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Psychosocial Stress and HPA Functioning: No Evidence for a Reduced Resilience in Healthy Elderly Men

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In order to investigate if HPA functioning is altered with age, the present study was conducted. Fifteen healthy elderly men (60–76 years; mean age 66.5 ± 1.48 yrs.) and 12 younger adults (20–29 years; mean age 25.6 ± 0.77 yrs.) collected salivary free cortisol profiles after awakening for basal HPA activity. Then, all subjects were exposed to the "Trier Social Stress Test" (TSST). This psychosocial stress protocol consists of a free speech and a mental arithmetic task of 13 minutes duration performed in front of an audience. Beside the assessment of endocrine and cardiovascular responses to the stressful task ratings of depression, mood and perceived stressfulness were obtained.

Results show that younger and elderly men had similar morning cortisol profiles after awakening with both groups showing the expected rise after awakening (P=0.004). The TSST induced significant increases in ACTH, total plasma cortisol, saliva free cortisol, and heart rates (all P<0.0001). Regardless of age, both age groups showed comparable endocrine response patterns when confronted with the stressor. However, cardiovascular responses were significantly higher in younger men compared to elderly men (P=0.03). Catecholamine data revealed significant norepinephrine and epinephrine increases due to the stressor (both P<0.0001) with a trend toward elevated norepinephrine levels in elderly men (P=0.058).

In sum, the investigated basal and response parameters of HPA functioning neither support the idea of a reduced resilience in healthy aged humans nor do they appear to strengthen assumptions derived from the so called "glucocorticoid cascade hypothesis".

Keywords: HPA-axis, psychosocial stress, aging, saliva, catecholamines

INTRODUCTION

Aging is a process often associated with progressive disability and morbidity. In the last decades, several animal and human studies have investigated if aging affects the functioning of the hypothalamus-pituitary-adrenal axis (HPA) causing, for example, a reduced resilience (Seeman & Robbins, 1994) or less flexible functioning of this hormonal system. The HPA axis is among the most important endocrine response systems that helps the organism to maintain basal and stress-related homeostasis of the central

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nervous system as well as cardiovascular, metabolic, and immune functions (Chrousos & Gold, 1998; Chrousos, 1997). Studies of age-related alterations seem to be warranted because disregulations of the HPA axis are apparently associated with manifestations of several behavioral, endocrine/metabolic and immune disorders (Schnyder, 1960; Adams & Victor, 1989; Mason, 1991; Weiner, 1991; Buske-Kirschbaum et al., 1997; McEwen, 1998) as well as psychosomatic and psychiatric disorders (Gold et al., 1984; Sachar et al., 1970).

The "glucocorticoid cascade hypothesis", initially formulated in 1986 (Sapolsky, Krey & McEwen, 1986) attributes age-related changes in HPA functioning to structural alterations in the hippocampus. Following this intriguing model, older age or life long exposure to stress result in a reduced negative feedback function of the hippocampus leading to elevated basal hormone levels as well as increased HPA reactivity after stress. This may initiate a feedforward process with downregulation of glucocorticoid receptors in the hippocampus and further impaired hippocampus-mediated glucocorticoid feedback. These processes could finally lead to a vicious circle of a permanently deteriorating functioning of the HPA axis with age.

In contrast to this model, derived from animal studies, evidence from human studies suggest that there is no or only a moderate impact of age on basal HPA parameters (Zimmerman & Coryell, 1987; Seeman & Robbins, 1994; Van Cauter, Kupfer & Leproult, 1996; Kudielka et al., 1999). Investigations of HPA reactivity in humans using psychosocial stress protocols in order to stimulate the suprapituitary part of the axis are sparse. Although Gotthardt et al. (1995) reported significant age-related differences in HPA responses using a "signal detection task", the employed computer-based stress protocol may have been inappropriate for studying age effects in different age groups. Other studies employing standardized psychosocial stress paradigms either failed to evoke a solid endocrine stress response (Nicolson et al., 1997; Lindheim et al., 1992) or did not include a young comparison group (Seeman, Singer & Charpentier, 1995; Lupien et al., 1997).

Several animal (Kitay, 1961, 1963; Carey et al., 1995; Handa et al., 1994) as well as human studies (Kirschbaum et al., 1996; Burleson et al., 1998; Kudielka et al., 1998) showed that sex steroids exert a significant impact on HPA functioning. As aging is characterized by significantly decreasing gonadal steroid concentrations, measurement of absolute sex steroid levels are required. Beside the impact of sex steroids on HPA functioning, psychological parameters could underlie possible age-related differences in HPA reactivity.

The question if there is an age-related decline in the resilience of human HPA functioning (Seeman & Robbins, 1994) still remains unanswered. Therefore, the aim of the present study was to investigate HPA *activity* under basal conditions as well as HPA *reactivity* after confrontation with a psychosocial stress test in healthy elderly men and young counterparts.

METHODS

Subjects

A total of 27 healthy male volunteers between 20 and 76 years of age participated in this study. Younger subjects were recruited at the University of Trier, older subjects were recruited via newspaper announcements in the local newspaper. The final sample was composed of 15 elderly (60–76 yrs.; mean 66.5 ± 1.48) and 12 younger men (20–29 yrs.; mean 25.6 ± 0.77). Before entering the study all subjects had to provide written consent and underwent a comprehensive medical examination for past and current health problems. Subjects with psychiatric, endocrine cardiovascular, other chronic diseases or those medicated with psychoactive drugs, sex-steroids or glucocorticoids were excluded. Two elderly men were not admitted to the study due to health problems. No subject had to be excluded due to depression. SDS scores (see below) revealed that only one participant reported minor depressive symptoms which were not clinically relevant. Therefore, this subject was not excluded. Depression scores did not differ signifi-

cantly between age groups ($F_{1,25}$=1.01 P=0.33). The mean body mass index (BMI: kg/m^2) was 25.4 (± 0.66; SEM) for the older and 22.9 (± 0.59; SEM) for the younger participants. Comparing both groups, aged subjects had a significantly higher BMI than their young counterparts ($F_{1,25}$=7.54 P<0.01). Three elderly men, who reported to smoke less than 10 cigarettes a day were not excluded from participation. Table I provides the number of subjects, mean age, range of age, SDS-scores, BMI, sex steroid and corticosteroid binding globulin (CBG) levels for both age groups.

Experimental procedure

Laboratory sessions started between 3 pm and 4 pm. The first time subjects reported to the laboratory, they underwent a medical examination and completed a mood questionnaire (MDBF; Steyer et al., 1994) as well as a depression questionnaire (SDS; Zung 1965, 1985) (see below). Furthermore, each subject received 5 devices for saliva collection (Salivettes) with instructions for collection of a morning cortisol profile after awakening. For the assessment of this basal HPA parameter, saliva had to be sampled immediately after waking up, 15, 30, 45, and 60 minutes thereafter (Pruessner et al., 1997).

On the second appointment all subjects were exposed to the psychosocial stress test (TSST). The experimental session started with heart rate recording and insertion of an indwelling catheter. A mood questionnaire (MDBF; see below) was filled out before and after confrontation with the stressor (TSST). After a rest period of 45 min the first saliva and blood samples were collected. Thereafter subjects were confronted with the "TSST" (Kirschbaum et al., 1993) which consists of a free speech and mental arithmetic task of 13-minutes duration performed in front of an audience. Recently, this stress protocol has been found to induce significant endocrine and cardiovascular responses in elderly subjects, too (Kudielka et al., 1998; Kudielka et al., 1999). Additional blood and saliva samples were obtained 1, 10, 20, 30, 45, 60, and 90 minutes after cessation of stress. The perceived stressfulness was assessed after stress exposure by visual analog scales (VAS). The study protocol was approved by the local ethics committee of the University of Trier.

Psychological assessment: depression, mood changes and perceived stress

All subjects filled out four different psychological questionnaires in order to investigate the extent of depression, mood changes and perceived stressfulness of the TSST. The German Self Rating Depression Scale (SDS) (Zung, 1965, 1986) was applied to measure the extend of depression. Scores over 41 indicate slightly depressed mood, scores over 47 point at clear-cut depressive symptoms. Momentary mood was assessed using the MDBF ("Mehrdimensionaler Befindlichkeitsfragebogen" (Steyer et al., 1994). This questionnaire measures "elevated vs. depressed mood", "wakefulness vs. sleepiness", and "calmness vs. restlessness" on five-point rating scales ranging from 1= "not at all" to 5="very much". This questionnaire was submitted at the first appointment as well as at the second laboratory appointment before and after stress exposition. 14 visual analog scales were employed for participants' ratings of the stressfulness of the TSST. After cessation of the psychosocial stress task, subjects rated 1. how strenuous, 2. how difficult the free speech, 3. how difficult the mental arithmetic task, 4. how burdening, and 5. how challenging the situation was, 6. how much they were personally involved, 7. how novel, 8. how controllable, and 9. how threatening the situation was, 10. how much stress they experienced due to failure, 11. how much stress they experienced due to time pressure, 12. how much they typically feel stressed in examination-tasks, 13. how much they were contented with their performance in the speech task and 14. how much they were contented with their performance in the mental arithmetic task.

Blood, saliva sampling and biochemical analyses

Basal blood samples were used to measure total testosterone, free testosterone, estradiol (all by RIA, Biermann, Bad Nauheim, Germany), and CBG (by RIA, IBL, Hamburg, Germany). CBG was measured due to its binding capability for cortisol. Therefore, CBG influences the proportion of total and free cortisol levels.

TABLE I Number of experimental groups, mean age, range of age, body-mass-index (BMI), sex steroid levels and CBG levels in younger men and elderly men

	younger men	*elderly men*	*P*
N	12	15	
Age (yrs.)	25.6 ± 0.77	66.5 ± 1.48	
Age – range (yrs.)	20–29	60–76	
BMI	22.9 ± 0.59	25.4 ± 0.65	P = 0.01
SDS	30.2 ± 1.30	32.2 ± 1.49	P = 0.33
Total testosterone (ng/ml): 1st appointment	4.98 ± 0.31	3.33 ± 0.23	P = 0.0002[a]
Total testosterone (ng/ml): 2nd appointment	4.58 ± 0.38	2.99 ± 0.18	P = 0.03[b]
			P = 0.82[c]
Free testosterone (pg/ml): 1st appointment	21.2 ± 1.53	12.6 ± 1.04	P < 0.0001[a]
Free testosterone (pg/ml): 2nd appointment	17.5 ± 1.89	10.9 ± 0.76	P < 0.0001[b]
			P = 0.12[c]
Estradiol (ng/ml): 1st appointment	47.2 ± 3.05	25.8 ± 2.68	P < 0.0001[a]
Estradiol (ng/ml): 2nd appointment	51.8 ± 4.00	26.4 ± 2.63	P = 0.32[b]
			P = 0.34[c]
CBG (µg/ml): 1st appointment	29.3 ± 1.47	36.9 ± 2.55	P = 0.04[a]
CBG (µg/ml): 2nd appointment	30.2 ± 1.57	33.6 ± 1.70	P = 0.43[b]
			P = 0.18[c]

a. Main effect group.
b. Main effect time.
c. Interaction effect group by time.

Further blood and saliva samples were collected directly before starting the psychosocial stress experiment as well as 1, 10, 20, 30, 45, 60, and 90 minutes after cessation of the task. Saliva was collected by the participants, using Salivette collection devices (Sarstedt, Rommelsdorf, Germany). Saliva free cortisol concentrations were determined using a time-resolved immunoassay with fluorometric detection as described in detail by Dressendörfer and co-workers. (Dressendörfer et al., 1992). Total plasma cortisol was measured with a RIA (IBL, Hamburg, Germany). ACTH was analyzed with a two-site chemiluminescence assay (Nichols Institute, Bad Nauheim, Germany). Total plasma cortisol is a marker of cortisol secretion whereas saliva cortisol reflects the biological active, free (biologically active) fraction of cortisol. Norepinephrine and epinephrine were assayed by a high performance liquid chromatography, as previously described (Smedes et al., 1982). Inter- and intraassay coefficients of variance were below 12% for all analytes. Total plasma and free salivary cortisol were analyzed in all 8 samples. ACTH was assayed before as well as 1, 10, 20, and 90 minutes after the task, whereas catecholamines were analyzed directly before the test as well as 1, 10, 20 and 30 minutes after test cessation.

Heart rate

A wireless Polar Sport Tester (Sport Tester Profi, Polar Instruments, Gross-Gerau, Germany) was used to record continuous heart rate profiles of 1-minutes intervals with EKC precision. The profiles were computed from 10 minutes before stress exposition until 10 minutes after cessation of stress. Heart rate was measured as a continuous estimate of sympathetic (re)activity.

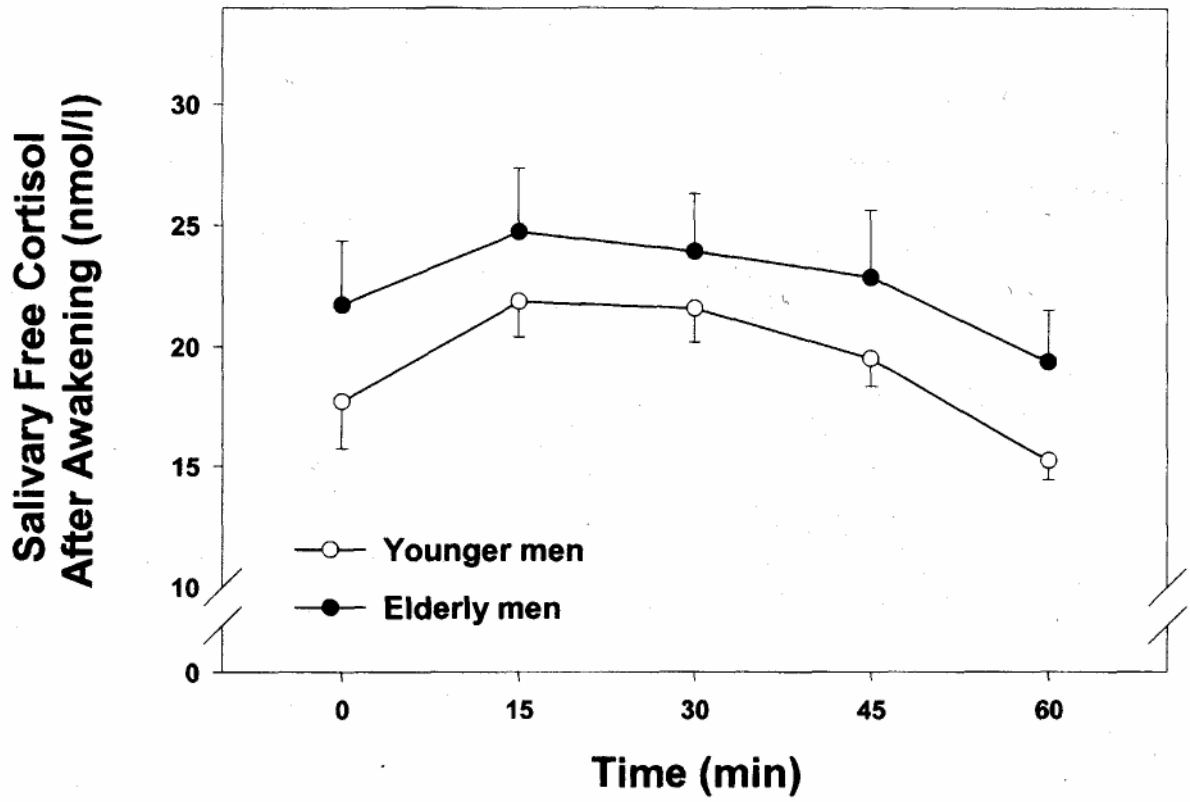

FIGURE 1 Salivary free cortisol profiles after awakening in younger and older men (mean ± SEM)

Statistical analyses

Endocrine and heart rate responses to the stressor were computed using ANOVAs for repeated measures. For all reported results Greenhouse-Geisser correction was applied where appropriate, resulting in corrected degrees of freedom and adjusted p-levels. The significance level was $\alpha = 0.05$. Test power, which reflects the probability of finding an effect in the experiment when the effect actually exists in the "real world", was analyzed by a computer program called GPower (Faul & Erdfelder, 1992). For multiple comparisons the nominal α-level was adjusted by Bonferroni correction. The scale "stressfulness" based on items of the VAS was created via factor analyses (principal component, Varimax oblique) and reliability analyses. Correlation analyses were computed using Pearson product-moment correlations. All results shown are the mean ± standard error of mean (SEM).

RESULTS

Analyses of the basal HPA parameter "morning cortisol after awakening" did not reveal age-related differences (main effect group: $F(1,25)=1.56$ n.s; interaction group by time: $F_{4,100}=0.18$ n.s.) with both age groups showing the typical rise with a peak 15 min after awakening (time effect: $F_{1.67,41.9}=7.51$ $P=0.003$).

ANOVAs revealed that the TSST induced significant increases in ACTH (time effect: $F_{1.28,39.5}=31.56$

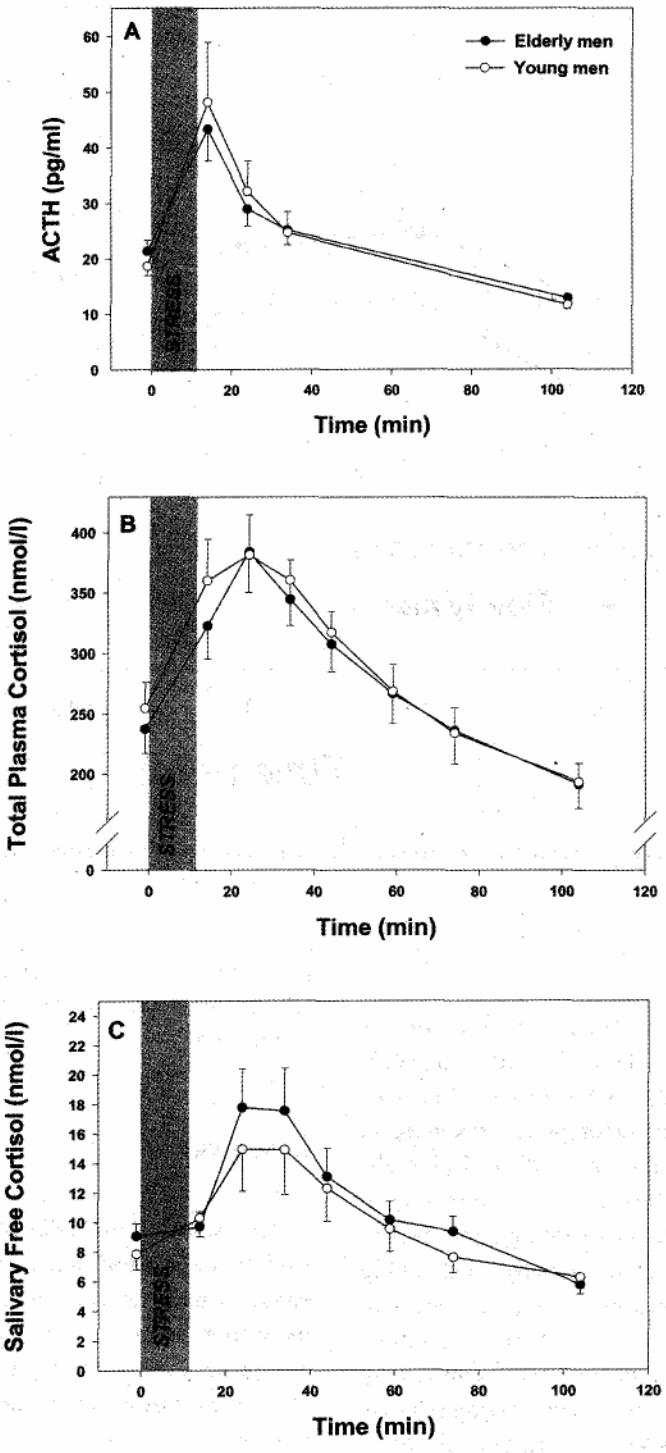

FIGURE 2 Mean ACTH (A), total plasma cortisol (B), and free salivary cortisol (C) levels (± SEM) in younger and older men before and after stress (TSST). The shaded areas indicate the period of stress exposure

P<0.0001), total plasma cortisol (time effect: $F_{3.03,94.1}$=87.88 P<0.0001) and salivary free cortisol (time effect: $F_{1.38,40.0}$=28.38 P<0.0001) in both groups with no differences between younger and older participants (all n.s.). The same result was obtained for the increase (increase: all F<1 all n.s.). Hormonal increases were about 1.5 to 2.5-fold from baseline values. ACTH peaked 1 minute after cessation of stress, whereas total plasma cortisol and free salivary cortisol peaked 10 minutes after stress provocation.

A different picture emerged for heart rate responses. While heart rates increased significantly in both groups due to the psychosocial stressor (time effect: $F_{7.80,226.2}$=29.76 P<0.0001), aged subjects showed an attenuated profile compared to their younger counterparts (interaction effect group by time: $F_{7.80,226.2}$=3.18 P=0.0021). Whereas mean heart rate increased by 23 beats/min for younger participants, a mean increase of 14 beats/min was observed for aged subjects. Norepinephrine and epinephrine both showed significant stress responses with peak levels one minute after cessation of the TSST (time effect: norepinephrine $F_{3.37,84.23}$=33.49 P<0.0001; epinephrine $F_{1.81,43.46}$=21.08 P<0.0001). There was a slight trend toward higher norepinephrine levels in elderly compared to younger participants but no such effect was observed for epinephrine (group effect: norepinephrine $F_{1.25}$=3,93 P=0.058; epinephrine $F_{4.96}$=0.92 n.s.). Group by time interactions showed no significance (interaction effect group by time: norepinephrine $F_{4,100}$=0.55 n.s.; epinephrine $F_{4.96}$=0.92 n.s.). In the present study, the test power was 83% for main effects and 99% for interactions given a big effect of interest. Correlation analyses between basal HPA activity (increase in morning cortisol of awakening) and endocrine HPA reactivity (increase) showed no significant associations (all n.s.).

Mood, wakefulness, and calmness measured by the three dimensional questionnaire MDBF changed similarly in younger and older participants (mood: $F_{1.25}$=0.14 n.s., wakefulness: F(1,25)=0.22 n.s., calmness: F(1,25)=0.36 n.s.). Correlation analyses showed significant correlations for mood change and ACTH increase (r=0.59 P=0.016), indicating that more decrease in mood was associated with higher ACTH responses.

Subjective ratings of the stressfulness of the TSST, measured by 14 visual analog scales, showed that the stress task was relatively novel for elderly men compared to younger men (mean score of older men 53.4, mean score of younger men 23.5; F(1,24)=6.2 P=0.02). Taking into account an adjusted α=0.004 for 14 single comparisons, this result can only be regarded as a trend. A factor analysis clustered 11 VAS (No: 1, 2, 4, 7, 8, 9, 10, 11, 12, 13, 14) to a factor which was termed "perceived stressfulness of the TSST" with a reliability of α=0.92 (Cronbachs α). "Perceived stressfulness of the TSST" did not reveal any significant age-related differences in the stressfulness of the psychosocial stress task.

DISCUSSION

In this study basal as well as stimulated HPA parameters were investigated in order to illuminate the impact of age on HPA activity and reactivity. The present results do not support the idea of a reduced resilience of HPA functioning (Seeman & Robbins, 1994) and disagree with assumptions derived from the "glucocorticoid cascade model" which would predict elevated basal as well as stimulated hormone concentrations in aged subjects compared to a young control group. Results show that basal HPA *activity* measured by morning cortisol profiles after awakening did not differ between age groups as well as confrontation with the standardized psychosocial stress protocol (TSST) for the assessment of HPA *reactivity* evoked similar endocrine ACTH, total plasma and free salivary cortisol patterns in younger and older men. The analyses of the test power showed that the probability of revealing age-effects in HPA stress responses were 83% for main effects and 99% for interactions given a big effect of interest. The test power analyses underline that age effects could have been discovered with the present study design. It is possible that small or only moderate differences between age groups remained undiscovered in this

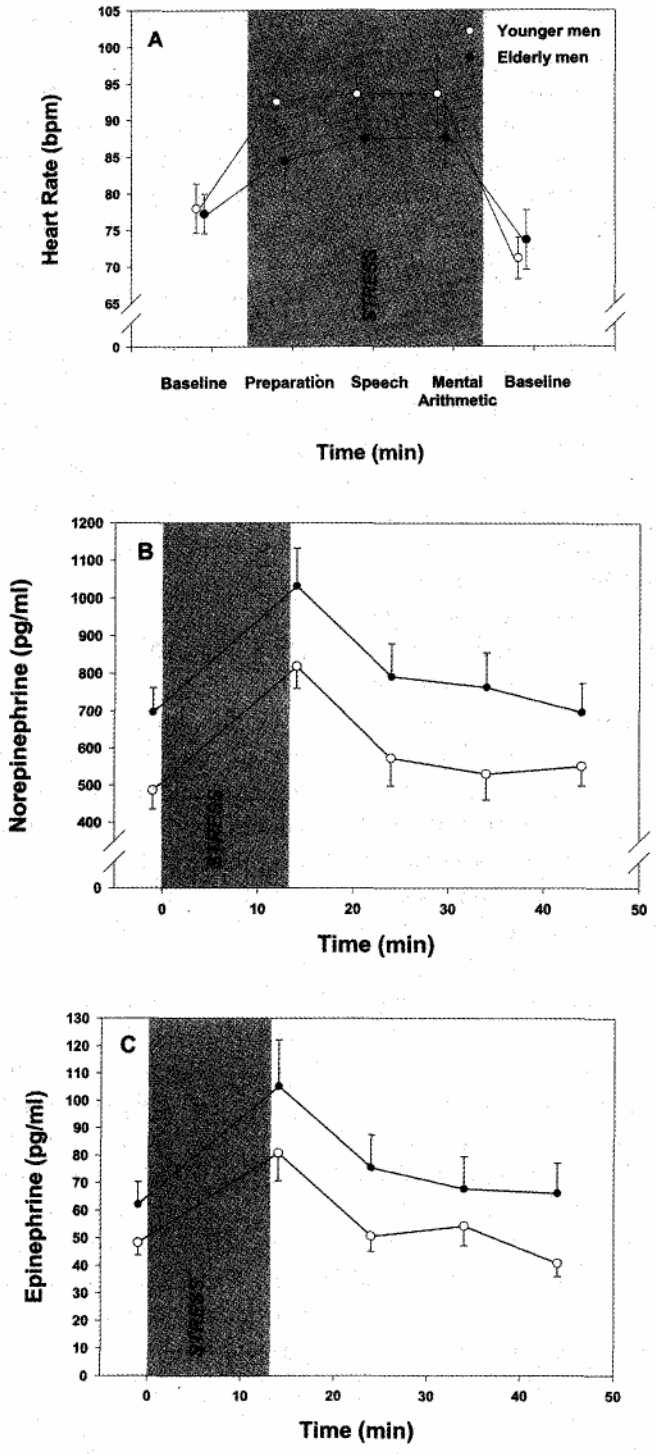

FIGURE 3 Mean heart rate (A), norepinephrine (B), and epinephrine (C) levels (± SEM) in younger and older men before and after stress (TSST). The shaded areas indicate the period of stress exposure

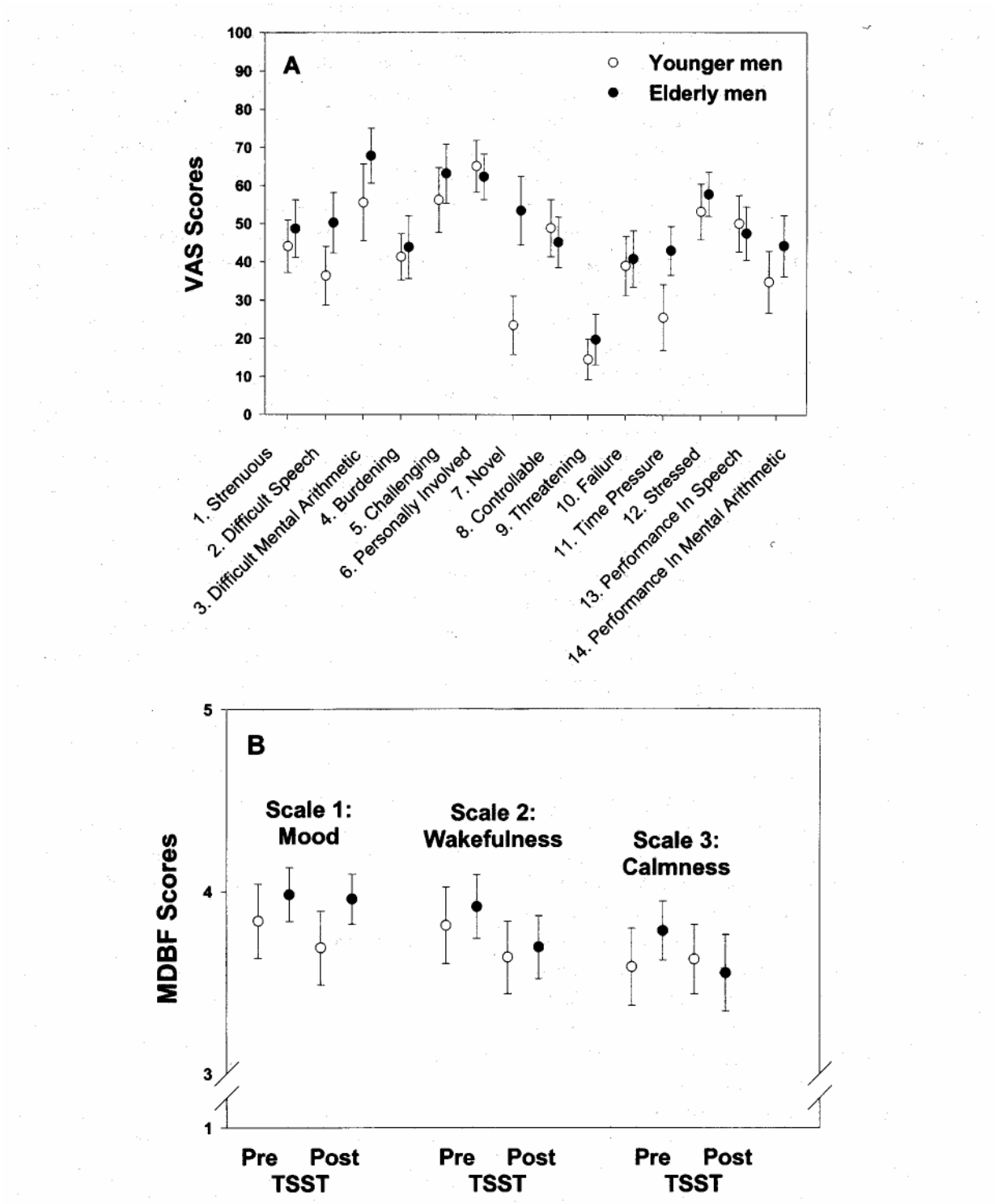

FIGURE 4 Perceived stressfulness of the TSST measured with 14 visual analog scales (A) as well as mood, wakefulness, and calmness before and after the TSST measured with the MDBF (B) in younger and elderly men (± SEM)

study. If such smaller effects are to be discovered much larger sample sizes are required. However, it remains to be discussed whether effects determining, e.g., only 1–3% of the total variance would justify a dramatic increase in financial and personnel efforts. In contrast to another study published earlier this year (Kirschbaum et al., 1999), the relatively small difference in CBG levels in the present study (although statistically significant) did not result in a blunted free cortisol response in the group of elderly men. Neither an overall heightened baseline level in these subjects was observed. Similar to endocrine data, subjective ratings of the stressfulness of the TSST were comparable in both generations, suggesting that there were no age-related differences at a subjective level of processing. Also, changes in mood, wakefulness and calmness due to stress provocation via TSST did not result in age-related differences.

Nevertheless, alternative explanations for the observed endocrine data have to be taken in consideration. First, possibly existing age-related changes could be compensated or masked by other HPA modulators, e.g. vasopressin, which themselves could be altered with progressive age (Lucassen et al., 1997; Calza et al., 1997; Mooradian et al., 1988). Furthermore, the hypothesis of "corticosteroid receptor balance" formulated by De Kloet and coworkers (1991, 1991, 1998) postulates that a new compensatory balance of glucocorticoid receptors type I and type II (glucocorticoid and mineralocorticoid receptors) arises with age which establish a new homeostatic control. Interpreting the present data, it must also be taken into consideration that only 60 to 76 year old subjects with a relatively good health status entered the study in order to avoid confoundation with specific diseases or medication typically used by individuals of advanced age. In line with this reasoning, Heuser and coworkers (Heuser et al., 1994) point at the necessity of studies with truly old participants (>80 years) to elucidate the question of age-related HPA alterations.

In contrast to HPA-axis reactivity, heart rate responses to the TSST were significantly higher in younger men compared to the elderlies. This observation is in accordance with many other studies investigating heart rate responses to physical as well as psychological stressors in younger and elderly men (Furchtgott & Busemeyer, 1979; Garwood et al., 1982; Gintner et al., 1986). Underlying mechanisms probably include alterations in the sensitivity of baroreceptors, neurotransmitter transmission, and adrenergic receptors (Pfeifer et al., 1983; Lakatta, 1990). More- over, there was a trend towards elevated norepinephrine concentrations in aged men compared to younger men which is in line with animal data showing higher plasma norepinephrine concentrations in aged compared to younger adult rats due to both a decrease in norepinephrine clearance and an increase in norepinephrine spillover (McCarty et al., 1997). Human data point at higher basal norepinephrine concentrations as well as higher stress-related norepinephrine increases in elderly men compared to younger men (Hoeldtke & Cilmi, 1985; Taylor et al., 1992; Barnes et al., 1982; Esler et al., 1995). Esler et al. (1995) showed that elevated plasma-norepinephrine levels in men of advanced age after stress exposition could be attributed to an increased norepinephrine secretion with a simultaneous decreased metabolic clearance. However, in the present study no age difference could be observed in norepinephrine reactivity.

In sum, in this study younger and elderly men showed similar HPA patterns under basal as well as stimulated conditions. Although based on a rather small study sample, the present results do not appear to support the idea of a reduced resilience in HPA activity or HPA reactivity in healthy elderly men.

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