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Characterization of Stress Reactions to the Stroop Color Word Test

J. H. M. TULEN,*[1] P. MOLEMAN,* H. G. VAN STEENIS† AND F. BOOMSMA‡

*Departments of *Psychiatry, †Clinical Neurophysiology and ‡Internal Medicine I*
Academic Hospital Dijkzigt and Erasmus University Rotterdam, The Netherlands

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TULEN, J. H. M., P. MOLEMAN, H. G. VAN STEENIS AND F. BOOMSMA. *Characterization of stress reactions to the Stroop Color Word Test.* PHARMACOL BIOCHEM BEHAV 32(1) 9–15, 1989.—Sympatho-adrenal activation induced by stress contributes to the development of pathological states such as hypertension and anxiety disorders. The Stroop Color Word Test (CWT) is evaluated as a test for the study of stress-induced sympathetic effects, on the basis of psychological, physiological and biochemical responses. The CWT induced increases in plasma and urinary adrenaline, heart rate, respiration rate, electrodermal activity, electromyography, feelings of anxiety, and decreased finger pulse amplitude.

Mental stress Sympatho-adrenal activation Catecholamines Cortisol Prolactin Heart rate
Respiration rate Electrodermal activity Vascular activity Electromyography

MANY normal and abnormal psychic and somatic human behavioral states are associated with altered patterns of sympatho-adrenal activity. Altered sympatho-adrenal activity, especially abnormal catecholamine release, is believed to be of importance in the pathogenesis of hypertension, orthostatic hypotension and anxiety states (3, 4, 9, 13).

Environmental events, which are subjectively perceived as stressors, evoke substantial increases in sympathetic nervous system activity and have been hypothesized to contribute to the development of the pathological states mentioned above. To characterize these sympathetic responses, i.e., how they may lead to pathological states and how they could be antagonized, tests are required that are capable of inducing: 1) psychological changes that indicate increased distress: the individual's perception and interpretation of the situation is of great importance for the observed physiological activation and variation, 2) physiological changes that indicate sympatho-adrenal activation as reflected in parameters such as heart rate, respiration rate, electrodermal activity, and peripheral blood flow, 3) muscular exertion as part of the fight-flight defence reaction (11,19), and 4) hormonal (and neuronal) changes as reflected in plasma and urinary catecholamines, and plasma cortisol and prolactin. In order to study these reactions, a controlled situation is needed and therefore it is necessary to induce anxiety or stress experimentally.

The present study was undertaken to investigate whether the Stroop Color Word Conflict Test meets all of the above-mentioned requirements. The Stroop Color Word Test, as presented in the version of Frankenhaeuser and Johansson (8), produces mental overstimulation as a result of cognitive conflict combined with time-pressure effects. This test has repeatedly been proven to increase plasma and urinary adrenaline concentrations (1,12). Although sympatho-adrenal activation during the Stroop test has been described before on the basis of psychological, physiological and biochemical parameters separately, it is necessary to combine these parameters in one study in order to evaluate the usefulness of this test for the above-mentioned purpose. The response patterns to the Stroop test were analysed in a group of young healthy male subjects and compared with measurements obtained during rest.

METHOD

Subjects and Procedure

Nine paid male volunteers (age range: 22–25; mean: 23.8) participated in two experimental sessions (a stress-session and a control-session) after giving informed consent. The experimental procedures were approved by the Medical Ethical Committee of the Erasmus University and Dijkzigt Hospital. All subjects were in good health; none reported excessive drinking or smoking habits. They were requested not to undertake unusual activities (exams, heavy training, abnormal drinking) for three days before the first recording session and the week between the two sessions. Of the two sessions, one was a stress-session and the other a control session. The stress- and control-sessions were recorded on separate days, one week apart. The stress-session was the first session for six of the nine subjects.

[1]Requests for reprints should be addressed to Dr. J. H. M. Tulen, Department of Psychiatry, Academic Hospital Dijkzigt, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

During the *stress-session*, the volunteers were subjected twice to a 20-min version of the Stroop Color Word Test (CWT), with 20-min periods of rest before and after each CWT. The *control-session* consisted of five successive periods of rest (20 min each). The subjects were asked to remain seated in the same position during the rest periods as during the presentation of the CWT, with their eyes kept open.

The CWT was presented on videotape and was constructed to conform to a previously reported description (8). In short, four words (red, green, blue and yellow) were presented at random in one of four colors (red, green, blue and yellow) with random intervals of 0.8–1.7 sec, while the duration of the stimuli varied at random between 0.4–1.0 sec. The subjects had to indicate the color of the words on an answer sheet. During the second CWT a double-conflict version was used: auditory stimuli were added by presenting the color word on video simultaneously with an incompatible color word on sound track. Beforehand, a two-minute practice session was administered so that the subjects would be familiar with the test requirements.

Before the start of each experimental session, the subjects were requested to void urine and to drink a glass of mineral water. Coffee, tea and smoking were not allowed before or during the recordings. Both recording sessions were performed between 9:15 and 12:00 a.m.; physiological, biochemical and psychological measurements were obtained during these periods. After the sessions, urine was collected and a standard light lunch was served. During the afternoons the subjects spent their time reading and/or studying in a quiet room; urine was collected twice during this period.

Measurements and Analyses

Psychological. A shortened version of the POMS (Profile Of Mood States) (16) and the ZBV (Dutch version of Spielberger's State Trait Anxiety Inventory) (21) self-rating scales were administered before and after the sessions to assess changes in mood and anxiety. The shortened version of the POMS has been validated for the Dutch population (25).

Biochemical. Forty-five minutes before the start of the recordings a catheter (Venflon) was inserted in a vein of the nondominant forearm. Immediately after each 20-minute period, 15 ml of venous blood was drawn and collected into two tubes, one containing 19 mg of EGTA and 12 mg of gluthathione for assay of catecholamines and one containing heparin for assay of cortisol and prolactin. The tubes were immediately placed on ice and centrifuged within 15 minutes. Plasma for assay of adrenaline and noradrenaline was subsequently frozen at −70°C. Catecholamines from 1 ml of plasma were concentrated by a liquid-liquid extraction method (24) and then measured by a radioenzymatic procedure (20). Plasma for assay of cortisol and prolactin was frozen at −20°C. Cortisol was estimated by radioimmunoassay, using kits supplied by Diagnostic Product Corporation (DPC, Los Angeles, CA), and prolactin was assayed using a modified version of the method published by Miles *et al.* (17). Urine for determination of catecholamines and their metabolites was collected immediately after the sessions, 3 and 6 hours later. Urinary catecholamines were extracted (24) and assayed by a HPLC procedure after fluorescence derivatization (18).

Physiological. A Siemens-Elema (EEG-mingograf) electroencephalograph was used to amplify, calibrate and monitor the physiological signals. The ECG was derived by placing one electrode on the sternum, one below the left breast and one (reference) electrode below the right breast. Thoracic and abdominal respiration were measured by means of two mercury strain gauges placed around the chest at the level of the nipples and the abdomen respectively. Skin resistance (Siemens Elema EMT 67) was recorded by two active Ag/AgCl electrodes with an effective area of 0.5 cm^2. The electrodes were attached to the medial phalanx of the index and ring finger of the nondominant hand. Peripheral vascular activity was monitored by means of a photoplethysmographic transducer, attached to the distal phalanx of the middle finger of the nondominant hand. EMG activity was derived from 2 Ag/AgCl electrodes, placed 3 cm apart, on the extensor muscles of the nondominant forearm. Before electrode placement the skin was cleaned with alcohol, and rubbed lightly with sandpaper; the interelectrode resistance had to be below 10 kOhm. All signals were recorded continuously during both sessions on a Racal instrumentation recorder.

Physiological analyses. The polygraphic recordings were checked visually for presence of artifacts. Because of technical shortcomings only six of the nine available GSR recordings of the stress-session were analyzed and seven of the control-session. All other recordings were of sufficient quality for further processing. Since the act of blood collection introduced movement artifacts and a short-lasting physiological arousal, only data collected after two minutes were used for analysis. All signals were sampled at 51.2 Hz on a MINC PDP-11/23 computer. The A/D conversion was performed at 4× real time speed. The data were further processed on a PDP-11/34 computer. Before A/D conversion, a level detector was used to trigger the incidence of the R-wave in the ECG. The output pulses of the trigger were fed into the computer for sampling. The time between the output pulses (the interbeat interval) was measured by a programmable clock and expressed in beats per minute. The EMG was full-wave rectified before processing; the digitized signal was integrated per minute for quantification. These integrated values were summed for the whole 20-minute period. The pulse amplitude of the plethysmogram was corrected for the amplification factor used for recording. Per R-R interval of the ECG, the amplitude of the pulse (max-min) was computed. Mean pulse amplitude was calculated per minute. GSR values (kOhms) were transformed to skin conductance values (micromho's). Mean SCL (Skin Conductance Level) was calculated per 20 minutes on basis of one sample per 10 sec. A logarithmic transformation was applied because of positive skewness of the distribution. Respiratory analyses were performed by means of a software program capable of computing different time and amplitude parameters per respiratory cycle. Only mean respiration rate per minute for the stress-session is presented here. Details of the respiratory changes during the Stroop test will be presented elsewhere.

Statistical Analyses

The data were averaged, per physiological parameter, to mean and SE (standard error of the mean) values per 20-minute period of rest or CWT. Results are presented for n=9, if not otherwise specified. Mean, median and range were defined for the nonparametric psychological data. Two-factor analyses of variance (ANOVA) for repeated measures (10) were used to assess the effects of the Stroop test (factor1:CWT/rest × factor2:subjects) with factor time as covariate. Two-tailed Student's *t*-tests were used for

TABLE 1

PSYCHOLOGICAL STATE, BEFORE AND AFTER THE STRESS- AND CONTROL-SESSION

		Stress-Session		Control-Session	
		Pre	Post	Pre	Post
POMS					
Depression	mean	8.1	8.3	8.2	8.6
	median	8.0	8.0	8.0	8.0
	range	1.0	2.0	1.0	2.0
Anger	mean	8.0	7.9	7.7	7.9
	median	7.0	7.0	7.0	7.0
	range	6.0	4.0	4.0	5.0
Fatigue	mean	6.9	8.3	7.6	8.6
	median	7.0	8.0	6.0	8.0
	range	3.0	5.0	7.0	11.0
Vigor	mean	17.7	16.6	18.0	15.1
	median	20.0	18.0	19.0	15.0
	range	12.0	10.0	14.0	9.0
Tension	mean	8.0	8.8	8.6	6.6
	median	8.0	8.0	8.0	6.0
	range	4.0	5.0	6.0	2.0
ZBV					
State-Anxiety	mean	32.7	34.7	32.8	28.4
	median	32.0	33.0	33.0	28.0
	range	16.0	25.0	27.0	18.0

Mean, median and range per subscale of the POMS and the ZBV, of the pre- and postsession measures of the stress- and the control-session.

pairwise comparisons. For the psychological data Wilcoxon tests were applied (23). Pearson correlation coefficients were used to analyze the relation between initial value during rest and response-magnitude during stress. Linear regression analyses for related measurements were performed in order to assess the presence of trends in the physiological and biochemical data of the control-sessions.

RESULTS

Psychological

Pre- and postsession values of the subscales of the POMS and the ZBV are presented in Table 1. The presession values of the stress- and control-session did not differ significantly on any factor. There was a significant decrease in tension after the control-session (Wilcoxon test: $Z=-2.02$; $p<0.05$), as well as a significant difference between postsession values of the stress- and control-session ($Z=-2.37$; $p<0.05$). The postsession values for state anxiety, as assessed by the ZBV, also differed significantly ($Z=-2.07$; $p<0.05$).

Biochemical

The stress-session. The CWT produced a significant rise in plasma adrenaline, as compared to the rest periods (Fig. 1, Table 2). Though the subjects clearly differed in baseline adrenaline level, all showed an increase as a result of mental stress. With regard to noradrenaline, the ANOVA indicated a significant interaction effect between the stress and subject factor. Two subjects showed a decrease in noradrenaline level after both CWT periods, while there was one nonresponder. The other six subjects showed noradrenaline increases after both CWT periods. No changes in cortisol or prolactin levels were observed. Over the whole stress-session a gradual declining trend was found for both hormones as indicated by the highly significant F-values of the covariate 'time' (Table 2). A significant difference between subjects was found for both cortisol and prolactin, indicating that the differences between subjects were consistent between the different stress and rest periods.

The control-session. The significant results on the ANOVA covariate 'time' of the stress-session suggest trends possibly due to habituation or circadian fluctuations. Regression analyses on the data of the control-session must show these trends. No significant regression was found in the adrenaline and prolactin data (Table 2); a significant increase with time was observed in the noradrenaline data and a significant decrease in plasma cortisol concentration. These effects were not the same for all subjects, since between subjects differences in regression were significant in noradrenaline, cortisol and prolactin concentration. For adrenaline and cortisol, these results are in agreement with the findings on the ANOVA covariate 'time' of the stress-session. Noradrenaline, however, showed a positive trend in the control-session, not present in the stress-session. For prolactin, no trend was observed in the control-session, although a clear time-related effect was present in the stress-session (Table 2, Fig. 1). There were no significant differences between the first rest periods of the stress- and control-session with regard to adrenaline (t-test: $t=-0.99$; $p=0.35$), noradrenaline ($t=1.24$; $p=0.25$), cortisol ($t=0.82$; $p=0.44$) and prolactin ($t=0.03$; $p=0.98$), indicating comparable initial baselines in both situations.

Urinary catecholamines. In order to analyze the effect of the CWT on the urinary catecholamines, data of the stress-session were compared with data of the control-session (Table 3). A significant increase in urinary adrenaline concentration during the stress-session was found for the first collection period (9–12 hours) (paired t-test: $t=2.64$; $p<0.05$) and the total collection period (9–18 hours) ($t=2.39$; $p<0.05$). With regard to urinary noradrenaline concentration, we found no significant differences between stress- and control-session.

Physiological

The stress-session. The CWT caused a significant increase in heart rate, EMG activity, skin conductance and a significant decrease in pulse amplitude (Fig. 2; Table 2). Respiration rate per minute showed an increase during the CWT (Rest1: mean=14.53, SE=0.76; CWT1: mean=17.56, SE=0.77; Rest2: mean=14.64, SE=0.71; CWT2: mean=17.41, SE=0.69; Rest3: mean=14.59, SE=0.72). With regard to respiration rate the ANOVA showed a significant CWT effect ($F=353.24$; $p<0.001$), a significant subject effect ($F=80.56$; $p<0.001$) as well as a significant interaction effect ($F=12.76$; $p<0.001$), but the covariate time was not significant ($F=0.07$). The two-factor ANOVA also indicated interaction effects for heart rate, EMG and SCL (Table 2). Since these interactions might be due to a relation between magnitude of response and prestimulus level (26), we calculated product-moment correlation coefficients between the prestimulus values and the response-magnitudes to the CWT for all physiological parameters. We did this both for each rest and CWT period of the stress-session separately as well

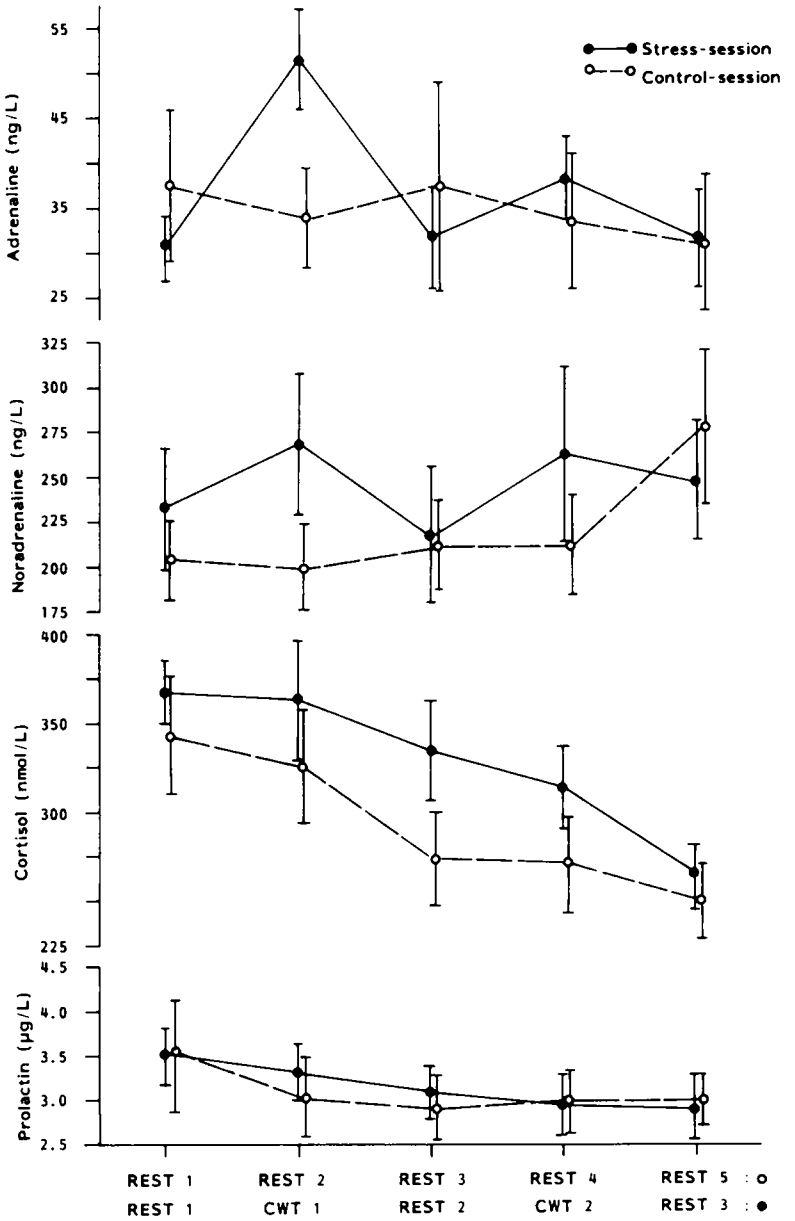

FIG. 1. Mean±SE values per rest or CWT periods of the endocrine plasma parameters during the stress- and control-session.

as for the averaged rest and CWT periods of the stress-session. Significant negative correlations were found for skin conductance level ($r=-.916$; $p=0.01$, $n=6$) and respiratory frequency ($r=-.736$; $p=0.04$) for the averaged data. The interaction effects found for SCL and respiratory frequency can thus be explained by the initial values during rest. A high prestimulus level results in a small response to stress. For heart rate and EMG activity the correlations were $-.337$ ($p=0.34$) and .004 ($p=0.99$) respectively. Therefore, the interaction could not be explained on basis of the initial values.

Reproducibility and individual differences. Response-magnitudes to the first and second CWT were compared, based on mean values per period and minute-to-minute changes within periods. The second CWT consisted of a double-conflict task, introducing visual and auditory conflict. All parameters showed reduced response-magnitudes to the second CWT (Fig. 2). With regard to the biochemical data, plasma adrenaline also showed a reduced response-magnitude during the second CWT. Plasma noradrenaline showed an increased average response-magnitude to the second CWT, which was, however, accompanied by an increased variability, due to an absence of response or negative response in three of the subjects. Double-conflict, therefore, did not appear to lead to more sympathetic activation, compared to the first CWT. As a matter of fact, the subjects reported not to be distracted by the auditory stimulation.

TABLE 2

ANOVA AND REGRESSION ANALYSES ON THE BIOCHEMICAL AND PHYSIOLOGICAL DATA

	Stress-Session ANOVA (F-values)				Control-Session Regression		
	CWT	Subject	Inter-action	Covariate Time	Mean Slope/Period	F(1)	F(2)
Plasma							
Adrenaline	26.00‡	11.12‡	0.60	1.35	−1.39	1.73	0.36
Noradre-naline	8.07†	39.13‡	2.64*	0.35	16.09	32.01‡	7.46‡
Cortisol	0.58	5.06‡	1.29	19.07‡	−24.26	53.91‡	3.99†
Prolactin	0.39	56.92‡	1.25	31.09‡	−0.10	4.20	3.31*
Physiological							
Heart rate	153.71‡	90.42‡	8.43‡	34.65‡	−1.35	78.54‡	1.85
EMG	118.78‡	22.66‡	29.36‡	10.79†	−0.15	5.62*	0.76
Finger pulse amplitude	27.66‡	10.36‡	1.09	11.77†	−0.11	62.97‡	6.22‡
SCL	33.85‡	23.87‡	4.24†	13.90†	−0.01	1.87	1.71

Stress-session: ANOVA F-values for the covariate 'time,' the main factors (CWT/rest and subjects) and the interaction effect between the two factors are indicated per biochemical and physiological parameter. Control-session: Regression analyses per biochemical and physiological parameter: F(1): F-values of the regression analyses indicating the presence of increasing or declining regression in the data; F(2): F-values indicating differences in regression between the subjects. (*$p<0.05$; †$p<0.01$; ‡$p<0.001$.)

TABLE 3

URINARY CATECHOLAMINES DURING AND AFTER THE STRESS- AND CONTROL-SESSION

	Collection Period				
Urinary excretion	9–12 hr		12–15 hr	15–18 hr	Total 9–18 hr	
Adrenaline						
stress-session: mean	6.42	*	1.98	2.92	3.92	*
s.e.	1.55		0.50	0.88	0.97	
control-session: mean	3.98		2.07	2.36	2.91	
s.e.	0.76		0.49	0.68	0.65	
Noradrenaline						
stress-session: mean	17.30		14.60	9.20	14.14	
s.e.	1.86		2.13	0.88	1.53	
control-session: mean	14.61		13.43	8.81	12.72	
s.e.	2.34		1.57	1.64	1.67	

Mean and SE values of the urinary catecholamines, expressed in metabolite/creatine × 10 (n=8) (*paired t-test: $p<0.05$).

Differences between individuals in stress response were observed for nearly all variables, as can be concluded from the interaction effects of the ANOVAs: the degree of inter-subject variability was highly significant for most variables, with the exception of adrenaline and peripheral blood flow. Noradrenaline was the only parameter which showed both positive and negative responses to the CWT.

The control-session. No significant differences were found between the first rest periods of the stress- and control-session with regard to heart rate ($t=-0.27$; $p=0.79$), EMG ($t=0.55$; $p=0.60$), finger pulse amplitude ($t=0.40$; $p=0.70$) or SCL ($t=0.36$; $p=0.74$, n=6), indicating comparability of initial baselines in both situations. The physiological data of the control-session were also analyzed for trends, attributable to habituation or circadian fluctuations. A significant decline was found for heart rate, EMG activity and pulse amplitude, and a significant difference in regression between subjects for pulse amplitude only (Table 2). With regard to heart rate, EMG, and pulse amplitude, these findings are in agreement with the ANOVA results of the stress-session: the same trend is present during both sessions. SCL showed no significant regression during the control-session,

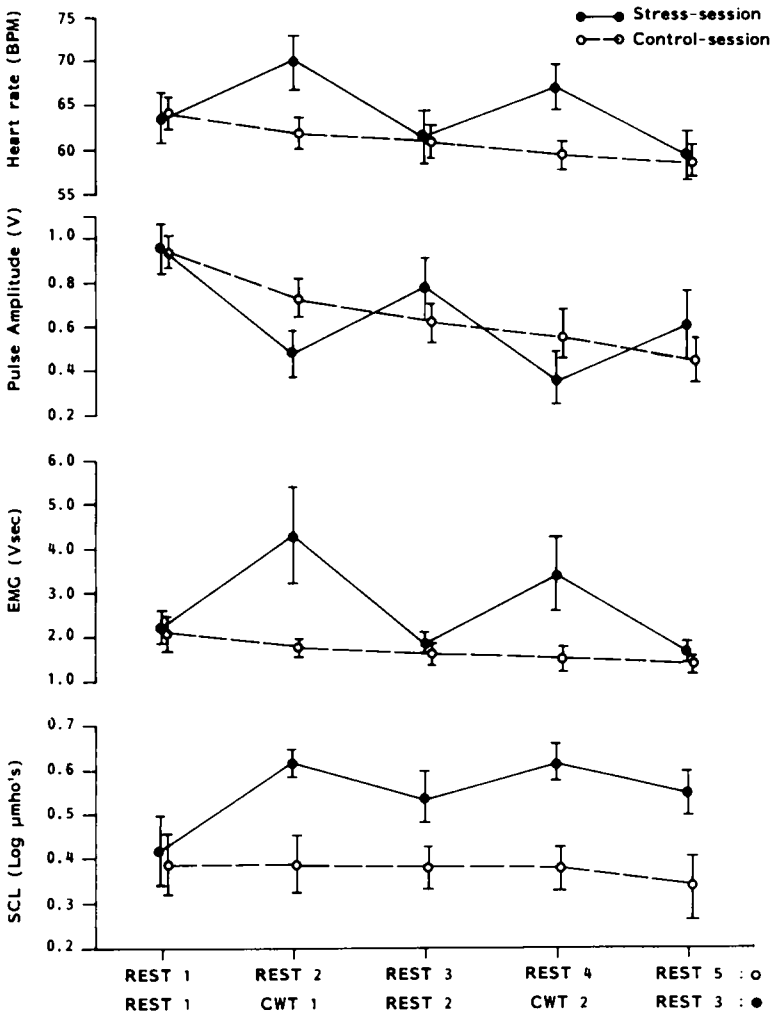

FIG. 2. Mean±SE values per rest or CWT periods of the physiological parameters during the stress- and control-session.

while for the stress-session the covariate 'time' was significant. Recovery to baseline values after the CWT was apparently much slower for SCL than for the other parameters.

DISCUSSION

The aim of the present study was to test whether the CWT induces simultaneously four types of reactions required for a suitable stress test.

1) With regard to psychological effects, significant differences in subjective feelings of anxiety and tension were observed between the stress- and control-session. Increased feelings of discomfort and distress have been observed previously in response to the CWT, measured by ratio estimations comparing situational aspects with a 'standard' situation, or analogue ratings on alertness, distress and irritation (6–8). A sharp decrease in subjective arousal was observed when the test was presented five times with intervals of one week, indicating that increased subjective arousal was primarily observed during the first CWT session (6).

2) For all physiological parameters significant changes were observed. Although we observed a clear increase in heart rate during the CWT (6–7 beats/min), a much larger response was reported before (12) (28 beats/min). One explanation for this difference might be the fact that we used a relatively homogeneous group of young subjects, all of about the same age, most of whom reported to be trained sportsmen. The response to the double-conflict CWT did not show the increased activation as observed by Frankenhaeuser *et al.* (8). However, they used a between-group design, while we used a within-group design.

3) EMG activity in the extensor muscles of the forearm was increased during the CWT. This has not been reported before, but is an essential requirement for a suitable stress-test.

4) The CWT induced a significant increase in adrenaline concentration and variable results with regard to noradrenaline concentration for both plasma and urinary data. This is in agreement with previous findings (1,12). A significant increase in urinary cortisol secretion during the Stroop CWT has been reported in a group of male engineering students (5), while no significant responses of plasma cortisol and prolactin secretion were found in a group of women with

oligomenorrhea (15). These last findings were confirmed by our study with healthy male volunteers. The CWT has, apparently, specific effects on the sympathetic-adreno-medullary system and not on the pituitary-adreno-cortical system (2).

The results, therefore, show that the CWT induces all four types of responses that are necessary for a test suitable to study stress-responses. However, it is not clear at this moment by what mechanism these responses are evoked. It could be argued that the CWT mainly increases arousal. Increases, specifically in adrenaline, in reaction to the CWT [(14); this study] have been interpreted as indicating increased arousal. On the other hand, arousal during an ongoing task most often lasts for 10 minutes or less (22) and increased adrenaline after 20 minutes of CWT could be interpreted as an increase in effort, especially since effort may be closely connected to cognitive conflict presented with the CWT. Whether the CWT induces stress in the sense of a seriously overloaded effort mechanism (22) remains to be established. One way of exploring such mechanisms would be by administering anxiolytic drugs such as benzodiazepines and evaluating their effect on all reactions to the CWT. In the design of such an experiment habituation effects, response-magnitude in relation to initial values and spontaneous changes of basal values over time, as reported here, must be accounted for.

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