The ‘Trier Social Stress Test’ –
A Tool for Investigating
Psychobiological Stress Responses in a Laboratory Setting

Abstract
This paper describes a protocol for induction of moderate psychological stress in a laboratory setting and evaluates its effects on physiological responses. The ‘Trier Social Stress Test’ (TSST) mainly consists of an anticipation period (10 min) and a test period (10 min) in which the subjects have to deliver a free speech and perform mental arithmetic in front of an audience. In six independent studies this protocol has been found to induce considerable changes in the concentration of ACTH, cortisol (serum and saliva), GH, prolactin as well as significant increases in heart rate. As for salivary cortisol levels, the TSST reliably led to 2- to 4-fold elevations above baseline with similar peak cortisol concentrations. Studies are summarized in which TSST-induced cortisol increases elucidated some of the multiple variables contributing to the interindividual variation in adrenocortical stress responses. The results suggest that gender, genetics and nicotine consumption can influence the individual’s stress responsiveness to psychological stress while personality traits showed no correlation with cortisol responses to TSST stimulation. From these data we conclude that the TSST can serve as a tool for psychobiological research.

Introduction
Endocrine responses to psychological stress have been frequently described in the literature. In general, distressing psychosocial stimuli are associated with increases in several hormones including adrenocorticotropic hormone (ACTH), cortisol, vasopressin, β-endorphin, epinephrine and growth hormone (GH) while concomitant decreases can be observed in other endocrine circuits, e.g. luteinizing hormone (LH) and testosterone. While unspecific in nature with respect to the affected physiological systems, one feature of endocrine responses to psychological stress appears to be uniformly present. The large interindividual variability of responses is a prominent and well documented phenomenon in psychoendocrine studies [1]. Experiments aimed at unravelling variables which contrib-
ute to this variability in hypothalamus-pituitary adrenal (HPA) function obtained inconsistent results [2]. This was partly due to the use of stress paradigms which yielded unreliable or insufficient stimulation of the axis. In an attempt to elucidate factors and variables responsible for the interindividual HPA response variability as a major research goal, our laboratory established a simple psychological stress protocol for induction of HPA activation in the majority of subjects tested. In the following, we report the responses of ACTH, GH, prolactin, total serum, salivary (‘free’) cortisol, and heart rate responses to the ‘Trier Social Stress Test’ (TSST). Furthermore, we summarize data previously obtained with the TSST in order to evaluate (a) the reliability of TSST-induced changes in saliva cortisol, and (b) briefly discuss variables responsible for interindividual variation in adrenocortical stress responses on the basis of those of our studies that have been published separately elsewhere [3–6].

Methods

Since the major focus of the present paper is the response of pituitary and peripheral hormones as well as heart rate changes to TSST, the general study outline along with a more elaborate description of the TSST is given herein.

Subjects

In study 1, 20 healthy male volunteers with a mean age of 24.7 ± 3.3 years (mean ± SD) were paid for their participation. Prior to testing they underwent a brief medical examination to exclude volunteers with acute or chronic diseases. A total of 155 subjects of both sexes were investigated in four additional independent studies. The age of the volunteers ranged from 15 to 33 years. They were recruited among students of the University of Trier and in part paid for participation (four studies). In one study mainly nonacademical volunteers were recruited by an announcement in a local newspaper or by direct contact (i.e., letter). They received no monetary incentive for participation. All subjects were medication-free and refrained from smoking, physical exercise, meals, alcoholic beverages, and low pH soft drinks at least 1 h prior to testing.

TSST Protocol

Experimental sessions were run between 9 a.m.–1 p.m. or 4–7 p.m., respectively. After arrival at the laboratory, subjects rested for 30 min (after insertion of an intravenous catheter) or 10 min (no blood sampling) in room A. At time 0 min they were taken to a second room (room B) and introduced to the task they would have to perform subsequently. In room B, 3 persons were already sitting at a table, and a video camera and a tape recorder were installed. The subject was asked to stand at a microphone in front of the 3 persons. Next, the investigator asked the subject to take over the role of a job applicant who was invited for a personal interview with the company’s staff managers (selection committee). They were told that after a preparation period they should introduce themselves to the managers in a free speech of 5 min duration and convince the managers that he/she was the perfect applicant for the vacant position. The managers were introduced as being specially trained to monitor nonverbal behavior. Furthermore, it was announced that a voice frequency analysis of nonverbal behavior would be performed on the tape-recorded talk and that a video analysis of the subject’s performance would also be conducted. The selection committee was always comprised of males and females.

Following these instructions, the subjects returned to room A and were given 10 min to prepare their talks. They were provided with paper and pencils for outlining their talks, however, they were not allowed to use the written concept for their speech. At time +10 min the volunteers were guided back to room B by the investigator who left the room before the subjects delivered their speeches. Now one of the managers welcomed the job applicant and asked him/her to deliver the talk during the next 5 min. Whenever the subjects finished their speeches in less than 5 min, the managers responded in a standardized way. First they told the volunteer ‘You still have some time left. Please continue!’ Should the subjects finish a second time before the 5 min were over, the managers were quiet for 20 s and then asked prepared questions. At time +15 min, the selection committee asked the subject to serially subtract the number 13 from 1,022 as fast and as accurately as possible. On every failure the subjects had to restart at 1,022 with one member of the committee interfering ‘Stop. 1,022.’

At time +20 min the task was stopped and the volunteer was taken back to room A by the investigator who then debriefed the subject about the goal of the study and that neither a voice frequency nor a video analysis would be performed. Thereafter, subjects rested for 30–70 min depending on the hormones measured in the particular study.

Control Setting

In study 1, the subjects also attended a control session for monitoring possible setting effects (coming to a hospital for psychological testing, insertion of a venous catheter, blood sampling etc.) on endocrine parameters and heart rates (see below). In this session, physiological saline was injected at time 0 min and no subsequent test was performed.

Sampling and Biochemical Analysis

Blood samples or saliva samples were obtained at 10- to 30-min intervals for subsequent analysis of cortisol in serum and saliva. For blood-borne hormone analysis, an indwelling catheter was inserted and kept patent by saline infusion. Saliva samples were obtained using the ‘Salivette’ for quick and hygienic sampling [7]. ACTH, GH, prolactin, and cortisol (serum and saliva) were measured in study 1, while only cortisol in saliva was determined in the four other experiments. Cortisol in saliva was measured with a time-resolved fluorescence immunoassay [8], and commercially available assay kits were used for ACTH, serum cortisol, prolactin, and GH.

Heart Rate Measurement

In one study, heart rate was monitored continuously employing a wireless signal transmission device (Sport Profi, Polar Instruments, FRG). Heart rates were averaged across 1-min intervals.

Statistical Analysis

Analyses of variance (ANOVA) for repeated measures were performed to reveal possible time and/or treatment effects with individual baseline levels serving as covariates. Taking into account the
Fig. 1. GH, prolactin, ACTH, serum cortisol, and saliva cortisol responses to TSST and saline injection in 20 healthy subjects.

sphericity assumption, degrees of freedom were adjusted employing the Greenhouse-Geisser approach where appropriate. Endocrine and heart rate stress responses were correlated using Pearson's correlation coefficient.

Results

Figure 1 summarizes endocrine responses to TSST and after injection of physiological saline in study 1. All hormones were significantly elevated in response to the psychological stress of public speaking and mental arithmetic. The mean increases above baseline levels ranged between 30% (prolactin) and 700% (GH). While prolactin, ACTH, and serum cortisol concentrations peaked immediately after cessation of stress, cortisol in saliva peaked 10 min and GH 40 min later. Comparing the mean salivary cortisol responses to TSST stimulation in five different studies (fig. 2), a high reproducibility was observed across the studies. From baseline salivary cortisol concentrations ranging between 4 and 9 nmol/l, mean absolute increases ranged from 5.3 to 8.2 nmol/l peaking 10 min after the end of the stress. Cortisol levels were down to prestimulation values 90 min after the start of the TSST procedure. In each study more than 70% of the volunteers responded with an increase of at least 2.5 nmol/l above baseline. Also, heart rate was significantly elevated under TSST stimulation (fig. 3). From a mean of 70.5 bpm, mean peak heart rates reached 96.5 bpm during the stressful task. Shortly after cessation of the stressor, heart rates dropped to baseline. Interestingly, the heart rate response to TSST was not significantly correlated with any of the endocrine responses.
Fig. 2. Consistency of saliva cortisol responses to TSST in five independent studies.

Fig. 3. Heart rate responses to TSST and saline injection in 20 healthy volunteers.
Discussion

The finding of increased concentrations of several hormones following public speaking or mental arithmetic as reported in this paper is not new. In fact, numerous research groups used either task to induce a psychologically distressing situation [9–15]. However, with respect to HPA-axis activity these protocols led only to small or moderate increases in cortisol and/or ACTH values. Only little information is available concerning the simultaneous effect on different hormonal systems as well as on the reproducibility of activation using these protocols.

The TSST had a profound effect on ACTH, GH, prolactin, serum and salivary cortisol. Additionally it was found to reliably induce 2- to 4-fold increases in salivary cortisol levels with similar peak concentrations in different populations studied.

It should be noted that the TSST protocol consists of several components (e.g. public speaking, mental arithmetic, audience, anticipatory period) all of which may contribute to the observed physiological responses. Since in previous studies we observed only small and inconsistent results with protocols employing only one type of performance task [16], the TSST protocol has been set up in an attempt to elicit significant and reproducible effects in the majority of subjects tested. Therefore, an evaluation of the contribution of the different components to the physiological responses has not been a primary goal in these studies. However, it seems worthwhile to elucidate the separate effects in future investigations. It can only be suspected that a high degree of ego involvement along with an anticipation of negative consequences, as realized with the introduction of a small group of confederates interacting with the subjects under study, is at least partly responsible for the large and consistent endocrine stress responses observed in the TSST. The importance of ego involvement and anticipation of negative consequences for significant cortisol responses to psychological stress has long been known [1].

Using the TSST as a tool in investigations of interindividual differences in cortisol stress response variability, we observed consistent sex differences [5]. While both sexes responded similarly to injection of corticotropin-releasing hormone or to exhausting physical exercise, male subjects displayed higher cortisol responses to the psychological stress of TSST. Moreover, we found evidence for genetic factors contributing to the variability of cortisol changes with greater resemblance of cortisol profiles in monozygotic twins compared to dizygotic pairs [4]. A third variable influencing the individual’s HPA-axis responses to TSST may be chronic nicotine consumption. In a recent study, smokers showed lower cortisol responses to TSST than nonsmokers [6], possibly indicating an effect of chronic stimulation of hypothalamic CRH-containing neurons by nicotine. On the other hand, personality traits as measured by the Eysenck Personality Questionnaire, the Strelau Temperament Inventory, and the Zuckerman Sensation Seeking Scale, showed no significant correlation with either baseline, peak or area under the curve cortisol levels in response to TSST in two independent studies [3].

In conclusion, the TSST has proven a useful tool in basic studies of cortisol responses to psychological stimulation in healthy volunteers. Future projects will have to assess the usefulness and the possible restrictions of this protocol in clinical populations.

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References


Trier Social Stress Test

Kirschbaum/Pirke/Hellhammer


