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Evaluation of an app-based stress protocol

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Abstract: Stress is a major influence on the quality of life in our fast-moving society. This paper describes a standardized and contemporary protocol that is capable of inducing moderate psychological stress in a laboratory setting. Furthermore, it evaluates its effects on physiological biomarkers. The protocol called “THM-Stresstest” mainly consists of a rest period (30 min), an app-based stress test under the surveillance of an audience (4 min) and a regeneration period (32 min). We investigated 12 subjects to evaluate the developed protocol. We could show significant changes in heart rate variability, electromyography, electro dermal activity and salivary cortisol and α -amylase. From this data we conclude that the THM-Stresstest can serve as a psychobiological tool for provoking responses in the cardiovascular-, the endocrine and exocrine system as well as the sympathetic part of the central nervous system.

Keywords: amylase; cortisol; EDA; EMG; HPA-axis; HRV; SAM-axis; stress protocol; stroop; THM-Stresstest.

1 Introduction

At the beginning of the century, the World Health Organization (WHO) predicted stress to become one of the major health problems for our generation [1]. Studies of German health insurances confirm that assumption. In Germany 70–80% of the insured complain about occasional stress and 20–30% about frequent stress [2], tendency increasing [3]. A recent study of the “Robert Koch Institut” shows the influence of stress on psychological disturbances like

burn-out, depression or sleep-disorder [4]. As a consequence of the manifold influence, the WHO forecasts that by the year 2020 stress could cause every second job absenteeism [5]. This indicates the immense increase of costs for primary and secondary care of stress related psychological, psychiatric and physical health issues. Thus, there is an urgent need for research in the context of stress and health. Besides the individual, subjective sense of stress there are two major physiological stress response systems: the hypothalamus-pituitary-adrenal-axis (HPA-axis) and the sympathetic-adrenal-medullary-axis (SAM-axis). The HPA-axis as a part of the neuroendocrine system triggers a cascade of hormones which leads to the release of stress related hormones. Cortisol e.g. plays an important role in this slow regulation of stress. The SAM-axis as the sympathetic part of the central nervous system activates the cardio-vascular system through a release of adrenalin and noradrenalin resulting in a quick reaction on sudden stress. Moreover, the sympathetic nerve innervates parts of the exocrine system. Thus, the production of specific enzymes like salivary α -amylase increases. A proper reaction to stress caused by the named systems is indispensable for physiological health, since alterations can cause diseases or enforce disease progression. Stress protocols can be used to examine the capability of a subject’s stress response system. However, most available laboratory protocols are not capable of inducing significant changes in both endocrine and cardiovascular parameters [6]. Meta-analysis show that protocols based on motivated performance tasks reveal success if they are uncontrollable and go along with a social component [7]. A few protocols like the “Trier social stress test” (TSST) apply that paradigm but seem to be very time-consuming for the trial staff concerning preparation and execution. We designed and evaluated a simple and contemporary stress protocol called “THM-Stresstest” based on a tablet application. Our concept is intended to induce moderate psychological stress in a laboratory setting on both HPA- and SAM-axis. For the procedure we elaborated an easy to follow storyboard that can be adapted to different scenarios. For evaluation purposes, it contains measurements of electrocardiography (ECG), electro dermal activity (EDA), electromyography (EMG) and salivary biomarkers.

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2 Material and methods

Subject collective: Twelve male subjects of ages ranging from 21 to 37 (mean age 26.4 ± 4.6) participated in the study. The mean weight and height of the participants were 83.7 ± 15.8 kg and 1.8 ± 0.1 m, respectively. Participants had the chance to win 20 Euros if they made the best performance in the test and a 10 Euro raffle took place between all participants. All subjects gave their written informed consent for the test. The exclusion criteria were regular consumption of nicotine, color blindness, drug or alcohol dependence, steroids or medicine intake with an effect on the function of cognitive and emotional state or with an influence on the hormone levels. Additional contraindications were any endocrine, exocrine, neurological or psychological system related diseases. The participants were instructed not to ingest any food or sugary drinks for at least 6 h, not to work out for 24 h and not to brush teeth for 2 h before the test.

Study design: The study was carried out in March 2016 within 2 weeks between 1 p.m. and 5 p.m. This time frame considers the circadian rhythm of cortisol [8]. Our study protocol followed basic advisements proposed by Kudielka et al. [9]. Figure 1 illustrates the principal periods of the protocol.

In the preparation period, the subject was instructed about the measuring process. In addition, sensor systems were prepared and reference and/or calibration measures were conducted. Subsequently, a 30-min rest period followed. During this time the subject was able to relax in order to minimize potential effects of prior stressful events on both SAM- and especially HPA-axis [7]. In the following stress period the subject performed a 4-min stress test in form of a tablet application. The stress test protocol was based on three key components pointed out in [7]: a motivated performance task, uncontrollability for the subject and a social evaluative threat. Motivation was ensured by a high-score and a monetary incentive. The stress application was designed with an increasing difficulty which made it almost impossible to control the task and reach a good score. The performance was evaluated by an audience which was introduced to the subject as

a committee of experts. To enforce the social threat, the subject's actions were projected on a screen for the audience and recorded on tape. In the proceeding regeneration period of about 30 min the high latency cortisol level could build up and reach its stress induced peak [10].

Stress test application: The stress test application called "THM-Stress"-App represents a modified Stroop-color test to provoke acute stress. This app was developed for Android 5 (and later) and installed on a Samsung Nexus 10 Tablet (Samsung, Samsung Town, Seocho-gu, Seoul, South Korea) with Android 5.1.1. After clicking the start-button, a 5 s countdown is displayed. During the stress test, a color-word and seven differently colored buttons are presented on the screen changing in a specific rhythm. The challenge is to press the correct button, corresponding to the word meaning, regardless of the font color. The words and the font colors are generated randomly. In the beginning of the stress test the word appears for 1.6 s. The display duration decreases down to 0.9 s. If time runs out or a wrong button is pressed a red cross signals the failure. At the end of the stress test, the number of right and wrong answers are displayed.

Data acquisition: During the rest and stress period the following electrical biosignals were recorded: EMG (M. trapezius), ECG (proximal to heart) and EDA. For the acquisition we used a Biopac MP 36 (BIOPAC® Systems, Inc., Goleta, CA, USA) and the accompanying software Biopac BSL 4.0 MP36 (BIOPAC® Systems, Inc.). The recordings were sampled with a frequency of 1000 Hz. The filter configuration was applied as described in [11]. We used Ag/AgCl ECG electrodes (Teqler, NetMed S.à.r.l., Wasserbillig, Luxembourg). The ECG and EMG electrodes were placed as described in [11]. The signals were recorded with shielded lead sets of type SS2LB (BIOPAC® Systems Inc.). For each subject a calibration of the EMG was performed as depicted in [11]. The EDA electrodes were positioned on the palm of the subject's non-dominant hand over the thenar and hypothenar. EDA was recorded with a lead set of type SS57L (BIOPAC® Systems Inc.) and amplified by factor 2000. For EDA reference the subject was requested to breathe in and out deeply for 30 s. This maneuver should be reflected in the signal waveform of EDA [12]. During the measuring process we collected five saliva samples to reconstruct the cortisol time response (see Figure 1). For data selection we considered individual hormone levels and the effects of prior potentially stressful events. For saliva collection oral fluid collector sets (IPRO Interactive Ltd, Wallingford, UK) were used consisting of a swap and a buffer solution. The sampling was performed in accordance with IPRO guidelines. During the protocol execution marks were set with a button switch of type

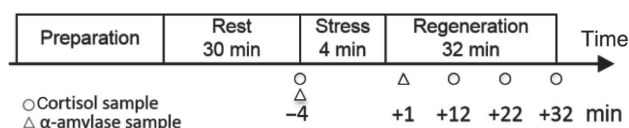


Figure 1: Abbreviated timeline of the THM-Stresstest protocol. Time indications refer to the end of the stress period, dots (cortisol) and triangles (α-amylase) mark the saliva sampling.

SS10L (BIOPAC® Systems Inc.) at characteristic points of time. These marks helped to cut and analyze the different periods of the measure.

Data-analysis: All following algorithms were implemented with Matlab® (The MathWorks, Inc., Natick, MA, USA). The EMG was checked for artefacts and band-pass filtered from 40 to 300 Hz [11]. The algorithm from Hof [13] was applied to reduce the ECG in the EMG. After pre-processing, we subdivided the EMG in 200-ms intervals, calculated the root-mean-square for each interval (IEMG), and computed the mean of these IEMGs for both, rest and stress period [11]. For HRV analysis NN-intervals were identified in ECG. Irregular beats, electrical artefacts and premature ventricular beats were rejected manually. Afterwards, the inter-beat interval was resampled to 1000 Hz. The power of low frequencies (LF) from 0.04–0.15 Hz and high frequencies (HF) from 0.15–0.40 Hz were extracted from the power spectrum of ECG for rest and stress period. We computed the ratio LF/HF as an indicator for change in sympathetic activity as described in [14]. We analyzed the skin conductance response (SCR), using the EDA. We calculated the frequency of nonspecific and event related SCRs, as described in [12], to distinguish between rest and stress period. The SCR-rate for both rest and stress period was determined semi-automatically. In our protocol, event related SCRs were triggered by changing words and negative feedback in the stress test application as well as feedback from the committee. All saliva samples were sent to IPRO Interactive and were analyzed with ELISA method.

Normal distribution was tested for all biomarkers using Shapiro-Wilk-test. For non-parametric variables we used a Wilcoxon rank sum test and for parametric variables the two-tailed paired-sample student's t-test. The significance level was set to $\alpha = 0.05$.

3 Results

For the analysis of biosignals in the rest period, only the last 10 min were taken into account. If artifacts were present in the examined signal, an equally long alternative window was selected. Figure 2 shows the analyzed biomarkers for SAM-axis for each subject and illustrates the change in value from rest to stress period. Shapiro-Wilk-test showed that only the frequencies of SCRs are normally distributed. Figure 2A–C depicts the biosignal features SCR, mean EMG activity and LF/HF-ratio and Figure 2D the biochemical analysis of salivary α -amylase. The EDA signal (see Figure 2A) of one subject reached

saturation during the stress period and was therefore excluded from SCR-analysis. The increase of SCR-rate from rest level (median = 6.7 1/min) to stress level (median = 22 1/min) is significant ($p < 0.01$). The mean EMG activity (see Figure 2B) rises with significance ($p < 0.01$) from rest level (median = $0.85 \mu V^2$ rms) to stress level (median = $4.05 \mu V^2$ rms). The increase of the LF/HF-ratio (Figure 2C) from rest level (median = 6.7 1/min) to stress level (median = 22 1/min) is significant ($p < 0.01$).

In Figure 2D the salivary α -amylase levels for all 12 subjects are plotted for rest (median = 89 $\mu g/ml$) and stress period (median = 137 $\mu g/ml$). One subject shows a decrease in concentration contrary to the other subjects.

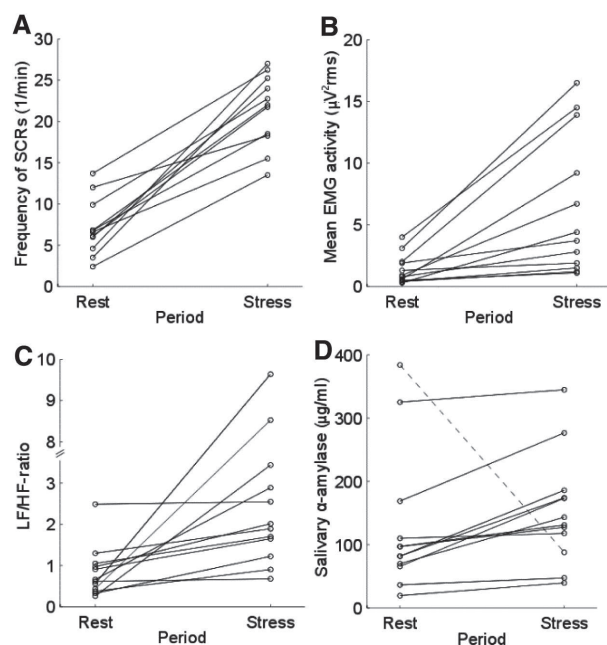


Figure 2: Stress induced changes on SAM-axis from rest to stress condition in frequencies of SCRs (A), mean EMG activity (B), LF/HF-ratio (C) and salivary α -amylase with dashed outlier (D).

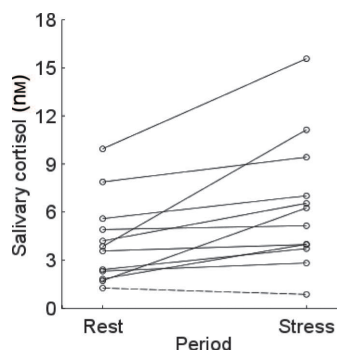


Figure 3: Stress induced change on HPA-axis from rest to stress condition in salivary cortisol.

The values of salivary α -amylase were excluded from statistical analysis and marked as outliers. The increase for all other subjects is significant ($p < 0.05$).

Figure 3 illustrates the effect of the THM-Stresstest on the HPA-axis of each subject which is reflected in salivary cortisol levels. For one subject there is a decrease in cortisol level from rest to stress period. Overall the level of cortisol from rest period (median = 4.0 nM) to stress period (median = 6.4 nM) is significant ($p < 0.01$).

4 Discussion

We could show that the THM-Stresstest induces moderate psychological stress in a laboratory setting on both HPA- and SAM-axis. All measured biomarkers differ significantly between rest and stress period. From a usability and feasibility perspective, the stress protocol is adaptable and intuitive to perform. Therefore, it is a versatile research tool to investigate physiological and biopsychological responses to acute stress. Considering the other three SAM-axis parameters, the results of the α -amylase outlier are implausible and therefore excluded. Thus, we assume an error of measurement in saliva sampling. In contrast to the slight increase of cortisol during Stroop-color tests in [10] we achieved a significant increase. Therefore, we conclude that HPA-axis is better stimulated by our protocol. The duration of rest period does not guarantee that stressful events prior to the stress test have no influences on the measurement of the slow reacting HPA-axis. Prospectively, this period could be prolonged to avoid these influences and confirm our results [6]. For scientific issues, regarding the SAM-axis only, the protocol can be reduced to the stress period using the THM-Stress-App only. The app logs events and will allow a synchronization with bio signals in future. Thereby, an event-related analyses will be possible. Based on this paper, we are planning to validate the THM-Stresstest in a more representative and larger population.

Author's Statement

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Material and methods: Informed consent: Informed consent has been obtained from all individuals included in this study.
Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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