middle finger of the left hand, which was kept at heart level. Beat-to-beat systolic and diastolic blood pressure were determined offline with the help of WinCPRS software.

2.4. Saliva sampling and cortisol analysis

Saliva was collected by the subjects themselves using standard Eppendorf tubes (1.5 ml, Eppendorf, Hamburg; Germany), stored at room temperature until completion of the session, and then kept at $-20\,^{\circ}\mathrm{C}$ until analysis. After thawing for biochemical analysis, the fraction of free cortisol in saliva (salivary cortisol) was determined using a time-resolved immunoassay with fluorometric detection, as described in detail elsewhere (Dressendorfer and Kirschbaum, 1992). Inter- and intra-assay coefficients of variance were below 9%.

2.5. Statistical analysis

Data were analyzed by χ^2-test and one-way or mixed-design ANOVA as appropriate. Significant main effects were further analyzed by Bonferroni adjusted post-hoc tests. Analyses include the partial η^2 as measure of effect size were appropriate. Following the conventions by Cohen (1988) partial $\eta^2 = 0.01$ is considered a small effect, partial $\eta^2 = 0.06$ a medium-sized and partial $\eta^2 = 0.14$ a large effect. Cortisol concentrations were first analyzed by a mixed-design ANOVA. Thereafter, we computed the area under the curve with respect to increase (AUCinc; Pruessner et al., 2003) and subjected the AUCinc values to a one-way ANOVA. This was done to avoid multiple testing when assessing treatment effects on the time course of cortisol (i.e. when analyzing the treatment $\times$ time interaction effect).

We split participants, on a post-hoc basis, into cortisol responders and cortisol non-responders to assess whether the inclusion of social evaluative elements increases the proportion of subjects that show cortisol elevations in response to the cold pressor. Subjects were classified as cortisol responder if they showed 20 or 30 min after cessation of the stress manipulation (when the cortisol peak can be expected) an increase in cortisol of at least 2 nmol/l relative to the individual baseline, otherwise they were categorized as cortisol non-responder (Fehm-Wolfsdorf et al., 1993). For the present sample, an increase of 2 nmol/l relative to baseline is equivalent to an increase of 40%.

3. Results

3.1. Cortisol response

Adding social-evaluative elements to the CPT increased cortisol responses significantly. As shown in Figure 1 the SECPT (vs. warm water test and socially evaluated warm water test: both p's<0.01) but not the standard CPT (vs. warm water test and socially evaluated warm water test: both p's>0.30) elicited significantly higher cortisol elevations than the two warm water conditions (treatment: $F_{3,66} = 6.96$, $p<0.001$, $\eta^2 = 0.16$; treatment $\times$ time: $F_{18,396} = 4.77$, $p<0.001$, $\eta^2 = 0.18$). A one-way ANOVA on the AUCinc revealed that the cortisol increase in response to the SECPT was significantly higher than to the other three conditions ($F_{3,66} = 7.45$, $p<.001$, $\eta^2 = 0.27$; Bonferroni adjusted post-hoc tests: p's<0.01), whereas the latter did not differ (p's>0.60). Importantly, the SECPT increased not only the averaged cortisol response, but also the number of cortisol responders, defined as participants that show a cortisol increase of at least 2 nmol/l in response to the treatment (χ^2 3 = 13.71, $p = 0.003$; percent cortisol responders per group: SECPT (70%) > CPT (40%) > socially evaluated warm water task (27%) > warm water task (7%); SECPT vs. all other groups: p's<0.05; CPT vs. warm water task: $p<0.05$; other comparisons: p's>0.40). Within the cortisol responders, the SECPT group showed significantly higher cortisol elevations than each of the other three groups (AUCinc: $F_{3,20} = 3.79$, $p = 0.03$; Bonferroni adjusted post-hoc tests: p's<0.01).

3.2. Cardiovascular stress responses

Significant effects of SECPT and CPT were found for blood pressure, but not for heart rate. A mixed-design ANOVA indicated significant time $\times$ treatment interactions for systolic ($F_{6,120} = 7.43$, $p<0.001$, $\eta^2 = 0.27$; treatment: $F_{3,66} = 1.35$, $p = 0.27$, $\eta^2 = 0.04$) and diastolic ($F_{6,120} = 11.45$, $p<0.001$, $\eta^2 = 0.37$; treatment: $F_{3,66} = 1.92$, $p = 0.14$, $\eta^2 = 0.09$) blood pressure. Analyses for each time point revealed significant differences only during hand immersion (systolic blood pressure: $F_{3,66} = 9.25$, $p<0.001$, $\eta^2 = 0.29$; diastolic blood pressure: $F_{3,66} = 14.52$, $p<0.001$, $\eta^2 = 0.43$) with highest blood pressure in the SECPT and CPT groups (see Table 1). For heart rate, we obtained neither a treatment effect nor a time $\times$ treatment interaction (both Fs<1.5, both p's>0.40). However, heart rate was reduced after the hand was removed from the water both in the SECPT and CPT groups (time: $F_{2,120} = 13.69$, $p<0.001$; see Table 1).

3.3. Subjective stress ratings

Groups differed significantly in their subjective stress ratings (all F's >12, all p's<0.001). Participants in the SECPT and CPT groups experienced the stress manipulation as significantly more unpleasant, stressful and painful than participants in the warm water groups; the SECPT and CPT groups did not differ in their stress ratings (Table 1).

4. Discussion

The CPT is a frequently used laboratory stressor (al' Absi et al., 2002; Buchanan et al., 2006; Duncko et al., 2007). In the present study, we asked whether the inclusion of social-evaluative elements can increase the ability of the CPT to activate the HPA axis. Indeed, cortisol responses were

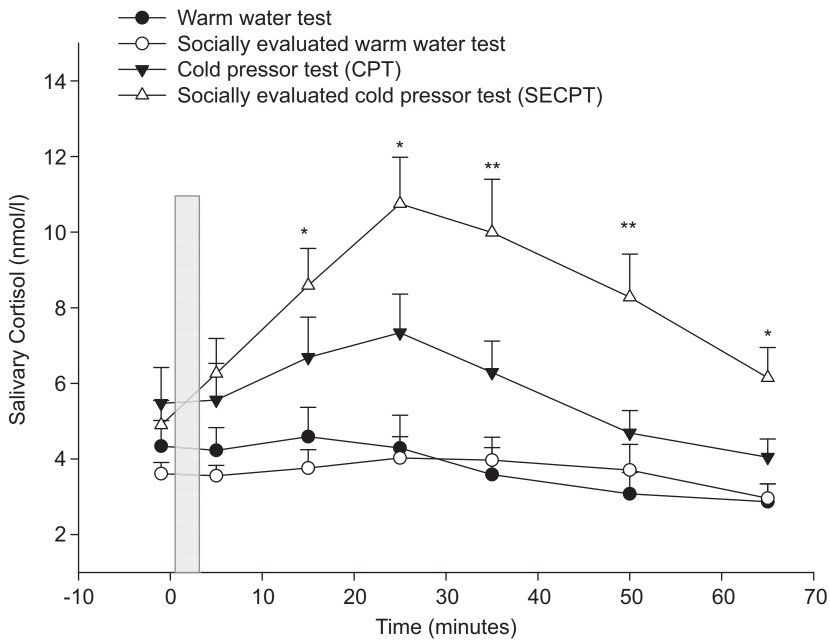

Figure 1 Salivary cortisol in nanomoles per liter ($M \pm$SEM) at several time points across the experiment. Cortisol responses were significantly increased in the socially evaluated cold-pressor test (SECPT) group but not in participants exposed to the standard cold-pressor test (CPT). The gray bar represents the time of the stress and control manipulation, respectively. *Significant difference between SECPT group and each of the two warm water groups ($p<0.05$); **Significant difference between SECPT group and each of the three other groups ($p<0.05$).

Table 1 Heart rate (beats per minute), systolic and diastolic blood pressure (mm/Hg) before (pre), during and after (post) hand immersion in warm or cold water as well as subjective stress ratings in the four treatment groups.

	Warm water test	Socially evaluated warm water test	Cold pressor test	Socially evaluated cold pressor test
Heart rate				
Pre	72.3±2.1	67.8±2.4	68.0±1.9	69.1±2.6
During	73.0±2.6	67.2±2.2	69.6±1.5	71.0±2.6
Post	71.1±2.6	66.8±1.8	**65.1±1.7**	**65.9±2.3**
Systolic blood pressure				
Pre	126.1±4.2	125.8±3.3	125.1±4.4	121.0±6.5
During	127.4±3.5	**141.2±5.4**	**149.4±3.2***	**154.2±5.1***
Post	127.9±4.2	125.3±4.7	128.5±5.1	128.1±5.8
Diastolic blood pressure				
Pre	72.2±2.0	73.6±2.9	74.2±2.2	68.5±2.8
During	72.8±2.0	76.3±1.9	**87.6±2.6***	**87.9±1.6***
Post	72.5±2.5	75.3±2.5	75.9±2.4	73.9±2.2
Subjective stress ratings				
Unpleasant	10.0±3.9	20.1±3.7	55.6±6.5**	46.7±6.3**
Stressful	4.0±1.3	14.0±3.5	42.8±6.6**	36.1±5.6**
Painful	1.3±0.9	7.0±0.7	58.9±4.7**	56.7±5.9**

*$p<0.01$ compared to warm-water task. **$p<0.01$ compared to warm-water task and socially evaluated warm-water task; **bold**—significant difference within the warm-water test, socially evaluated warm-water test, cold-pressor test and socially evaluated cold-pressor test group, respectively ($p<0.05$). Data represent $M \pm$SEM.

increased significantly when male subjects were watched by a woman and videotaped during hand immersion into ice water. Peak cortisol levels were increased by 45%, cortisol responder rates by 75% in the SECPT compared to the standard CPT. Corroborating the findings of the meta-analysis by Dickerson and Kemeny (2004), we found large effect sizes for the effect of social evaluation. Importantly, although we observed increases in systolic blood pressure and stress ratings in the socially evaluated warm water test, the increased HPA axis responses were not produced by the social-evaluative components alone but required both a challenge (cold pressor) and social evaluation.

Our findings are comparable to those of Gruenewald et al. (2004), who reported similar cardiovascular responses to evaluative and non-evaluative conditions of the TSST, while cortisol elevations were observed in the social evaluative condition only. Thus, the fact that we found sizeable cortisol responses in the SECPT but not in the socially evaluated warm water task appears to be rather independent of the physical properties of the CPT, but instead could mean that social evaluation is especially likely to elicit HPA axis responses under conditions when individuals worry about self-presentation. Social evaluation in the context of any situation that raises the potential for negative evaluation and threatens one's social value may be the key ingredient. The CPT might be just a convenient laboratory stimulus for inducing such a context.

Interestingly, the addition of social-evaluative elements appeared to increase HPA axis responses selectively. Cardiovascular and subjective stress responses were comparable in the SECPT and the standard CPT. Both stress tests elicited significant increases in systolic and diastolic blood pressure as well as in subjective ratings of stressfulness, painfulness and unpleasantness. At first glance, it might be surprising that heart rate did not increase significantly in response to SECPT and CPT. This, however, is due to the nature of the cold pressor stress. Cold stress causes vasoconstriction. Consequently, blood pressure is elevated and baroreceptors are activated which induce heart rate deceleration. This heart rate deceleration was still visible in the two ice water groups in the post-stress measurement. Furthermore, the absence of an increase in heart rate could be due to the type of stress response triggered. The CPT is a passive coping task; it does not allow subjects to exert control over aversive outcomes but requires passive tolerance. Such tasks were described as eliciting a vascular response pattern without increases in heart rate (e.g. Bosch et al., 2001).

Previous studies demonstrated significant sex differences in stress responses. In the TSST, for instance, men show usually higher HPA axis responses than women (Kirschbaum et al., 1993; Kirschbaum et al., 1999). Here, we examined stress responses to the SECPT in young men only. Thus, future studies will have to corroborate our findings in female subjects.

While a potential disadvantage of the SECPT might be seen in the fact that some people (e.g. people with skin diseases) are excluded from participation, its advantages are at hand: it takes only 3 min and requires only one experimenter. Thus, it is a very quick and efficient method to induce stress.

The SECPT elicited blood pressure and cortisol responses which are comparable to those observed in response to the TSST (Kirschbaum et al., 1993; Maheu et al., 2005; Schwabe et al., 2007). While the TSST is a well-established laboratory stressor, the SECPT might represent a very economic alternative tool in stress research.

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Conflict of interest

All authors report no conflict of interest.

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