

Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

## Biological Psychology

journal homepage: [www.elsevier.com/locate/biopsycho](http://www.elsevier.com/locate/biopsycho)

## Salivary alpha-amylase response to acute psychosocial stress: The impact of age

Mercedes Almela<sup>a,\*</sup>, Vanesa Hidalgo<sup>a</sup>, Carolina Villada<sup>a</sup>, Leander van der Meij<sup>a</sup>,  
Laura Espín<sup>b</sup>, Jesús Gómez-Amor<sup>b</sup>, Alicia Salvador<sup>a</sup><sup>a</sup> Laboratory of Social Neuroscience, University of Valencia, 46010 Valencia, Spain<sup>b</sup> Department of Human Anatomy and Psychobiology, University of Murcia, 30100 Murcia, Spain

## ARTICLE INFO

## Article history:

Received 29 December 2010

Accepted 25 May 2011

Available online 12 June 2011

## Keywords:

Salivary alpha-amylase

Cortisol

HPA axis

Middle-aged persons

Psychosocial stress

Age differences

## ABSTRACT

The impact of stress on health varies across the different stages of human life. Aging is associated with psychobiological changes that could limit our ability to cope with stressors. Therefore, it is crucial to clarify the physiological mechanisms that underlie the stress response and the changes that occur in them as we age. Our aim was to investigate age differences in the salivary alpha amylase (sAA) response to stress, and its relationship with other typical stress biomarkers such as cortisol and heart rate (HR). Sixty-two participants divided into two age groups (younger group:  $N = 31$ , age range: 18–35 years; older group:  $N = 31$ , age range: 54–71 years) were exposed to the Trier Social Stress Test and a control condition in a crossover design. No age differences were found in the sAA or HR responses to stress. However, the sAA global output was higher in older than younger adults. Additionally, in the stress condition, the total amount of cortisol released was positively related to the total sAA released, while the HR increase was positively related to the sAA increase. Our results do not support the existence of an attenuated autonomic nervous system response to stress in older adults, but rather a heightened sympathetic tone. Furthermore, we found further evidence of the coordination between the hypothalamus–pituitary–adrenal system and the autonomic nervous system in their response to acute psychosocial stress.

© 2011 Elsevier B.V. All rights reserved.

## 1. Introduction

Lifetime exposure to stress can have important consequences for health. Stress has been related to a large number of pathologies that have a higher incidence in old age, such as cardiovascular disease, atherosclerosis, cancer or Type 2 diabetes (Chrousos and Kino, 2007; Steptoe, 1991). Aging is associated with several psychobiological changes, such as increased vulnerability to oxidative stress, imbalances in central neurotransmitter pathways, and changes in emotional regulation (Alameida et al., 2011; Ferrari and Magri, 2008; Salmon et al., 2010), which could limit our ability to cope with stressors (Pardon, 2007). Therefore, it is important to clarify the physiological mechanisms that underlie the stress response, as well as the changes that occur in them as we age.

Two main body systems are involved in the stress response, the autonomic nervous system (ANS) and the hypothalamus–pituitary–adrenal axis (HPA-axis). Recently, salivary alpha amylase (sAA), an oral cavity enzyme, has been identified as a possible biomarker of ANS reactivity to stress (for reviews see: Nater and

Rohleder, 2009; Rohleder and Nater, 2009). This enzyme increases rapidly in response to physiological and psychosocial stress conditions, such as exercise and written examinations (Chatterton et al., 1996), the cold pressor stress test (van Stegeren et al., 2008), and the Trier Social Stress Test (Nater et al., 2005, 2006; Rohleder et al., 2004). Several studies have been performed to profile the sAA response to stress mainly in children (Granger et al., 2006; Räikkönen et al., 2010; Spinrad et al., 2009), adolescents (Gordis et al., 2006; Sumter et al., 2010; Susman et al., 2010) and young adults (Nater et al., 2005, 2006; Rohleder et al., 2006; Schoofs et al., 2008). However, data available on older people are very sparse and the results are mixed. Previous studies have suggested that old age has no effect on basal sAA levels (Aguirre et al., 1987; Pajukoski et al., 1997; Salvolini et al., 1999), although a more recent study has shown that older adults have higher overall sAA output throughout the day (Strahler et al., 2010a). To our knowledge, only one published study investigated the effect of old age on the acute sAA response to stress, finding an attenuated response in older adults (59–61 years) compared to young adults (20–31 years) (Strahler et al., 2010b).

More studies have been performed to examine the impact of aging on other ANS biomarker responses to stress, such as heart rate, heart rate variability or plasma epinephrine and norepinephrine. According to an exhaustive review by Seals and Dineno (2004), it appears that the primary effect of aging on

\* Corresponding author at: Department of Psychobiology, University of Valencia, Blasco Ibañez 21, 46010 Valencia, Spain. Tel.: +34 651 651 380; fax: +34 963 864 668.

E-mail address: [Mercedes.Almela@uv.es](mailto:Mercedes.Almela@uv.es) (M. Almela).

the human ANS is an elevation in the tonic sympathetic activity. However, the influence of aging on the ANS response to stress remains controversial. For example, several studies have reported no changes (Esler et al., 1995; Wood et al., 2002), a decreased response (Kudielka et al., 2004a; Strahler et al., 2010b), or even an enhanced response to stress with older age (Pascualy et al., 1999; Uchino et al., 1999).

In contrast to sAA, the impact of aging on the end product of HPA-axis activation, cortisol, has been investigated more extensively (for reviews and a meta-analysis see: Kudielka et al., 2009; Otte et al., 2005; Seeman and Robbins, 1994). Studies using pharmacological stimulation of the HPA-axis have consistently shown that older adults have an elevated HPA-axis response compared to young adults (e.g. Born et al., 1995; Heuser et al., 1994; Kudielka et al., 1999; Luisi et al., 1998). Nevertheless, results are mixed when studying the effects of aging on the cortisol response to different kinds of stressors (Kudielka et al., 2009). While several studies did not find any effect of aging on the cortisol response to psychosocial stressors (Kudielka et al., 1999, 2000; Nicolson et al., 1997; Rohleder et al., 2002), others found a higher cortisol response with increasing age (Gotthardt et al., 1995; Kudielka et al., 2004b; Seeman et al., 2001; Strahler et al., 2010b; Traustadóttir et al., 2005).

In the current study, we subjected a group of young and older participants to both a psychosocial stressor (TSST, Kirschbaum et al., 1993) and a control situation in a crossover design. Although no sex differences have been found in basal sAA levels (Nater et al., 2007; Rantonen and Meurman, 2000), or in the acute sAA response to stressors (Kivlighan and Granger, 2006; Takai et al., 2007), the HPA-axis shows sexual dimorphism. In fact, the cortisol response to stress is up to twice as high in men as it is in women, regardless of age (Kudielka et al., 2009). Furthermore, this response is dependent on the phase of women's menstrual cycle (Kirschbaum et al., 1999). For these reasons, the current study included men and women in equal numbers in each age group. Additionally, as it has been shown that taking oral contraceptives does not alter basal sAA levels or sAA responses to stress (Laine et al., 1991; Schoofs et al., 2008), and in order to avoid the effect of menstrual cycle phase on cortisol concentrations, we decided to select only young women taking oral contraceptives. Before, during and after the stress task, we measured cortisol and sAA concentrations and, as a complementary measure of the ANS, heart rate (HR). Following the only study that has assessed the effect of aging on the sAA response to stress (Strahler et al., 2010b), we expected attenuated sAA and HR responses to stress in the older group. Furthermore, due to the mixed results regarding the effect of older age on basal sAA levels and cortisol reactivity to stress, we investigated whether the sAA overall output and the stress-induced cortisol response were different between age groups.

## 2. Methods

### 2.1. Participants

The sample was composed of sixty-two participants divided into two age groups: older adults ( $N=31$ ; 16 men and 15 women; age range: 54–71 years) and young adults ( $N=31$ ; 16 men and 15 women; age range: 18–35 years). Within both

age groups, there were no sex differences in age, body mass index (BMI), subjective socioeconomic status (subjective SES scale: Adler et al., 2000) or education level (for all  $p > 0.11$ ) (see Table 1).

Most of the young participants (90%) were university students from a wide range of college studies, such as Psychology, Medicine, History, and unemployed (90%). Most of the older participants were retired (90%) and belonged to a study program at the University of Valencia for people over 50 years of age (84%). For subject recruitment, announcements were posted and informative talks were held in the various departments of the University campus. Volunteers were interviewed by trained psychologists and completed an extensive questionnaire to check whether they met the study prerequisites. Ninety-two volunteers were excluded from participation. The criteria for exclusion were: alcohol or other drug abuse, dental, visual or hearing problems, presence of cardiovascular, endocrine, neurological or psychiatric disease, and the presence of a stressful life event during the last year. Participants were excluded if they were using any medication directly related to emotional or cognitive function, or one that was able to influence hormonal and sAA levels, such as glucocorticoids,  $\beta$ -blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies, and psychotropic substances. Vitamins, sporadic use of painkillers, and natural therapies were allowed. All the older women were postmenopausal, having had their last menstrual period at least four years before, and none of them were receiving estrogen replacement therapy. All the young women were taking oral contraceptives (monophasic formulas). The use of contraceptives is widespread in Western society; therefore, women using this medication constitute an interesting research group in itself. None of the participants were habitual smokers, although in each age group two participants reported sporadic smoking (less than 10 cigarettes a week).

Participants meeting the criteria were contacted by telephone and asked to attend two sessions that took place in a laboratory at the Faculty of Psychology. Before each session, participants were asked to maintain their general habits, sleep as long as usual, refrain from heavy physical activity the day before the session, and not consume alcohol since the night before the session. Additionally, they were instructed to drink only water, not eat, smoke or take any stimulants, such as coffee, cola, caffeine, tea or chocolate, 2 h prior to the session, and not brush their teeth at least 1 h prior to the session. The study was conducted in accordance with the Declaration of Helsinki, and the protocol and conduct were approved by the Ethics Research Committee of the University of Valencia. All the participants received verbal and written information about the study and signed an informed consent form.

### 2.2. Procedure

This study employed a within-subject design with two completely randomized and counterbalanced conditions in two separate sessions: a stress condition and a control condition, with about two weeks between sessions. The sessions consisted of several phases of equal duration for both conditions. Each session took 1 h and 15 min to complete, and they were always held between 16.00 and 20.00 h. Each participant started his or her two sessions at the same hour. Upon arrival at the laboratory, the weight and height of the participants were measured, and the experimenter checked to see whether they had followed the instructions given previously (see Section 2.1).

#### 2.2.1. Stress condition

To produce stress, we subjected the participants to the Trier Social Stress Test (TSST). The stress task consisted of 5 min of free speech (job interview) and a 5 min arithmetic task, performed in front of a committee composed of a man and a woman. The participants remained standing at a distance of 1.5 m from the committee. Additionally, a video camera and a microphone were clearly visible. Both the speech and arithmetic tasks were filmed.

The protocol started with a habituation phase of 15 min to allow the participants to adapt to the laboratory setting. During this phase, the participants remained seated. Five minutes after the start of this phase, they completed the mood questionnaire (PANAS Pre-task). After the habituation phase, the introduction phase started (duration 5 min). In this phase the participants were informed about the procedure for the stress task. They received the instructions in front of the committee in the same room where the task took place. Next, the participants had 10 min to prepare for the speech at hand. Following the preparation phase, the stress task was car-

**Table 1**  
Descriptive statistics (mean  $\pm$  SEM) of younger ( $N=31$ ) and older groups ( $N=31$ ).

	Younger group			Older group		
	Total	Men	Women	Total	Men	Women
Age (years)	22.4 (0.8)	22.1(1.4)	22.7(0.8)	61.8 (0.8)	60.5 (1.2)	63.1 (1.0)
BMI (kg/m <sup>2</sup> )	23.4 (0.5)	24.1 (0.8)	22.7 (0.5)	26.6 (0.5)	27.0 (0.5)	26.1 (1.0)
SES <sup>a</sup>	6.5 (0.2)	6.4 (0.2)	6.6 (0.2)	6.1 (0.2)	6.1 (0.3)	6.1 (0.3)
Education level <sup>b</sup>	2.5 (0.1)	2.4 (0.2)	2.5 (0.2)	2.9 (0.2)	2.7 (0.3)	3.0 (0.3)

<sup>a</sup> SES: Subjective Socio-Economic Status scale, ranging from 1 (lowest SES) to 10 (highest SES) (Adler et al., 2000).

<sup>b</sup> Range: 0 = no studies, 1 = primary school, 2 = secondary education, 3 = university and higher education, 4 = postgraduate (Master, PhD). Values are means ( $\pm$ SEM).

ried out. Subjects had 35 min to recover afterwards, during which they answered some questionnaires, including the mood questionnaire (PANAS Post-task). Participants were not allowed to distract themselves during this period, and when they had completed the questionnaires they waited alone for the remainder of the time.

The timing of the saliva sampling was different for the cortisol and sAA samples according to the time course of their responses to stress induction. The cortisol response to stress, as a reflection of HPA-axis activation, is slower than that of sAA, which reflects ANS activation (see Granger et al., 2007). Therefore, the first saliva sample employed to measure cortisol was taken 25 min after the participant's arrival at the laboratory (0 min Pre-task). The second cortisol sample was collected 20 min after the onset of the stress task, and the third one 45 min after. To measure sAA the first saliva sample was collected 10 min before the onset of the stress task (–10 min), with the second one taken immediately before the speech (0 min), followed by one sample every 5 min after the onset of the task (5 min, 10 min and 15 min).

### 2.2.2. Control condition

The control condition was similar to the experimental condition, except that the stressful task was replaced by a control task. The control task was designed to be similar to the stress task in mental workload and global physical activity, but without the main components capable of provoking stress, such as evaluative threat and uncontrollability (Dickerson and Kemeny, 2004). The control task was composed of 5 min of reading aloud and 5 min of counting without being in front of an audience. In the preparation phase, the participants read a book with a neutral content. To provoke the same orthostatic stress as in the stress condition, participants had to stand and walk at the same time points and for the same amount of time as in the stress condition. The timing of the saliva samples, the questionnaires used, and the phase durations were the same for the two conditions.

### 2.3. Affect questionnaire

Affect was evaluated by the Spanish version (Sandín et al., 1999) of the PANAS (Positive and Negative Affect Schedule, Watson et al., 1988). This 20-item questionnaire assesses affect according to two dimensions: positive affect (PA: *interested, excited, strong, enthusiastic*, etc.) and negative affect (NA: *distressed, upset, guilty, scared*, etc.), with 10 items measuring each state. Participants were asked to complete the questionnaire twice, immediately before (Pre-task) and immediately after the stress/control task (Post-task). They gave their answers based on how they felt at that particular moment. They responded using a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely). Sandín et al. (1999) reported a high internal consistency for the Spanish version, with a Cronbach's alpha for PA ranging from 0.87 to 0.89 and for NA from 0.89 to 0.91.

### 2.4. Heart rate

Heart rate was measured using an HR monitor (Suunto, model T6, Suunto Oy, Vantaa, Finlandia), which consists of a chest belt for detection and transmission of the heartbeats and a "watch" for collection and storage of the data. The heart-beat detection is performed with an accuracy of 1 ms, and these types of monitors have shown good validity (Radespiel-Troger et al., 2003; Roy et al., 2009). Every heartbeat is transmitted and stored in the flash memory of the watch. HR was monitored continuously during the entire session, but the recorded periods when the participants were changing their positions (sitting/standing up) and walking were removed. After eliminating the artifacts, the HR mean for each phase was computed. The HR monitor failed to register the heart rate of one participant in the older group.

### 2.5. Biochemical analyses

#### 2.5.1. Cortisol

Participants provided three saliva samples by depositing 3 ml of saliva in plastic vials. They took approximately 5 min to fill the vial. The samples were frozen at  $-80^{\circ}\text{C}$  until the analyses were done. The samples were analyzed by a competitive solid phase radioimmunoassay (tube coated) using the commercial kit Coat-A-Count Cortisol (DPC, Siemens Medical Solutions Diagnostics). For each subject, all the sam-

ples were analyzed in the same trial. The within and inter assay variation coefficients were all below 8%.

#### 2.5.2. Alpha-amylase

Saliva was collected using salivettes (Sarstedt, Nümbrecht, Germany). Participants were instructed to introduce the cotton swab into their mouths for exactly 1 min, not chew the cotton, and move the swab around in a circular pattern to collect saliva from all the salivary glands (Rohleder and Nater, 2009). The samples were frozen at  $-20^{\circ}\text{C}$  from the completion of the session until the analyses took place. The samples were shipped to Dresden and analyzed at the Kirschbaum lab, Technical University of Dresden. Concentration of alpha-amylase in saliva was measured by an enzyme kinetic method according to the protocol specified in Rohleder et al. (2006). Inter- and intra-assay variation was below 10%. Analyses of sAA failed to detect the sAA concentrations in the samples of one young woman, one older woman and one older man; therefore, these people were excluded from the statistical analyses regarding sAA.

### 2.6. Statistical analyses

Data were tested for normal distribution and homogeneity of variance using Kolmogorov–Smirnov and Levene's tests before statistical procedures were applied. These analyses revealed significant deviations of some sAA and cortisol values; therefore, they were square root transformed. We analyzed whether the application of the TSST in the first or second session had an effect on all the variables measured, by conducting repeated-measures ANOVAs with Order as a within-subject factor. Only in cortisol analysis did Order have a significant effect ( $p < 0.05$ ); therefore, we included Order as a covariate in all the statistical analyses involving the cortisol variables.

Student's *t*-tests were used to investigate age and sex differences in the demographic variables. We used repeated-measures ANOVAs with Condition (stress vs. control) as a within-subject factor to evaluate baseline differences between the stress and control conditions in all the variables measured.

For sAA, cortisol and HR we calculated the areas under the total response curve with respect to the ground ( $\text{AUC}_g$ ) and with respect to the increase ( $\text{AUC}_i$ ), using the trapezoid formula specified in Pruessner et al. (2003). We also computed the delta change in PANAS positive and negative affect scores (PostTask–PreTask).

Repeated-measures ANOVAs were used to investigate the effect of the TSST on positive and negative affect, HR and the summary indices (i.e.  $\text{AUC}_g$ ,  $\text{AUC}_i$ ) of sAA and cortisol. We used condition (stress vs. control) and time (positive and negative affect: Pre vs. Post-task; HR: each phase of the stress and control condition) as within-subject factors and age and sex as between-subject factors.

We investigated whether sAA indices ( $\text{AUC}_g$  and  $\text{AUC}_i$ ) were related to cortisol and HR indices, using hierarchical regression and controlling for age, sex, BMI, order and delta change in negative affect.

We used the Greenhouse–Geisser procedure when the requirement of sphericity in the ANOVA for repeated measures was violated. Post hoc planned comparisons were performed using the Bonferroni adjustments for the *p*-values. All *p*-values reported are two-tailed, and the level of significance was marked at  $<0.05$ . For significant results, partial eta squared is reported as a measure for effect size. When not otherwise specified, results shown are means  $\pm$  standard error of means (SEM). We used SPSS 17.0 to perform the statistical analyses.

## 3. Results

### 3.1. Positive and negative affect

The participants' positive affect was not influenced by the experimental procedure (all  $p > 0.17$ ) (see Fig. 1A). However, the stress induction had an effect on their negative affect (NA) (condition:  $F(1,58) = 32.595$   $p < 0.001$ ,  $\eta_p^2 = 0.360$ ; Condition  $\times$  Time:  $F(1,58) = 30.890$   $p < 0.001$ ,  $\eta_p^2 = 0.348$ ). Baseline NA was similar

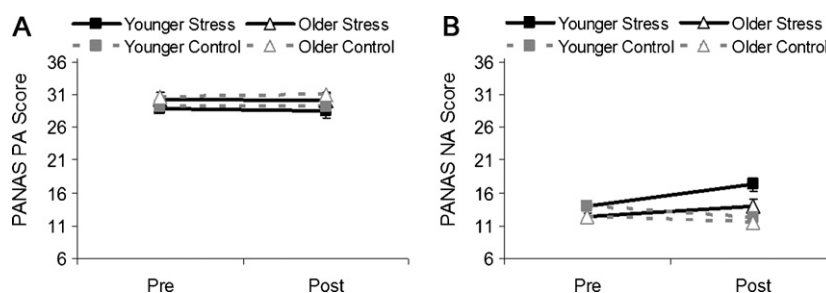


Fig. 1. Means of (A) positive and negative affect (B) scores (PANAS) ( $\pm$ SEM) before and after the TSST and the control task in the age groups (younger:  $N = 31$ ; older:  $N = 31$ ).

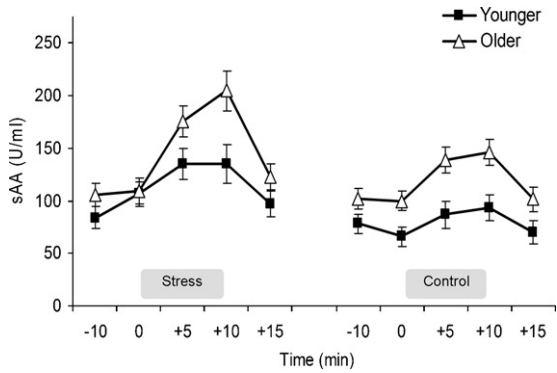


Fig. 2. Means of salivary alpha amylase concentrations ( $\pm$ SEM) in the stress (left) and control (right) conditions in the age groups (younger:  $N=30$ ; older:  $N=29$ ).

between conditions ( $p=0.745$ ). The TSST provoked an increase in NA ( $p=0.001$ ,  $\eta_p^2 = 0.183$ ), while after the control task the NA scores decreased,  $p < 0.001$ ,  $\eta_p^2 = 0.357$ . Consequently, the NA scores were higher after the stress than after the control task,  $p < 0.001$ ,  $\eta_p^2 = 0.493$ .

A main effect of age was found for NA scores ( $F(1,58)=5.892$ ,  $p=0.018$ ,  $\eta_p^2 = 0.092$ ), showing that the younger group had higher NA scores than the older group across both conditions (see Fig. 1B). However, the stress-induced NA increase was not different between the two age groups ( $p > 0.3$ ). The factor sex was not significant, nor were there any interactions between sex and the other factors, for all  $p > 0.5$ .

### 3.2. Salivary alpha-amylase

Fig. 2 shows the means of sAA concentrations ( $\pm$ SEM) for both age groups in the stress and control conditions. Baseline sAA concentrations did not differ between conditions,  $p > 0.5$ . The total sAA increase (AUCi) and the overall sAA output (AUCg) were higher in the stress than in the control condition (AUCi:  $F(1,55)=17.084$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.237$ ; AUCg:  $F(1,55)=21.512$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.281$ ).

A main effect of age was found for both AUCi and AUCg ( $F(1,55)=4.620$ ,  $p=0.036$ ,  $\eta_p^2 = 0.077$ ; and  $F(1,55)=4.374$ ,  $p=0.041$ ,

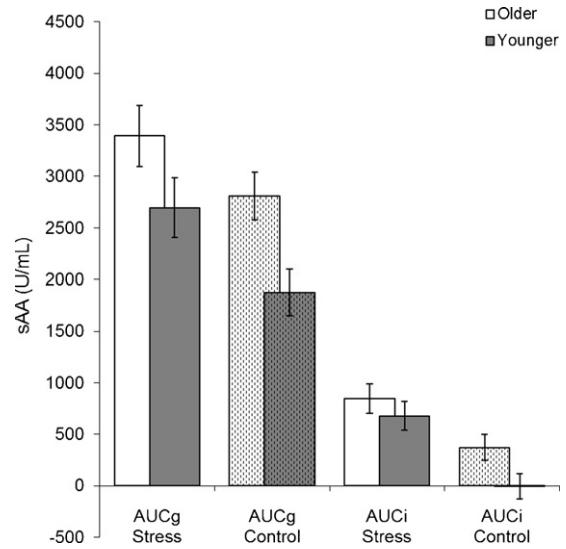


Fig. 3. Means of salivary alpha amylase AUCg and AUCi in the stress and control conditions in (A) the total sample ( $N=59$ ) and (B) the age groups (younger:  $N=30$ ; older:  $N=29$ ).

$\eta_p^2 = 0.074$ , respectively). Regardless of the condition, the older participants increased their sAA concentrations more, and their overall sAA output was higher, than the younger participants (see Fig. 3). The interaction between Condition and Age was not significant,  $p > 0.2$ . The factor Sex was not significant nor were there any interactions between sex and the other factors, for all  $p > 0.2$ .

### 3.3. Salivary cortisol

Fig. 4A shows the means ( $\pm$ SEM) of cortisol released by men and women from both age groups in the stress and control conditions. Baseline cortisol concentrations did not differ between conditions,  $p > 0.6$ . The cortisol concentrations increased in the stress condition but decreased in the control condition (AUCi:  $F(1,54)=12.501$ ,  $p=0.001$ ,  $\eta_p^2 = 0.188$ ). Consequently, the overall cortisol output

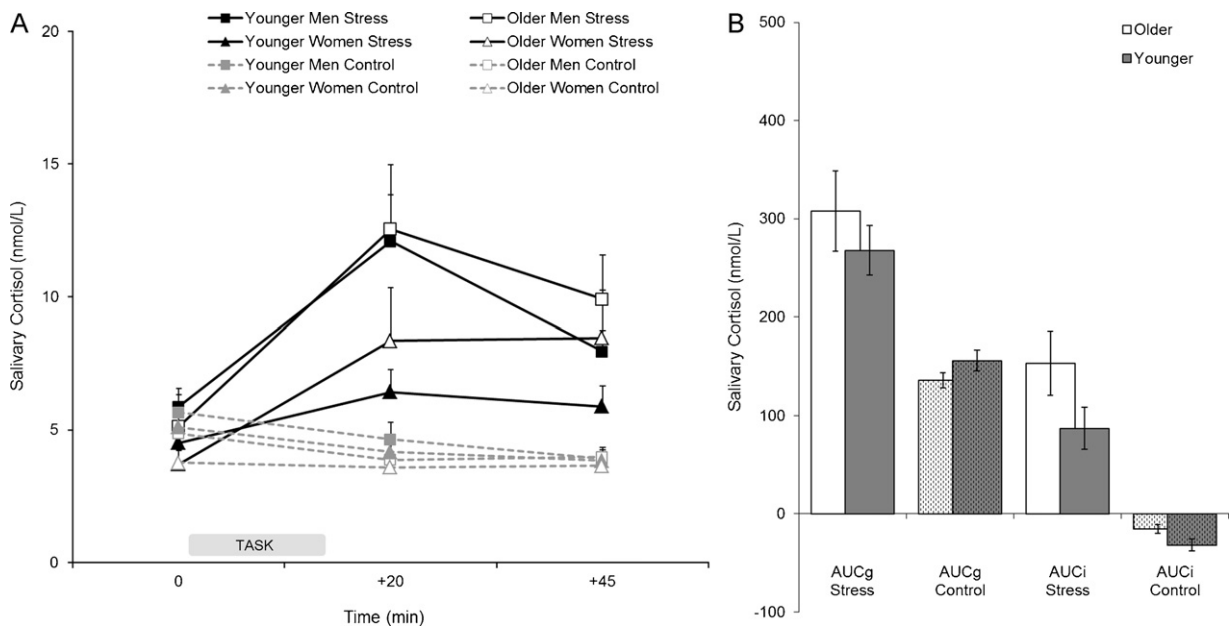


Fig. 4. Means of (A) salivary cortisol concentrations ( $\pm$ SEM) and cortisol AUCg and AUCi in the stress and control conditions, in the younger men ( $N=14$ ), younger women ( $N=15$ ), older men ( $N=15$ ) and older women ( $N=15$ ).

(AUCg) was higher in the stress condition than in the control condition,  $F(1,54) = 13.122$ ,  $p = 0.001$ ,  $\eta_p^2 = 0.195$ .

A main effect of age was found for cortisol AUCi ( $F(1,54) = 4.537$ ,  $p = 0.038$ ,  $\eta_p^2 = 0.077$ ), showing that regardless of the condition the older group had higher cortisol AUCi than the younger group (see Fig. 4B). The interaction between Condition and Age was not significant,  $p > 0.2$ .

An interaction between Condition and Sex was found for both AUCi and AUCg ( $F(1,54) = 4.336$ ,  $p = 0.042$ ,  $\eta_p^2 = 0.074$  and  $F(1,54) = 6.038$ ,  $p = 0.017$ ,  $\eta_p^2 = 0.101$ , respectively). Men had higher overall cortisol output in the stress condition than women ( $p = 0.010$ ,  $\eta_p^2 = 0.117$ ), but not in the control condition,  $p = 0.290$ . Furthermore, although non significant, on average the stress-induced cortisol increase was higher in men than in women ( $p = 0.091$ ), and men had a larger decrease in their cortisol concentrations in the control condition than women did,  $p = 0.064$ .

For the above analysis the cortisol data of 3 participants (2 young men and 1 older man) were removed, as their concentrations differed by more than 3 standard deviations from the rest of the sample. However, including them did not change the statistical conclusions in any way.

### 3.4. Heart rate

Fig. 5 shows the means of HR ( $\pm$ SEM) in the stress and control conditions for both age groups. The repeated-measures ANOVA with HR as a dependent variable showed the main effects of Condition ( $F(1,57) = 36.882$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.393$ ), Time ( $F(2.76,157.63) = 162.116$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.740$ ), and the interaction between the two factors,  $F(2.62,149.27) = 32.175$ ,  $p < 0.001$ ,  $\eta_p^2 = 0.361$ . Baseline HR did not differ between conditions,  $p = 0.519$ . In the stress condition, HR progressively increased from baseline to the speech phase (for all  $p < 0.001$ ), and steadily decreased from the speech phase to baseline levels at the recovery phase,  $p > 0.99$ . Although the control task provoked a HR increase, HR was lower in the control condition than in the stress condition in all phases (for all  $p < 0.001$ ), except the habituation phase.

The following interactions were also significant: Condition  $\times$  Time  $\times$  Sex ( $F(2.62,149.27) = 3.427$ ,  $p = 0.024$ ,  $\eta_p^2 = 0.057$ ), Condition  $\times$  Time  $\times$  Age ( $F(2.62,149.27) = 3.846$ ,  $p = 0.015$ ,  $\eta_p^2 = 0.063$ ), Time  $\times$  Age  $\times$  Sex ( $F(2.76,157.63) = 3.000$ ,  $p = 0.036$ ,  $\eta_p^2 = 0.050$ ), and Age  $\times$  Sex,  $F(1,57) = 4.332$ ,  $p = 0.042$ ,  $\eta_p^2 = 0.071$ . HR of men and women did not differ in the stress condition (for all  $p > 0.12$ ), but in the control condition women had higher HR than men in all phases (for all  $p < 0.03$ ), except the preparation phase. Exploring the interaction between Condition, Time and Age, we

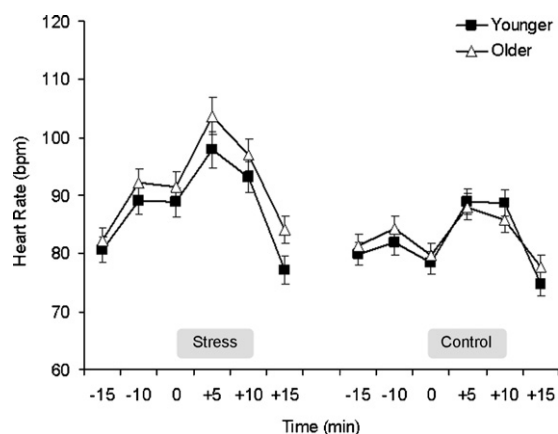


Fig. 5. Means of heart rate ( $\pm$ SEM) in the stress (left) and control (right) conditions in the age groups (younger:  $N = 31$ ; older:  $N = 30$ ).

found that older participants had higher HR than younger participants in the recovery phase of the stress condition,  $p = 0.038$ .

### 3.5. Relationships between sAA and the other physiological indices

We computed hierarchical linear regression analyses to test whether the sAA summary indices were related to HR and cortisol indices. For each analysis, in Step 1 we controlled for age, sex, BMI, delta change of negative mood, and order (whether the stress or control condition was performed first).

In the regression analysis with  $AUC_{g_{sAA}}$  as a dependent variable, entering  $AUC_{g_{HR}}$  in Step 2 did not increase the amount of variance explained,  $\Delta F(1,49) = 2.397$ ,  $p = 0.128$ . However, entering  $AUC_{g_{Cortisol}}$  in Step 3 increased the amount of variance explained,  $\Delta F(1,48) = 8.425$ ,  $p = 0.006$ , adjusted  $R^2 = 15.5\%$ ,  $\Delta R^2 = 12.9\%$ . In this model, only  $AUC_{g_{Cortisol}}$  was significantly related to  $AUC_{g_{sAA}}$ ,  $\beta = 0.423$ ,  $p = 0.006$ .

In the regression analysis with  $AUC_{i_{sAA}}$  as a dependent variable, entering  $AUC_{i_{HR}}$  in Step 2 increased the amount of variance explained,  $\Delta F(1,49) = 5.595$ ,  $p = 0.022$ , adjusted  $R^2 = 2.2\%$ ,  $\Delta R^2 = 9.9\%$ . In this model, only  $AUC_{i_{HR}}$  was significantly related to  $AUC_{i_{sAA}}$ ,  $\beta = 0.324$ ,  $p = 0.022$ . Entering  $AUC_{i_{Cortisol}}$  in Step 3 did not increase the amount of variance explained in  $AUC_{i_{sAA}}$ ,  $\Delta F(1,48) = 0.074$ ,  $p = 0.787$ .

## 4. Discussion

In the current study, a group of young and older adults was exposed to psychosocial stress and a control situation in a crossover design. The experimental procedure was indeed able to induce stress, since the exposure to the TSST produced an increase in negative mood, cortisol, sAA and HR. We did not find evidence of an attenuated ANS response to stress in older adults in either sAA or HR. However, regardless of the condition, the older group had a higher sAA global output and increased their sAA concentrations more than the younger group. Finally, we found that in the stress condition, the total amount of cortisol released was positively related to the total sAA released, and, interestingly, the HR increase was positively related to the sAA increase.

In contrast with our hypothesis, we did not find age differences in HR and sAA reactivity to stress. Our results coincide with other studies that did not find age differences in HR reactivity to psychosocial stress (Esler et al., 1995; Uchino et al., 1999). However, using the same procedure to provoke stress (i.e. TSST), but without a control condition, it has been shown that HR (Kudielka et al., 2004a) and sAA (Strahler et al., 2010b) responses to stress were attenuated in elderly people. Although the TSST is a standardized procedure to provoke stress, small modifications in this procedure could partially explain this discrepancy. We employed a preparation period of 10 min, while this period in the other studies lasted only 3 min. It is possible that having more time to prepare and think about the speech could be more stressful and provoke a higher ANS response in the older participants. The time of sAA sampling was also different. Strahler et al. (2010b) took one saliva sample 1 min after the arithmetic task and, therefore, measured sAA reactivity of both the speech and arithmetic tasks combined. However, in our study we took one sample immediately after the speech task and another one immediately after the arithmetic task, while both of these saliva samples were provided in front of the committee. It is likely that we more accurately captured the individual profile of sAA secretion. In fact, 24 participants (41% of the current sample) had their maximum sAA concentration after the speech task; therefore, these participants started to recover to baseline sAA lev-

els in subsequent samples, including the sample taken after the arithmetic task.

Moreover, age differences in the relevance of laboratory-based stressors may affect physiological reactivity (Uchino et al., 2010). For this reason, we tried to maximize the stressfulness of the TSST by making the committee up of university professors older than 50 years of age. In addition, the interaction between the committee and the participant was always performed by the committee member of the opposite sex. Unfortunately, information about the composition of the committee in other studies (Kudielka et al., 2004a; Strahler et al., 2010b) is not available, and, therefore, we are hesitant to draw any conclusions.

Although we did not find age differences in the stress-induced sAA increase, overall older adults had higher sAA global output (AUC<sub>g</sub>) irrespective of psychosocial stress. It has been well established that age increases basal sympathoneural activity, while sympathoadrenomedullary activity appears to decrease or remain unchanged (Seals and Dinunno, 2004). In our opinion, the observed higher sAA concentrations among the older participants across both conditions could reflect this increased basal sympathoneural activity. This finding reinforces the suggestion made by Ehlert et al. (2006) that sAA secretion reflects central norepinephrine release instead of peripheral norepinephrine secretion. They conclude this after showing that the increase in sAA after yohimbine infusion (an alpha-2 adrenoceptor antagonist) was not related to plasma levels of catecholamines. Furthermore, concentrations of cerebrospinal fluid norepinephrine are higher in older people (Elrod et al., 1997; Raskind et al., 1988), and older people react with greater increases in cerebrospinal fluid norepinephrine after yohimbine challenge than younger people (Peskind et al., 1995; Raskind et al., 1999). Research in the future should help clarify this matter by studying whether sAA is more reactive to yohimbine challenge in older people than in younger people.

Our findings regarding the increase in sAA (AUC<sub>i</sub>) were similar to the results regarding sAA global output. Irrespective of psychosocial stress, the older group increased their sAA levels more than the younger one. This difference was more evident when looking at the control condition (see Fig. 3). In this condition, the total amount of sAA secreted was higher in the older group than in the younger one, and while the younger participants hardly changed their sAA levels, among the older participants the sAA concentrations increased. Since negative affect and cortisol declined in the older group after the control task, it is not likely that this increase in sAA concentrations was provoked by stress. Moreover, the control task also provoked an increase in HR that was similar in both age groups. Another possible explanation for these changes in sAA and HR during the control condition could be related to orthostatic challenge. During the control condition, the participants were asked to stand up and walk at the same moments and for the same length of time as in the stress condition. In line with our results, Nater et al. (2006), using a similar experimental design that emulated a comparable orthostatic challenge in the control and stress conditions, found that the sAA of a young sample was not affected by the control condition, but plasma norepinephrine release was. The results of our study suggest that aging increases the sensitivity of sAA to postural and small changes in global physical activity.

Regarding HPA-axis activity, age was related to a higher increase in cortisol (AUC<sub>i</sub>) across both conditions. In the stress condition cortisol levels increased, but in the control condition they decreased following the natural cortisol circadian rhythm. In this particular case, the AUC<sub>i</sub> is an index of decrease (Pruessner et al., 2003, p. 921). Therefore, in our study, the older group increased their cortisol levels more in the stress condition, but at the same time they decreased them less in the control condition than the younger group (see Fig. 4B). Previous studies have related increasing age with both increased stress-induced cortisol responses and decreased cortisol

variability in the late afternoon (van Cauter et al., 1996; Deuschle et al., 1997; Gotthardt et al., 1995; Kudielka et al., 2004b; Seeman et al., 2001; Strahler et al., 2010b; Traustadóttir et al., 2005). Both effects are consistent with the loss of HPA-axis feedback sensitivity that has been hypothesized to occur with aging (Seeman and Robbins, 1994). In addition to the effects of age on cortisol release, we found sex differences in the cortisol response to stress. Men responded to stress with a higher increase in cortisol concentrations than women, as has been shown consistently in the literature (Kudielka et al., 2009). Several attempts to explain these sex differences have pointed to sexual dimorphisms in brain structures and functioning, but they have also pointed to the circulating levels of corticosteroid binding globulin protein (CBG) (Kudielka and Kirschbaum, 2005). The use of oral contraceptives has been shown to produce an increase in CBG release (Wiegratz et al., 2003), and in the same way, Kudielka et al. (2004b) found higher CBG levels in older women compared to older men. As the cortisol concentrations measured in saliva reflect the free portion of cortisol (not bound with CBG), the blunted cortisol response observed in women can partly be explained by higher concentrations of CBG, leading to a greater percentage of secreted cortisol bound with it (Kajantie and Phillips, 2006; Kumsta et al., 2007).

The overall stress-induced cortisol output (AUC<sub>gCortisol</sub>) was associated with more overall sAA release (AUC<sub>gSAA</sub>), but it was not related to the overall sAA increase (AUC<sub>iSAA</sub>). However, the overall sAA increase (AUC<sub>iSAA</sub>) was associated with a higher HR increase (AUC<sub>iHR</sub>). Previously, correlations between stress-induced sAA and cortisol responses had rarely been reported (Gordis et al., 2006), and the majority of studies did not find any correlation between sAA and cortisol responses to stress (Nater et al., 2005, 2006; Strahler et al., 2010b). The relationship observed between the HR increase and the sAA increase in response to stress is in some way not surprising, as both are biomarkers of the ANS. Associations between sAA and HR or other cardiovascular indices have been reported previously. For example, sAA reactivity to stress has been positively related to HR reactivity and the low frequency/high frequency ratio (an index of sympathetic tone), and negatively with the left ventricular ejection time and the RMSSD (root mean square of successive differences of normal-to-normal intervals, an index of parasympathetic tone) (Bosch et al., 2003; Nater et al., 2006). Some studies have also found positive relationships between sAA and other ANS biomarkers, such as plasma norepinephrine and epinephrine (Chatterton et al., 1996; Rohleder et al., 2004), although others failed to find such associations (Nater et al., 2006).

The positive relationship found between the cortisol and sAA total output reflects the coordination between the two main physiological stress systems. As Chrousos and Gold (1992) discuss, the HPA-axis and ANS could interact at many potential central sites to coordinate the stress response, leading the activation of one system to produce the activation of the other as well. The neurons that release corticotropin releasing factor (CRF) project from the lateral paraventricular nuclei (PVN) in the hypothalamus to sympathetic hindbrain regions (Nauta and Feirtag, 1986; Saper et al., 1976), and, conversely, catecholaminergic fibers from the locus coeruleus (LC)–noradrenergic system project to the PVN (Cunningham and Sawchenko, 1988; Cunningham et al., 1990; Saper and Loewy, 1980). Furthermore, the administration of CRF onto LC neurons increases the LC firing rate (Dunn and Berridge, 1990; Jedema and Grace, 2004), while norepinephrine is a potent stimulus for the release of CRF (Calogero et al., 1988; Cunningham et al., 1990). Our findings support the coordination of both the HPA-axis and the ANS in generating the physiological stress response (Granger et al., 2007).

Some limitations have to be considered in order to interpret the results of the current study. The use of sAA as a biomarker of ANS activation is relatively novel, and the biochemical and physio-

logical properties of this enzyme are still under investigation. The saliva in this study was collected using salivettes, and recently it has been shown that unstimulated and stimulated saliva collection yield different sAA concentrations (DeCaro, 2008). Although we did not instruct the participants to chew the cotton roll, we did ask them to move it around in their mouths in order to collect saliva from all the salivary glands (Rohleder and Nater, 2009). It has been shown that sAA stress-induced increases are independent of salivary flow rate (Rohleder et al., 2006); however, the results observed in the current study cannot be compared with studies that have collected saliva using non stimulating methods. In addition, age could affect salivary gland physiology and, therefore, saliva composition, since the parenchyma of the salivary glands is gradually replaced by fat, connective tissue and oncocytes with age (Nagler, 2004). However, until now, there has been no evidence that aging per se leads to a reduction in the capacity of salivary glands to produce saliva (Dobrosielski-Vergona, 1993) or produce changes in the saliva composition in healthy subjects (Aguirre et al., 1987; Fox et al., 1987). Moreover, it is important to note that all the young women in this study were taking oral contraceptives. Although these pills have not been reported to affect sAA release, they do have an effect on salivary free cortisol. The use of oral contraceptives is very widespread, and, thus, the women using this medication constitute an interesting research group in itself. However, our results cannot be generalized to premenopausal women with natural cycles, and it would, therefore, be interesting to include them in future research. Finally, we used a stressor (TSST) that is mainly based on social-evaluative threat, and it is plausible that previous experiences with social evaluative challenges moderated the observed stress response. However, it seems unlikely that previous experiences with social stress influenced our main findings since our age groups were quite homogenous. For example, participants were all recruited within university programs, and for all participants it was their first exposure to a standardized lab stressor. Nevertheless, future research should control for previous experiences with social evaluative challenges, and investigate whether it affects age differences in the psycho-physiological response to lab stressors.

Taken together, our results add new knowledge about the effect of age on stress-induced sAA release. Our results do not support the existence of an attenuated ANS response to psychosocial stress in older adults, but rather a heightened sympathetic tone. Furthermore, our findings give support to the coordination between the two main stress systems, the HPA-axis and ANS, beyond kinetic differences in their responses to psychosocial stress.

### Role of the funding source

This research study was supported by the Spanish Education and Science Ministry with grants no. SEJ2004-07191/PSIC, SEJ2006-14086/PSIC, SEJ2007-62019/PSIC, Generalitat Valenciana no. ACOMP/2009/032, ACOMP/2010/131, PROMETEO2011-048 and grant no. FPU/00195. These grants had no further role in the study design; in the collection, analysis and interpretation data; in the writing of the report; and in the decision to submit the paper for publication.

### Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

### Acknowledgements

The authors wish to thank Prof. Dr. Clemens Kirschbaum for his suggestions on the control task, Dr. Ferran Suay for his support in

the research process and Ms. Cindy DePoy for the revision of the English text.

### References

- Adler, N.E., Epel, E.S., Castellazzo, G., Ickovics, J.R., 2000. Relationship of subjective and objective social status with psychological and physiological functioning: preliminary data in healthy, white women. *Health Psychology* 19 (6), 586–592.
- Aguirre, A., Levine, M.J., Cohen, R.E., Tabak, L.A., 1987. Immunochemical quantitation of alpha-amylase and secretory IgA in parotid saliva from people of various ages. *Archives of Oral Biology* 32 (4), 297–301.
- Alameida, D.M., Piazza, J.R., Stawski, R.S., Klein, L.C., 2011. The speedometer of life: stress, health and aging. In: Schaie, K.W., Willis, S.L. (Eds.), *Handbook of the Psychology of Aging*, 7th edition, pp. 191–206.
- Born, J., Ditschuneit, I., Schreiber, M., Dodt, C., Fehm, H.L., 1995. Effects of age and gender on pituitary-adrenocortical responsiveness in humans. *European Journal of Endocrinology* 132 (6), 705–711.
- Bosch, J.A., De Greus, E.J.C., Veerman, E.C.I., Hoogstraten, J., Nieuw Amerongen, A.V., 2003. Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. *Psychosomatic Medicine* 65 (2), 245–258.
- Calogero, A.E., Gallucci, W.T., Chrousos, G.P., Gold, P.W., 1988. Catecholamine effects upon rat hypothalamic corticotropin-releasing hormone secretion in vitro. *Journal of Clinical Investigation* 82 (3), 839–846.
- Chatterton Jr., R.T., Vogelsong, K.M., Lu, Y.C., Ellman, A.B., Hudgens, G.A., 1996. Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clinical Physiology* 16 (4), 433–448.
- Chrousos, G.P., Gold, P.W., 1992. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* 267 (9), 1244–1252.
- Chrousos, G.P., Kino, T., 2007. Glucocorticoid action networks and complex psychiatric and/or somatic disorders. *Stress* 10 (2), 213–219.
- Cunningham Jr., E.T., Bohn, M.C., Sawchenko, P.E., 1990. Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *Journal of Comparative Neurology* 292 (4), 651–667.
- Cunningham Jr., E.T., Sawchenko, P.E., 1988. Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. *Journal of Comparative Neurology* 274 (1), 60–76.
- DeCaro, J.A., 2008. Methodological considerations in the use of salivary alpha-amylase as a stress marker in field research. *American Journal of Human Biology* 20 (5), 617–619.
- Deuschle, M., Gotthardt, U., Schweiger, U., Weber, B., Korner, A., Schmider, J., Standhardt, H., Lammers, C.H., Heuser, I., 1997. With aging in humans the activity of the hypothalamus-pituitary-adrenal system increases and its diurnal amplitude flattens. *Life Sciences* 61 (22), 2239–2246.
- Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychological Bulletin* 130 (3), 355–391.
- Dobrosielski-Vergona, K., 1993. Age-related changes in the function of salivary glands. In: Dobrosielski-Vergona, K. (Ed.), *Biology of the Salivary Glands*. CRC press, Boca Raton, pp. 431–433.
- Dunn, A.J., Berridge, C.W., 1990. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Research: Brain Research Reviews* 15 (2), 71–100.
- Ehler, U., Erni, K., Hebisch, G., Nater, U., 2006. Salivary alpha-amylase levels after Yohimbine challenge in healthy men. *JCEM* 91 (12), 5130–5133.
- Elrod, R., Peskind, E.R., DiGiacomo, L., Brodtkin, K.I., Veith, R.C., Raskind, M.A., 1997. Effects of Alzheimer's disease severity on cerebrospinal fluid norepinephrine concentration. *American Journal of Psychiatry* 154 (1), 25–30.
- Esler, M.D., Thompson, J.M., Kaye, D.M., Turner, A.G., Jennings, G.L., Cox, H.S., et al., 1995. Effects of aging on the responsiveness of the human cardiac sympathetic nerves to stressors. *Circulation* 91 (2), 351–358.
- Ferrari, E., Magri, F., 2008. Role of neuroendocrine pathways in cognitive decline during aging. *Ageing Research Reviews* 7, 225–233.
- Fox, P.C., Busch, K.A., Baum, B.J., 1987. Subjective reports of xerostomia and objective measures of salivary gland performance. *Journal of the American Dental Association* 115 (4), 581–584.
- Gordis, E.B., Granger, D.A., Susman, E.J., Trickett, P.K., 2006. Asymmetry between salivary cortisol and alpha-amylase reactivity to stress: relation to aggressive behavior in adolescents. *Psychoneuroendocrinology* 31 (8), 976–987.
- Gotthardt, U., Schweiger, U., Fahrenberg, J., Lauer, C.J., Holsboer, F., Heuser, I., 1995. Cortisol, ACTH, and cardiovascular response to a cognitive challenge paradigm in aging and depression. *American Journal of Physiology* 268, R865–R873.
- Granger, D.A., Kivlighan, K.T., el-Sheikh, M., Gordis, E.B., Stroud, L.R., 2007. Salivary alpha-amylase in biobehavioral research: recent developments and applications. *Annals of the New York Academy of Sciences* 1098, 122–144.
- Granger, D.A., Kivlighan, K.T., Blair, C., El-Sheikh, M., Mize, J., Lisonbee, J.A., et al., 2006. Integrating the measurement of salivary (alpha)-amylase into studies of child health, development, and social relationships. *Journal of Social and Personal Relationships* 23 (2), 267–290.
- Heuser, I.J., Gotthardt, U., Schweiger, U., Schmider, J., Lammers, C.H., Dettling, M., et al., 1994. Age-associated changes of pituitary-adrenocortical hormone regulation in humans: importance of gender. *Neurobiology of Aging* 15 (2), 227–231.
- Jedema, H.P., Grace, A.A., 2004. Corticotropin-releasing hormone directly activates noradrenergic neurons of the locus ceruleus recorded in vitro. *Journal of Neuroscience* 24 (43), 9703–9713.



- Kajantie, E., Phillips, D.I.W., 2006. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology* 31 (2), 151–178.
- Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamic–pituitary–adrenal axis. *Psychosomatic Medicine* 61 (2), 154–162.
- Kirschbaum, C., Pirke, K., Hellhammer, D.H., 1993. The “trier social stress test”: a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28 (1–2), 76–81.
- Kivlighan, K.T., Granger, D.A., 2006. Salivary alpha-amylase response to competition: relation to gender, previous experience, and attitudes. *Psychoneuroendocrinology* 31 (6), 703–714.
- Kudielka, B.M., Hellhammer, D.H., Wust, S., 2009. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology* 34 (1), 2–18.
- Kudielka, B.M., Schmidt-Reinwald, A.K., Hellhammer, D.H., Kirschbaum, C., 1999. Psychological and endocrine responses to psychosocial stress and dexamethasone/corticotropin-releasing hormone in healthy postmenopausal women and young controls: the impact of age and a two-week estradiol treatment. *Neuroendocrinology* 70 (6), 422–430.
- Kudielka, B.M., Schmidt-Reinwald, A.K., Hellhammer, D.H., Schurmeyer, T., Kirschbaum, C., 2000. Psychosocial stress and HPA functioning: no evidence for a reduced resilience in healthy elderly men. *Stress* 3 (3), 229–240.
- Kudielka, B.M., Buske-Kirschbaum, A., Hellhammer, D.H., Kirschbaum, C., 2004a. Differential heart rate reactivity and recovery after psychosocial stress (TSST) in healthy children, younger adults, and elderly adults: the impact of age and gender. *International Journal of Behavioral Medicine* 11 (2), 116–121.
- Kudielka, B.M., Buske-Kirschbaum, A., Hellhammer, D.H., Kirschbaum, C., 2004b. HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. *Psychoneuroendocrinology* 29 (1), 83–98.
- Kudielka, B.M., Kirschbaum, C., 2005. Sex differences in HPA axis responses to stress: a review. *Biological Psychology* 69 (1), 113–132.
- Kumsta, R., Entringer, S., Hellhammer, D.H., Wust, S., 2007. Cortisol and ACTH responses to psychosocial stress are modulated by corticosteroid binding globulin levels. *Psychoneuroendocrinology* 32 (8–10), 1153–1157.
- Laine, M., Pienihakkinen, K., Ojanotko-Harri, A., Tenovuuo, J., 1991. Effects of low-dose oral contraceptives on female whole saliva. *Archives of Oral Biology* 36 (7), 549–552.
- Luisi, S., Tonetti, A., Bernardi, F., Casarosa, E., Florio, P., Monteleone, P., et al., 1998. Effect of acute corticotropin releasing factor on pituitary–adrenocortical responsiveness in elderly women and men. *Journal of Endocrinological Investigation* 21 (7), 449–453.
- Nagler, R.M., 2004. Salivary glands and the aging process: mechanistic aspects, health-status and medicinal-efficacy monitoring. *Biogerontology* 5 (4), 223–233.
- Nater, U.M., La Marca, R., Florin, L., Moses, A., Langhans, W., Koller, M.M., et al., 2006. Stress-induced changes in human salivary alpha-amylase activity—associations with adrenergic activity. *Psychoneuroendocrinology* 31 (1), 49–58.
- Nater, U.M., Rohleder, N., 2009. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology* 34 (4), 486–496.
- Nater, U.M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., et al., 2005. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journal of Psychophysiology* 55 (3), 333–342.
- Nater, U.M., Rohleder, N., Schlotz, W., Ehler, U., Kirschbaum, C., 2007. Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology* 32 (4), 392–401.
- Nauta, W.J.H., Feirtag, M., 1986. *Fundamental Neuroanatomy*. WH Freeman & Co, NY.
- Nicolson, N., Storms, C., Ponds, R., Sulon, J., 1997. Salivary cortisol levels and stress reactivity in human aging. *Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 52 (2), M68–M75.
- Otte, C., Hart, S., Neylan, T.C., Marmar, C.R., Yaffe, K., Mohr, D.C., 2005. A meta-analysis of cortisol response to challenge in human aging: importance of gender. *Psychoneuroendocrinology* 30 (1), 80–91.
- Pajukoski, H., Meurman, J.H., Snellman-Grohn, S., Keinanen, S., Sulkava, R., 1997. Salivary flow and composition in elderly patients referred to an acute care geriatric ward. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* 84 (3), 265–271.
- Pascualy, M., Petrie, E.C., Brodtkin, K., Peskind, E.R., Veith, R.C., Raskind, M.A., 1999. Effects of advanced aging on plasma catecholamine responses to the cold pressor test. *Neurobiology of Aging* 20 (6), 637–642.
- Pardon, 2007. Stress and ageing interactions: A paradox in the context of shared etiological and physiopathological processes. *Brain Research Reviews* 54 (2), 251–273.
- Peskind, E.R., Wingerson, D., Murray, S., Pascualy, M., Dobie, D.J., Le Corre, P., Le Verge, R., Veith, R.C., Raskind, M.A., 1995. Effects of Alzheimer's disease and normal aging on cerebrospinal fluid norepinephrine responses to yohimbine and clonidine. *Archives of General Psychiatry* 52 (9), 774–782.
- Pruessner, J.C., Kirschbaum, C., Meinschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 28 (7), 916–931.
- Radespiel-Troger, M., Rauh, R., Mahlke, C., Gottschalk, T., Muck-Weymann, M., 2003. Agreement of two different methods for measurement of heart rate variability. *Clinical Autonomic Research* 13 (2), 99–102.
- Räikkönen, K., Matthews, K.A., Pesonen, A.K., Pyhälä, R., Paavonen, E.J., Feldt, K., Jones, A., Phillips, D.I.W., Seckl, J.R., Heinonen, K., Lahti, J., Komsa, N., Järvenpää, A.L., Eriksson, J.G., Strandberg, T.E., Kajantie, E., 2010. Poor sleep and altered hypothalamic–pituitary–adrenocortical and sympathetic–adrenal–medullary system activity in children. *Journal of Clinical Endocrinology and Metabolism* 95 (5), 2254–2261.
- Rantonen, P.J., Meurman, J.H., 2000. Correlations between total protein, lysozyme, immunoglobulins, amylase, and albumin in stimulated whole saliva during daytime. *Acta Odontologica Scandinavica* 58 (4), 160–165.
- Raskind, M.A., Peskind, E.R., Veith, R.C., Beard, J.C., Gumbrecht, G., Halter, J.B., 1988. Increased plasma and cerebrospinal fluid norepinephrine in older men: differential suppression by clonidine. *Journal of Clinical Endocrinology and Metabolism* 66 (2), 438–443.
- Raskind, M.A., Peskind, E.R., Holmes, C., Goldstein, D.S., 1999. Patterns of cerebrospinal fluid catechols support increased central noradrenergic responsiveness in aging and Alzheimer's disease. *Biological Psychiatry* 46 (6), 756–765.
- Rohleder, N., Kudielka, B.M., Hellhammer, D.H., Wolf, J.M., Kirschbaum, C., 2002. Age and sex steroid-related changes in glucocorticoid sensitivity of pro-inflammatory cytokine production after psychosocial stress. *Journal of Neuroimmunology* 126 (1–2), 69–77.
- Rohleder, N., Nater, U.M., Wolf, J.M., Ehler, U., Kirschbaum, C., 2004. Psychosocial stress-induced activation of salivary alpha-amylase: an indicator of sympathetic activity? *Annals of the New York Academy of Sciences* 1032, 258–263.
- Rohleder, N., Wolf, J.M., Maldonado, E.F., Kirschbaum, C., 2006. The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. *Psychophysiology* 43 (6), 645–652.
- Rohleder, N., Nater, U.M., 2009. Determinants of salivary  $\alpha$ -amylase in humans and methodological considerations. *Psychoneuroendocrinology* 34 (4), 469–485.
- Roy, R.A., Boucher, J.P., Comtois, A.S., 2009. Heart rate variability modulation after manipulation in pain-free patients vs patients in pain. *Journal of Manipulative and Physiological Therapeutics* 32 (4), 277–286.
- Salmon, A.B., Richardson, A., Pérez, V.I., 2010. Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? *Free Radical Biology and Medicine* 48 (5), 642–655.
- Salvolini, E., Mazzanti, L., Martarelli, D., Di Giorgio, R., Fratto, G., Curatola, G., 1999. Changes in the composition of human unstimulated whole saliva with age. *Aging (Milano)* 11 (2), 119–122.
- Sandín, B., Chorot, P., Lostao, L., Joiner, T.E., Santed, M.A., Valiente, R.M., 1999. Escalas PANAS de afecto positivo y negativo: Validación factorial y convergencia transcultural./The PANAS scales of positive and negative affect: factor analytic validation and cross-cultural convergence. *Psicothema* 11 (1), 37–51.
- Saper, C.B., Loewy, A.D., 1980. Efferent connections of the parabrachial nucleus in the rat. *Brain Research* 197 (2), 291–317.
- Saper, C.B., Loewy, A.D., Swanson, L.W., Cowan, W.M., 1976. Direct hypothalamo-autonomic connections. *Brain Research* 117 (2), 305–312.
- Schoofs, D., Hartmann, R., Wolf, O.T., 2008. Neuroendocrine stress responses to an oral academic examination: no strong influence of sex, repeated participation and personality traits. *Stress* 11 (1), 52–61.
- Seals, D.R., Dinunno, F.A., 2004. Collateral damage: cardiovascular consequences of chronic sympathetic activation with human aging. *American Journal of Physiology: Heart and Circulatory Physiology* 287 (5), H1895–H1905.
- Seeman, T.E., Robbins, R.J., 1994. Aging and hypothalamic–pituitary–adrenal response to challenge in humans. *Endocrine Reviews* 15 (2), 233–260.
- Seeman, T.E., Singer, B., Wilkinson, C.W., McEwen, B., 2001. Gender differences in age-related changes in HPA axis reactivity. *Psychoneuroendocrinology* 26 (3), 225–240.
- Spinrad, T.L., Eisenberg, N., Granger, D.A., Eggum, N.D., Sallquist, J., Haugen, R.G., et al., 2009. Individual differences in preschoolers' salivary cortisol and alpha-amylase reactivity: relations to temperament and maladjustment. *Hormones and Behavior* 56 (1), 133–139.
- Stephens, A., 1991. The links between stress and illness. *Journal of Psychosomatic Research* 35 (6), 633–644.
- Strahler, J., Berndt, C., Kirschbaum, C., Rohleder, N., 2010a. Aging diurnal rhythms and chronic stress: distinct alteration of diurnal rhythmicity of salivary alpha-amylase and cortisol. *Biological Psychology* 84 (2), 248–256.
- Strahler, J., Mueller, A., Rosenlocher, F., Kirschbaum, C., Rohleder, N., 2010b. Salivary alpha-amylase stress reactivity across different age groups. *Psychophysiology* 47 (3), 587–595.
- Sumter, S.R., Bokhorst, C.L., Miers, A.C., Van Pelt, J., Westenberg, P.M., 2010. Age and puberty differences in stress responses during a public speaking task: do adolescents grow more sensitive to social evaluation? *Psychoneuroendocrinology* 35 (10), 1510–1516.
- Susman, E.J., Dockray, S., Granger, D.A., Blades, K.T., Randazzo, W., Heaton, J.A., et al., 2010. Cortisol and alpha amylase reactivity and timing of puberty: vulnerabilities for antisocial behaviour in young adolescents. *Psychoneuroendocrinology* 35 (4), 557–569.
- Takai, N., Yamaguchi, M., Aragaki, T., Eto, K., Uchihashi, K., Nishikawa, Y., 2007. Gender-specific differences in salivary biomarker responses to acute psychosocial stress. *Annals of the New York Academy of Sciences* 1098, 510–515.
- Traustadóttir, T., Bosch, P.R., Matt, K.S., 2005. The HPA axis response to stress in women: effects of aging and fitness. *Psychoneuroendocrinology* 30 (4), 392–402.

- Uchino, B.N., Uno, D., Holt-Lunstad, J., Flinders, J.B., 1999. Age-related differences in cardiovascular reactivity during acute psychological stress in men and women. *Journals of Gerontology Series B: Psychological sciences and Social sciences* 54 (6), 339–346.
- Uchino, B.N., Birmingham, W., Berg, C.A., 2010. Are older adults less or more physiologically reactive? A meta-analysis of age-related differences in cardiovascular reactivity to laboratory tasks. *Journals of Gerontology Series B: Psychological sciences* 65 (2), 154–162.
- van Cauter, E., Leproult, R., Kupfer, D.J., 1996. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *Journal of Clinical Endocrinology and Metabolism* 81 (7), 2468–2473.
- van Stegeren, A.H., Wolf, O.T., Kindt, M., 2008. Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex. *International Journal of Psychophysiology* 69 (1), 33–40.
- Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology* 54 (6), 1063–1070.
- Wiegatz, I., Kutschera, E., Lee, J.H., Moore, C., Mellinger, U., Winkler, U.H., et al., 2003. Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. *Contraception* 67 (1), 25–32.
- Wood, R., Maraj, B., Lee, C.M., Reyes, R., 2002. Short-term heart rate variability during a cognitive challenge in young and older adults. *Age Ageing* 31 (2), 131–135.