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Verbal performance during stress in healthy older people: Influence of dehydroepiandrosterone (DHEA) and cortisol reactivity

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ABSTRACT

The impact of stress on the dehydroepiandrosterone (DHEA) response in older population is understudied. This study investigated, in healthy older people, whether the DHEA and cortisol responses to the Trier Social Stress Test (TSST) was related to performance on this task. Both speech (rated by committee and self-rated) and arithmetic (number of mistakes) performance were assessed. Sixty-five participants (55–77 years old) were exposed to the TSST. Increases in negative affect, state anxiety, and cortisol levels could be observed, but there were no significant changes in positive affect or DHEA levels. Interestingly, a larger DHEA response was related to better verbal performance after controlling for the cortisol's reactivity. No relationships were found between hormonal responses and the arithmetic task performance. Our results suggest that, in healthy older people, an increase in DHEA levels in response to acute psychosocial stress may help them to cope with this stressor by increasing verbal performance.

1. Introduction

The endocrine response to acute psychosocial stress has been widely studied through the assessment of cortisol levels, the end-product of the hypothalamic-pituitary-adrenal (HPA) axis. Thus, it is well known that when an individual is facing a stressor, the corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus of the hypothalamus are secreted. These two hormones cause the adrenocorticotropic hormone (ACTH) to be released by the pituitary gland, which stimulates the adrenal glands and, consequently, leads to the secretion of cortisol, which is the most important glucocorticoid in humans (Ulrich-Lai & Herman, 2009). However, the ACTH not only leads to the production of cortisol in the zona fasciculate of the adrenal cortex, but it is also involved in dehydroepiandrosterone (DHEA) secretion in the zona reticularis of the adrenal cortex. Interestingly, although there are numerous studies on cortisol changes in response to stress (see review: Pulopulos, Hidalgo, Puig-Perez, & Salvador, 2018), only a few studies have investigated the DHEA response to acute psychosocial stress.

Various forms of acute psychosocial stress have been related to an increase in DHEA. For example, DHEA levels increased before a parachute jump (Oberbeck et al., 1998), during a public speaking task (Shirtcliff, Zahn-Waxler, Klimes-Dougan, & Slattery, 2007) or a stress interview and a mental task test (Pico-Alfonso et al., 2007), and on days when children were interviewed in front of a camera (Ponzi, Muehlenbein, Sgoifo, Geary, & Flinn, 2015). Nonetheless, to date, most of the studies assessing the DHEA response to acute psychosocial stress have used the Trier Social Stress Test (TSST), a standardized laboratory protocol where participants have to carry out an interview and an arithmetic task in front of a committee (Kirschbaum, Pirke, & Hellhammer, 1993). As in other stress protocols, studies using the TSST have consistently shown DHEA increases coinciding with the stress exposure (Izawa et al., 2008; Lennartsson, Kushnir, Bergquist, & Jonsdottir, 2012; Phan et al., 2017; Shields, Lam, Trainor, & Yonelinas, 2016; Shirotsuki et al., 2009). However, the majority of the studies assessing DHEA levels after the TSST have done so in young or middle-aged adults. Unfortunately, little is known about the DHEA response to the TSST in older people. Only one study assessed whether DHEA

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increased after the TSST in an older population (Fang et al., 2014), finding that postmenopausal women (aged 50–74 years) increased their DHEA levels when exposed to this task. However, to our knowledge, no research has assessed the DHEA response to acute psychosocial stress in both older men and women.

The study of the DHEA response to acute psychosocial stress could explain some of the mechanisms contributing to heterogeneity in aging (Maggio et al., 2013). There is clear evidence indicating that DHEA levels decline with age (Maggio et al., 2013; Nguyen et al., 2017). In fact, DHEA levels are highest in people from 20 to 30 years of age, and they decline by about 80% between the ages of 65 and 70 (as cited in Stárka, Dušková, & Hill, 2015). These age-related DHEA changes can be explained by several factors (see reviews: Kamin & Kertes, 2017; Wolf & Kirschbaum, 1999). First, it is possible that aging selectively impairs the zona reticularis of the adrenal cortex, resulting in a reduction in the number of functional cells in the zona reticularis, reduced ACTH sensitivity of these cells, and an altered zonation in the adrenal cortex. Second, aging could reduce the 17, 20-desmolase activity, which is involved in the conversion from 17-hydroxypregnenolone to DHEA. Third, aging influences the secretion of 3βHSD, an enzyme that is inversely associated with DHEA biosynthesis.

Overall, there is evidence that DHEA has beneficial effects on wellbeing and cognition across the lifespan (Maggio et al., 2015; Nguyen, 2017; Nguyen et al., 2017). DHEA exerts this effect on the brain because it easily crosses the brain-blood barrier, and once in the brain, it converts into testosterone, dihydrotestosterone, or estradiol, and it binds to androgen and estrogen receptors located throughout the brain. Thus, DHEA can affect mood, emotions, behavior, cognition, and immune reactions (Stárka et al., 2015). In fact, it has been involved in several brain functions such as neuroprotection, neurite growth, neurogenesis, neuronal survival, stimulation of apoptosis, catecholamine synthesis and secretion, and antioxidant, anti-inflammatory, and anti-glucocorticoid effects (for a review see: Maggio et al., 2015). In addition, it seems that stress-induced DHEA could be related to better coping during stress, given that it plays a protective role during the stress response, antagonizing the cortisol effects (Hechter, Grossman, & Chatterton, 1997). Moreover, it has beneficial psychological effects during acute stress. Thus, lower DHEA levels during the TSST have been related to an increase in negative mood, and a stress-induced DHEA increase has been found to contribute to reducing negative mood (Izawa et al., 2008). Therefore, based on these previous findings, another interesting question to investigate is whether the DHEA response to psychosocial stress helps an individual to successfully deal with the stressor he or she is facing.

Interestingly, only a few studies have investigated whether the psychophysiological stress response is related to how well people perform on the stress task. For example, regarding performance on a speech task, Saslow et al. (2014) found in young people that a greater heart rate and cortisol response to acute stress were related to low linguistic complexity, resulting in poor performance, whereas Regehr, LeBlanc, Jelley, and Barath (2008) showed a positive relationship between the cortisol response and specialized work performance. However, in adolescents, young people, and middle-aged people, performance was not related to the following indicators: cardiovascular activity (Regehr et al., 2008; Rith-Najarian, McLaughlin, Sheridan, & Nock, 2014; Villada, Hidalgo, Almela, & Salvador, 2018), anxiety (Regehr et al., 2008), mood or cortisol (Losiak, Blaut, Klosowska, & Slowik, 2016). Furthermore, another study investigating performance on an arithmetic task found that higher displacement behavior during the TSST, which is a behavioral indicator of the experience of stress, was associated with better performance in young men and poorer performance in young women (Mohiyeddini, Bauer, & Semple, 2013a). Despite these studies, to our knowledge, no published studies in older healthy people have shown that the release of DHEA and cortisol in a controlled and standardized laboratory setting is related to better or worse performance during that stressor.

Hence, the goals of the present study were to investigate whether older men and women show a DHEA response to acute psychosocial stress, and whether the stress response is related to the performance displayed on the speech and arithmetic tasks of the TSST. To do so, 30 older men (aged 55–77 yrs.) and 35 older postmenopausal women (aged 56–73 yrs.) were exposed to the TSST. DHEA and cortisol were measured during the entire experimental session with six saliva samples. Moreover, performance on the TSST was assessed by the TSST committee, who evaluated the speech task and the number of mistakes made during the arithmetic task. Participants also evaluated their own performance on the speech task. Following the only study that investigated the DHEA response to acute stress in older women (Fang et al., 2014), we expected that DHEA would increase in response to stress in women. Due to the lack of studies in older men, no hypotheses about the DHEA response to stress in men were formulated. Based on previous studies showing that stress-induced cortisol and DHEA responses were related to successful coping with stress, we expected that the cortisol and DHEA responses to stress would be related to better performance on the TSST task. Finally, because the DHEA/cortisol ratio has been related to the psychological stress response (Shirotsuki et al., 2009), we also investigated the possible relationship between the DHEA/cortisol ratio and performance on the TSST.

2. Materials and methods

2.1. Participants

The sample was composed of sixty-five subjects (30 men and 35 women) from 55 to 77 years old. No sex differences were found in age ($p = 0.665$); however, men had a higher Body Mass Index (BMI) than women ($p = 0.023$). Moreover, men scored marginally higher on their educational level ($p = 0.069$) and subjective socioeconomic status (SES) ($p = 0.064$) than women. Table 1 shows the mean and standard error of the mean for age, BMI, SES, and educational level.

All participants were recruited from university courses and seminars for retired people to participate in the present study. All of them completed a general questionnaire in order to check the exclusion criteria. These criteria were: smoking more than 10 cigarettes a day;

Table 1
Means and standard error of the mean (SEM) for age, SES, IMC, and educational level for the complete sample and for each sex group.

	Complete Sample			Men			Women			Sex Differences
	Range	Mean	SEM	Range	Mean	SEM	Range	Mean	SEM	$t_{(63)}$
Age	55–77	63.32	0.54	55–77	63.07	0.94	56–73	63.54	0.62	$p = 0.665$
SES[a]	4–8	6.22	0.12	4-8	6.47	0.20	5-8	6	0.14	$p = 0.064$
BMI[b]	19.88–39.31	26.64	0.47	19.88–39.31	27.80	0.72	20.66–34.42	25.65	0.59	$p = 0.023$
Education[c]	1–5	2.77	0.13	1-5	3.03	0.19	1-4	2.54	0.18	$p = 0.069$

[a] Subjective Socioeconomic Status was measured using the SES Ladder (Adler et al., 2000), where scores ranged from 1 (lowest SES) to 10 (highest SES).
[b] BMI: Body Mass Index.
[c] Education was measured according to the following scale: 1 Basic School; 2 Secondary School; 3 Technical Degree; 4 University Degree; 5 PhD.

intake of alcohol or other drug abuse; presence of dental, visual and hearing problems; presence of cardiovascular, endocrine, neurological, or psychiatric disease; and in the past year, having been under general anesthesia or having experienced a stressful life event. Volunteers who were using medication related to emotional, cognitive, or endocrine function were excluded. However, volunteers who were sporadically using painkillers and vitamins were included. None of the participants met the criteria for dementia, based on the criteria for Alzheimer's disease proposed by the DSM-IV and NINCDS-ADRDA. All the women were postmenopausal (last menstrual period at least one year before the study), and they were not receiving estrogen replacement therapy.

2.2. Procedure

Participants were invited by telephone to attend an experimental session in a laboratory at the Faculty of Psychology of the University of Valencia. Participants were given the following instructions: (i) maintain general habits, (ii) sleep as long as usual, (iii) refrain from heavy activity the day before the session, and (iv) refrain from consuming alcohol from the night before the session. They were also instructed to drink only water, not eat, not smoke, and not take any stimulants (e.g. coffee, cola, caffeine, tea, or chocolate) two hours prior to the onset of the session. In addition, they were instructed to not brush their teeth at least one hour prior to the session.

The experimental session consisted of an individual session that lasted 01:40 h, approximately, and took place in the afternoon in three different shifts, starting at either 16:00 h, 17:15 h, or 18:30 h, in order to minimize the circadian variations of the hormones. Men and women were distributed similarly in all three shifts ($p > 0.3$). Once in the lab, the participants received both verbal and written information about the study and signed an informed consent form. Moreover, they were asked whether the instructions previously given by phone had been followed, and the experimenter measured participants' weight and height.

The present study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Research Committee of the University of Valencia.

2.3. TSST

To provoke acute stress, the TSST was used. This stress task consisted of a free speech task and an arithmetic task (Kirschbaum et al., 1993). Both tasks, which lasted 5 min, were performed in front of a committee (a man and a woman) and filmed. Participants remained standing at a distance of 1.5 m from the committee. During the speech task, participants had to convince the committee that they were the best applicants for a vacant position of student representative by focusing on their personal characteristics.

The experimental session started with a habituation phase that lasted 15 min to allow participants to adapt to the laboratory setting. During this time, in Room A, participants remained seated. Five minutes before this phase ended, participants provided the first saliva sample (−20 min), and they completed the STAI and PANAS questionnaires (pre-task). Next, the introduction phase started. This phase lasted 5 min, during which participants received instructions about the stress task in front of a committee in the same room where the task would take place, and so they were accompanied to Room B. After this, participants came back to Room A, where they had 10 min to prepare the speech task in Room A. Immediately before the onset of the stress task, participants provided the second saliva sample (0 min). Following this phase, the stress task was carried out in Room B. Afterwards, participants rested for 60 min in Room C (recovery phase). Fifteen min after the end of the stress task, participants were instructed to complete the STAI and PANAS questionnaires (post-task) while collecting the third saliva sample (+25 min). In addition, they provided three more saliva samples (+40 min, +55 min, and +70 min) (Fig. 1). At 100 min, the session ended, and the participants were debriefed.

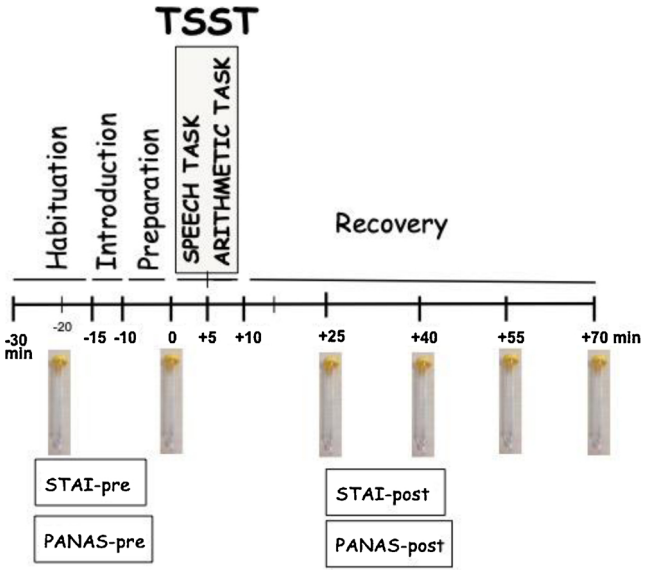

Fig. 1. Timeline of the procedure.

2.4. Psychological questionnaires

2.4.1. Mood

The participants' mood was assessed using the Spanish version (Sandín et al., 1999) of the Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1998). From this 20-item questionnaire, two dimensions (each measured by 10 items) were obtained: positive affect (PA: *interested*, *excited*, *strong*, *enthusiastic*, etc.) and negative affect (NA: *distressed*, *upset*, *guilty*, *scared*, etc.). Participants completed the questionnaire based on how they felt at that particular moment, using a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely). In the current study, the Cronbach's alphas were as follows: PANAS-pre task (PA: 0.79 and NA: 0.78) and PANAS-post task (PA: 0.84 and NA: 0.86).

2.4.2. Anxiety

The state anxiety of the participants was assessed using the Spanish version (Seisdedos, 1988) of the State Anxiety Inventory (STAI form S; Spielberger, Gorsuch, & Lushene, 1970). This questionnaire consisted of 20 items (e.g. '*I feel at ease*', '*I feel upset*') that evaluated how participants felt at that particular moment, rated on a 4-point Likert scale ranging from 0 (not at all) to 3 (extremely). In the present study, Cronbach's alphas were as follows: STAI-pre task (direct scale: 0.77 and indirect scale: 0.83) and STAI post-task (direct scale: 0.77 and indirect scale: 0.90).

2.5. TSST performance

2.5.1. Performance on speech task assessed by the committee

The committee was asked to fill out an evaluation sheet about each participant's speech performance. This sheet was completed by the two members of the committee using a 4-point Likert scale ranging from 1 (bad performance) to 4 (perfect performance), similarly to Villada et al. (2018) (see supplementary data 1). They rated the 10 features of the speech: *Introduction* (the participant introduces him/herself, briefly presents the contents of the speech, and gains the audience's attention); *Speech development* (coherent and logical speech); *Closing* (integration of the speech); *Content* (whether the participants link their personal characteristics to the hypothetical job); *Body posture and visual contact* (good body posture, relaxed and self-confident, makes eye contact with the two members of the committee); *Non-verbal communication* (facial expressions and body language generate strong interest and enthusiasm about continuing to listen to the speech); *Volume* (adequate volume to

Table 2
The Intracalass Correlation Coefficient (ICC) average between raters on the verbal and non-verbal features of the speech task during the TSST.

	Feature	ICC Average
Verbal	Introduction	0.814
	Speech Development	0.750
	Closing	0.699
	Content	0.803
	Verbal Fluency	0.789
	Comprehension	0.939
Non-Verbal	Body Posture and Visual Contact	0.679
	Non-verbal Communication	0.620
	Volume	−0.053
	Final Result	0.850

listen to the entire speech); *Verbal fluency* (no lengthy silences and/or crutches); *Comprehension* (no need to ask questions and uses all the allotted speech time); and *Final Result* (overall the speech was clear and to the point, and the person is a good candidate for the job). To assess the interrater reliability of the measurements, the Intraclass Correlation Coefficient (ICC) was computed (see Table 2). The ICC for the three features that asked about non-verbal competences did not reach the threshold of 0.7, and so these features were not taken into account in the analyses.

To assess verbal performance, the mean of the two ratings of each feature of the verbal performance speech was computed. To reduce the number of variables, exploratory factor analysis with varimax rotation with the verbal performance features were performed. The Kaiser criterion (dropping all components with eigenvalues < 1.0) and scree plot inspection were used to determine the number of factors. Only one factor was identified, *Verbal Performance*, which explained 64% of the total variance. Factor loadings ranged from 0.64 (Verbal Fluency) to 0.91 (Speech Development). The Kaiser-Meyer-Olkin (KMO) indicated a satisfactory relationship between sample size and the number of variables (0.889), and Bartlett's test indicated that the correlations between variables were sufficient to warrant factor analysis, $\chi^2_{(21)} = 285.890$, $p < 0.001$. Finally, the composite measure of Verbal Performance using the mean was computed. This variable consisted of the following features: Introduction, Speech Development, Closing, Content, Verbal Fluency, and Comprehension (Table 2).

2.5.2. Self-reported performance

Following Villada et al. (2018), after the TSST, the participants were asked for their opinion of their performance on the oral speech task, using the question "*How well do you think you performed?"* Participants answered this question on a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely). This variable was called self-reported performance.

2.5.3. Arithmetic task performance

After the oral speech, participants were asked to subtract thirteen from a specific number. Every time a mistake was made, the committee said: "ERROR, start from the beginning again". To assess performance on this task, the number of mistakes the participant made during the arithmetic task was coded (Mohiyeddini et al., 2013a; Mohiyeddini, Bauer, & Semple, 2013b). Agreement between the two members of committe was 100%.

2.6. Biochemical analyses

To assess cortisol and DHEA, six saliva samples were collected by means of passive drooling. For each saliva sample, participants deposited 3 ml of saliva in a plastic vial, which took approximately five minutes. They were instructed to collect as much saliva as possible from their mouths without forceful movements. During the saliva sample collection, participants could not drink water. All samples were frozen at -20 °C until the biochemical analyses were conducted by the first author at the Laboratory of Social Cognitive Neuroscience of the University of Valencia (Spain).

2.6.1. Salivary cortisol

To measure salivary cortisol concentrations the Spectria Cortisol RIA kit from Orion Diagnostica (Espoo, Finland) was used. Assay sensitivity was 0.8 nmol/l. Each saliva sample was determined in duplicate. Each participant's samples were analyzed in the same trial. The within and inter assay variation coefficients were all below 8%.

2.6.2. Salivary DHEA

To measure salivary DHEA concentrations, the DHEA Enzyme Immunoassay Kit from Salimetrics (Suffolk, UK) was used. Assay sensitivity was 5 pg/mL. Each saliva sample was determined in duplicate. Each participant's samples were analyzed in the same trial. The within and inter assay variation coefficients were all below 10%. DHEA concentrations were not detectable in 2 or more salivary samples of 13 participants (5 men and 8 women), and so they were excluded from the DHEA analyses. We explored whether there were differences in demographic variables between those with non-detectable DHEA versus those with detectable DHEA levels. Participants with non-detectable DHEA concentrations were older ($p = 0.019$) and had higher SES ($p = 0.052$) and education levels ($p = 0.045$) than participants with detectable DHEA levels. There were no differences between the two groups in BMI ($p = 0.903$).

2.7. Statistical analyses

Student's *t*-tests were performed to investigate sex differences in the demographic variables.

To investigate whether cortisol, DHEA, mood (positive, negative), and anxiety changed during the TSST, repeated-measures ANOVAs were performed. We included Time as a within-subject factor (for anxiety and mood: pre-task, post-task; for cortisol and DHEA: -20 min, 0 min, +25 min, +40 min, +55 min and +70 min), and Sex (man or woman) as a between-subject factor. Greenhouse-Geisser was used when the requirement of sphericity in the repeated-measures ANOVAs was violated. *Post-hoc* planned comparisons were performed using Bonferroni adjustments for the *p* values.

To investigate whether Cortisol and DHEA secretion were related to TSST performance, hierarchical regression analyses were performed. Separate analyses were conducted for each TSST performance outcome (i.e. Verbal Performance, Arithmetic Result, and Self-Performance). To study the associations between basal Cortisol and basal DHEA (cortisol or DHEA concentrations in the first saliva sample) and TSST performance, in step 1, Sex, Age, SES, and Education Level were included as control variables, and in step 2, basal Cortisol or DHEA was introduced. To study the relationships between Cortisol or DHEA reactivity (calculated by delta value MAX-basal) and TSST performance, in step 1, in addition to the control variables, basal Cortisol or basal DHEA was introduced, as suggested by Dalecky and Willits (1991), and in step 2, Cortisol or DHEA reactivity was included. Regression analyses with AUCg Cortisol, AUCi Cortisol, AUCg DHEA, and AUCi DHEA as predictors, and each outcome of TSST performance as dependent variables, are included in supplementary material 2.

Two outliers in the cortisol data (one man and one woman) and two outliers in the DHEA data (one man and one woman) were excluded from the analyses because their concentrations were more than 3 SD from the mean. One man's arithmetic task performance was also more than 3 SD from the mean, and so he was excluded from the arithmetic task performance analyses.

Salivary cortisol and DHEA values did not have a normal distribution and, therefore, were log transformed. All *p* values reported are two-tailed. The results shown are means ± standard error of mean (SEM). Statistical analyses were performed with SPSS 22.0. In order to aid the

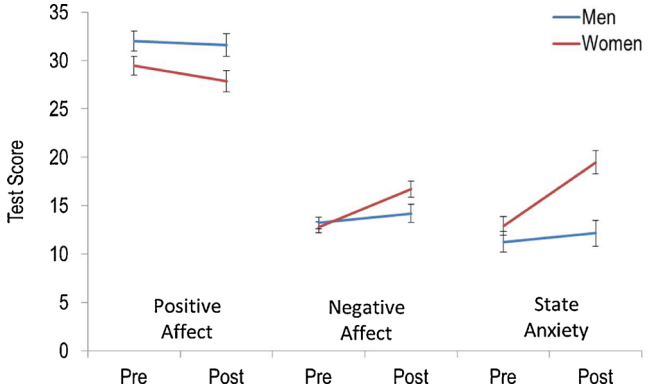

Fig. 2. Positive affect, negative affect, and state anxiety before (pre) and after (post) the TSST in men (n = 30) and women (n = 35).

interpretation of the figures, the values are presented as raw data.

3. Results

3.1. Psychological changes

Fig. 2 shows participants' scores on positive and negative affect and state anxiety before and after the TSST.

3.1.1. Mood

Results showed that positive mood did not change during the TSST (Time: $p = 0.104$, $\eta^2 = 0.042$), and that this lack of change did not differ between men and women (Time × Sex: $p = 0.321$, $\eta^2 = 0.016$). However, regardless of time, men did experience more positive mood than (Sex: $F_{(1, 63)} = 5.117$, $p = 0.027$, $\eta^2 = 0.075$). However, participants' negative mood increased during the TSST (Time: $F_{(1, 63)} = 13.339$, $p = 0.001$, $\eta^2 = 0.175$). There were no differences between men and women in overall negative mood (Sex: $F_{(1, 63)} = 1.650$, $p = 0.204$, $\eta^2 = 0.026$), but men and women did experience a different change in negative mood (Time × Sex: $F_{(1, 63)} = 4.775$, $p = 0.033$, $\eta^2 = 0.070$). Although men and women started the study with similar levels of negative mood ($p = 0.616$, $\eta^2 = 0.004$), after the TSST, negative mood was higher in women than in men ($p = 0.046$). In addition, negative mood increased in women (pre vs. post: $p < 0.001$, $\eta^2 = 0.227$), but it did not change in men (pre vs. post: $p = 0.321$, $\eta^2 = 0.016$).

3.1.2. Anxiety

Results showed that, overall, participants experienced an increase in anxiety during the TSST (Time: $F_{(1, 63)} = 19.508$, $p < 0.001$, $\eta^2 = 0.236$). Furthermore, overall, women experienced more anxiety than men (Sex: $F_{(1, 63)} = 10.384$, $p = 0.002$, $\eta^2 = 0.236$). Moreover, men and women experienced a different change in anxiety (Time × Sex: $F_{(1, 63)} = 11.291$, $p = 0.001$, $\eta^2 = 0.152$). Although men and women had similar baseline levels ($p = 0.257$, $\eta^2 = 0.020$), after the TSST, women experienced more anxiety than men ($p < 0.001$). Furthermore, in women, anxiety increased during the TSST (pre vs. post: $p < 0.001$, $\eta^2 = 0.342$), whereas in men it did not (pre vs. post: $p = 0.474$, $\eta^2 = 0.008$).

3.2. Physiological changes

3.2.1. Cortisol

Results showed that cortisol concentrations changed throughout the session (Time: $F_{(1.9, 116.6)} = 29.501$, $p < 0.001$, $\eta^2 = 0.326$), see Fig. 3. Men and women did not differ in their changes in cortisol (Time × Sex: $p = 0.670$, $\eta^2 = 0.006$), and overall cortisol concentrations did not differ between men and women (Sex: $p = 0.169$, $\eta^2 = 0.031$). Cortisol concentrations in the two samples provided before the speech task did not differ (-20 vs. 0: $p > 0.9$). The peak cortisol levels were reached 25 min after the onset of the speech task (+25 vs. -20 or 0: $p < 0.001$). Afterwards, cortisol concentrations decreased until reaching baseline levels in the last saliva sample (+70 vs. -20 or 0: $p > 0.4$).

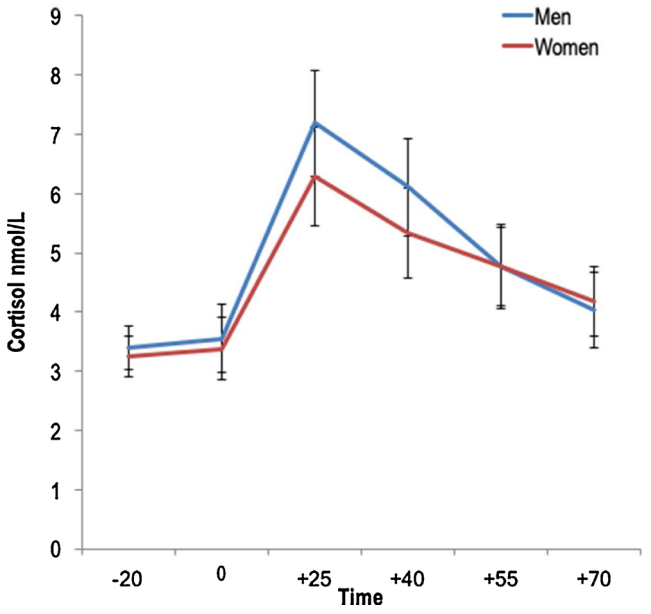

Fig. 3. Cortisol secretion in men and women across the entire session.

3.2.2. DHEA

Results showed a main effect of Time for DHEA ($F_{(3.6, 170.91)} = 15.263$, $p < 0.001$, $\eta^2 = 0.241$), see Fig. 4. However, Sex ($p = 0.589$, $\eta^2 = 0.006$) and the Time × Sex interaction ($p = 0.640$, $\eta^2 = 0.012$) were not significant. DHEA concentrations were similar between baseline and the sample provided immediately before the onset of the speech task (-20 vs. 0: $p > 0.9$). Participants reached their peak DHEA levels after the TSST, 25 min after the onset of the speech, although this sample (+25) was not statistically different from the DHEA concentrations in samples -20 or 0 (both $p > 0.4$). Furthermore, DHEA concentrations decreased to below baseline levels in the last saliva sample (+70 vs. -20: $p < 0.001$).

3.3. Relationship between hormonal levels and TSST performance

3.3.1. Cortisol levels as predictor of TSST performance

Regression analyses showed that basal cortisol levels were negatively related to the self-reported performance outcome. Thus, participants with higher basal cortisol levels thought their performance on the speech task was worse ($p = 0.034$). However, cortisol reactivity was positively related to verbal performance ($p = 0.041$). Finally, cortisol levels were not related to arithmetic task performance (both $p > 0.597$) (Table 3).

3.3.2. DHEA levels as predictor of TSST performance

Regression analyses for DHEA levels showed a negative association between DHEA baseline levels and the self-reported performance outcome. Thus, participants who had higher DHEA baseline levels at the onset of the session thought their performance on the speech task was worse ($p = 0.043$). In addition, a positive association was found between DHEA reactivity and the verbal performance outcome ($p = 0.007$). No associations were found between DHEA levels and arithmetic task performance (both $p > 0.649$) (Table 3).

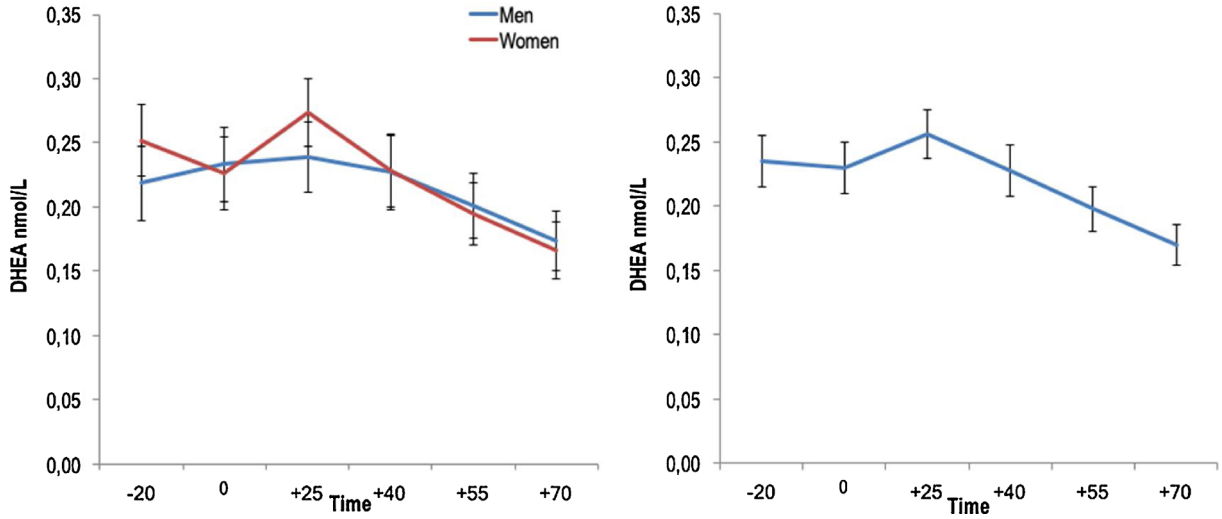

Fig. 4. DHEA secretion in men and women (left) and the complete sample (right) across the entire session.

Table 3
Regression analyses with cortisol and DHEA levels as predictors and TSST performance outcomes as dependent variables.

	Speech task		Arithmetic task
	Verbal Performance	Self-performance	
Cortisol Basal			
R^2	0.350	0.317	0.038
Adj R^2	0.294	0.259	−0.046
ΔR^2	0.018	0.055	0.001
β	−0.148	−0.257	0.038
p	0.204	**0.034**	0.788
Cortisol Reactivity			
R^2	0.383	0.301	0.042
Adj R^2	0.317	0.226	−0.062
ΔR^2	0.048	0.000	0.005
β	0.228	−0.005	0.073
p	**0.041**	0.967	0.597
DHEA Basal			
R^2	0.325	0.295	0.062
Adj R^2	0.248	0.215	−0.047
ΔR^2	0.002	0.069	0.002
β	−0.048	−0.280	0.049
p	0.717	**0.043**	0.756
DHEA Reactivity			
R^2	0.439	0.316	0.061
Adj R^2	0.359	0.219	−0.076
ΔR^2	0.107	0.026	0.005
β	0.364	0.178	0.082
p	**0.007**	0.217	0.649

3.3.3. Comparison of Cortisol and DHEA levels as joint predictors of TSST performance

Because both cortisol and DHEA were related to TSST performance, and each of these relationships may be influenced by the other hormone (Shields et al., 2016), we repeated the regression analyses controlling for basal levels of DHEA or cortisol in the basal cortisol or DHEA analysis, respectively; and controlling for DHEA or cortisol reactivity in the cortisol or DHEA reactivity analysis.

In these regression analyses, the negative associations between basal cortisol and basal DHEA and self-reported performance disappeared after controlling for the other hormone (both $p > 0.156$). Regarding DHEA reactivity, it continued to be a significant predictor of verbal performance ($\beta = 0.316$, $p = 0.038$) (see Fig. 5); by contrast, cortisol reactivity was no longer a significant predictor of this TSST performance outcome ($\beta = 0.131$, $p = 0.350$), see Table 4.

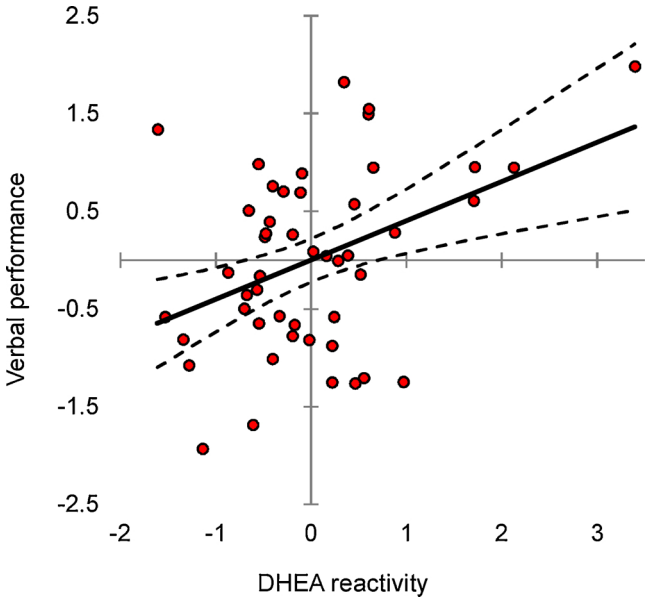

Fig. 5. Relationship between Verbal performance and DHEA reactivity after controlling for the cortisol reactivity.

3.4. Exploratory analyses

We also explored whether there was an interaction between DHEA and cortisol levels when relating them to verbal performance on the task. To test this, the procedure used by Shields et al. (2016) was followed and DHEA/cortisol basal and reactivity ratios were calculated. Results showed that basal DHEA/cortisol and DHEA/cortisol reactivity did not predict verbal performance ($\beta = 0.080$, $p = 0.515$ and $\beta = 0.160$, $p = 0.257$, respectively) or self-reported performance ($\beta = -0.009$, $p = 0.948$ and $\beta = -0.181$, $p = 0.231$, respectively).

4. Discussion

The aims of the present study were to investigate whether older men and women showed DHEA and cortisol responses to acute psychosocial stress, and whether these responses were related to performance on TSST tasks. To do so, 30 healthy older men and 35 healthy older postmenopausal women were exposed to the TSST. After the stress task, all the participants increased their negative mood, state anxiety, and

Table 4
Regression analyses with cortisol and DHEA basal levels (after controlling for DHEA or cortisol basal levels, respectively) and cortisol and DHEA reactivity (after controlling for DHEA or cortisol reactivity, respectively) as predictors and TSST performance outcomes as dependent variables.

	Speech task		Arithmetic task
	Verbal Performance	Self-performance	
Cortisol Basal			
R^2	0.347	0.330	0.069
Adj R^2	0.254	0.234	−0.068
ΔR^2	0.017	0.032	0.023
β	−0.150	−0.208	−0.175
p	0.305	0.162	0.318
Cortisol Reactivity			
R^2	0.448	0.332	0.062
Adj R^2	0.354	0.218	−0.103
ΔR^2	0.012	0.012	0.006
β	0.131	−0.131	−0.100
p	0.350	0.398	0.602
DHEA Basal			
R^2	0.347	0.330	0.069
Adj R^2	0.254	0.234	−0.068
ΔR^2	0.000	0.033	0.008
β	0.006	−0.206	0.103
p	0.967	0.156	0.549
DHEA Reactivity			
R^2	0.438	0.311	0.061
Adj R^2	0.340	0.191	−0.107
ΔR^2	0.065	0.044	0.006
β	0.316	0.260	0.106
p	**0.038**	0.118	0.619

cortisol levels, whereas positive mood and DHEA did not change. Moreover, basal DHEA and cortisol levels predicted self-reported performance, whereas DHEA and cortisol reactivity predicted verbal performance (rated by committee). However, after controlling for the other hormone's reactivity, only DHEA reactivity was related to verbal performance. No relationships were found between basal levels and reactivity of cortisol and DHEA and the arithmetic task performance.

Our findings confirm that the experimental procedure (i.e. TSST) induced stress at a psychological level, given the changes observed in negative mood and anxiety in our sample. Furthermore, only women experienced an increase in negative mood and anxiety, whereas men's scores did not change. We also observed these sex differences in a previous study by our group (Pulopulos et al., 2015).

At a physiological level, the TSST induced a significant cortisol response in men and women, but, unexpectedly, no sex differences were found. It is well known that in the cortisol response to acute stress there is a clear effect of sex, with men usually showing a greater response than women (for a review see: Kudielka, Hellhammer, & Wust, 2009; Pulopulos et al., 2018). One explanation for this discrepancy would be that, as can be observed, although the men in our sample experienced a stress-induced cortisol increase response, the magnitude of this cortisol response was moderate and similar to the women's cortisol response, a finding that is not comparable to other previous studies (Almela et al., 2011; Hidalgo, Almela, Villada, & Salvador, 2014, 2015; Pulopulos et al., 2013). A potential explanation for our findings is that men reported slightly higher SES and educational level than women, and so they may have had more psychological resources to deal with the stressful situation, and, thus, responded with a smaller cortisol increase (Derry et al., 2013).

With regard to the DHEA response to stress, our result does not support our hypothesis. Based on Fang et al.'s findings (Fang et al., 2014), we expected that DHEA levels would increase in response to the TSST in women. However, we failed to find a significant DHEA response to stress in men and women when the DHEA concentrations 25 min after the onset of the stressor were compared to those at the onset (0 min sample) and 20 min earlier. This result contrasts with studies investigating the DHEA response to the TSST in young people (mean: from 20 to 22 years old) (Izawa et al., 2008; Phan et al., 2017; Shields et al., 2016; Shirotsuki et al., 2009) and middle-aged people (mean: 37 years old) (Lennartsson et al., 2012; Lennartsson, Theorell, Kushnir, Bergquist, & Jonsdottir, 2013). To our knowledge, only one previous study investigated the impact of acute stress on the DHEA response in older people (Fang et al., 2014), finding, in contast to our results, a significant DHEA response to the TSST. Several methodological factors can explain these divergent results. First, in the present study we measured the DHEA levels by collecting saliva samples, whereas Fang et al. (2014) used blood samples, which could have caused an increased in DHEA levels in response to the blood extraction itself rather than exposure to the stressor. Second, we measured the DHEA levels with an enzyme immunoassay kit, whereas Fang et al. (2014) used a radioimmunoassay kit. Third, the age range of the Fang et al. (2014) sample was slightly broader and younger than our sample (50–74 vs. 56–73 age old, respectively).

It is possible that a DHEA response to stress has not been detected because overall DHEA secretion declines with older age (Carlström et al., 1988; Orentreich, Brind, Rizer, & Vogelman, 1984; Sulcova, Hill, Hampl, & Stárka, 1997). During aging, different changes occur, such as a reduction in the functional cells in the zona reticularis, reduced ACTH sensitivity of these cells, altered zonation in the adrenal cortex, and decreased 17,20-desmolase enzymatic activity (Wolf & Kirschbaum, 1999). These age-related alterations could lead to both lower baseline levels and a lower - or even non - response to stress in older populations. In support of this idea, a negative relationship between age and baseline DHEA levels (Fang et al., 2014) and the magnitude of the change in DHEA in response to TSST (Lennartsson et al., 2012, 2013) has been reported. These age-related changes could explain the absence of DHEA reactivity in response to the TSST in the current study. In addition, it is worth noting that, in our study, the participants with no detectable DHEA levels were older than the participants with detectable DHEA levels. Thus, more research is needed to shed light on the role of aging in the DHEA response to acute stress.

Sex differences in the DHEA response to acute stress have been understudied, given that previous studies have only included either men (Izawa et al., 2008; Oberbeck et al., 1998; Shirotsuki et al., 2009) or women (Fang et al., 2014; Pico-Alfonso et al., 2007). In the current study, although women seem to show a higher DHEA response to the TSST (see Fig. 4), no significant sex differences were found. This lack of a sex effect is consistent with previous studies carried out with young people (Lennartsson et al., 2012; Phan et al., 2017; Shields et al., 2016), and it supports the idea that, as occurred in young people, the DHEA response to acute stress may not be affected by sex in older people.

We also found that when cortisol and DHEA were considered in isolation, basal levels of cortisol and DHEA were negatively related to self-reported performance, whereas cortisol and DHEA reactivity were positively related to verbal performance assessed by the committee. However, when these hormones were considered simultaneously, we found that only DHEA reactivity predicted verbal performance. Thus, a higher DHEA response to TSST was related to better verbal performance, after controlling for cortisol reactivity. The mechanism underlying the positive effect of DHEA is unclear, although there are some possible explanations for this finding. For example, it has been well established that DHEA, as an anabolic hormone, has beneficial effects on wellbeing and cognition, given its neuroprotective, antioxidant, anti-inflammatory, and anti-glucocorticoid effects (for a review see: Maggio et al., 2015). Thus, it is possible that its beneficial effects extend to the way the individual copes with a stressful situation. In addition, the speech task could be considered a verbal memory task because the person has to speak about his/her personal characteristics. Because DHEA can modulate $GABA_A$, NMDA, and $sigma_1$ receptor functions (Maninger, Wolkowitz, Reus, Epel, & Mellon, 2009; Pérez-Neri, Montes, Ojeda-López, Ramírez-Bermúdez, & Ríos, 2008; Yabuki et al., 2015; Yadid, Sudai, Maayan, Gispan, & Weizman, 2010), which are involved

in cognitive function (for a review see: Wolf & Kirschbaum, 1999), it is possible that DHEA exerts its effect on this type of memory through its effects on these receptors. Another alternative explanation would be that participants considered the stressor to be a challenge, rather than a threat, and increased their DHEA levels during the stressor, leading to better performance (Crum, Akinola, Martin, & Fath, 2017).

Furthermore, unlike the verbal performance on the speech task, DHEA did not predict performance on the arithmetic task. There are several potential explanations for this finding. First, the nature of each task is different, and they involve different cognitive processes. It is possible that stress-induced DHEA affects the cognitive processes involved in speaking more and the processes involved in arithmetic calculations less. Another explanation would be that our way of assessing arithmetic performance was not sensitive enough to be able to detect a relationship with hormonal levels. We followed the procedure of Mohiyeddini et al. (2013a, 2013b) by counting the number of time participants made a mistake. However, as a reviewer pointed out, another method would be to also take math speed into account. In sum, more research is needed to fully understand the relationship between DHEA and the cortisol response to stress and arithmetic task performance.

We failed to find a relationship between cortisol and TSST performance after controlling for DHEA. It is possible that, as occurs with declarative (Hidalgo et al., 2014, 2015; Pulopulos et al., 2013) and working memory (i.e. executive component) (Pulopulos et al., 2015), older people are less sensitive to stress-induced cortisol on speech and arithmetic tasks. In our opinion, an age-related dysregulation of HPA-axis activity (Mizoguchi et al., 2009) and functional changes in the amygdala and hippocampus (Mather, 2006; Murthy et al., 2010; St. Jaques, Dolcos, & Cabeza, 2009) could underlie this lack of a relationship between cortisol and performance on these kinds of tasks in older people (for a review see: Hidalgo, Pulopulos, & Salvador, 2019).

It is important to mention several limitations of the present study. We cannot rule out the possibility that we failed to detect the DHEA response, although DHEA levels were measured twice before the stressor and four times after the stressor, based on previous knowledge. The age range of our population was relatively broad (56–73 age). Thus, future studies may find stronger effects of aging when considering even older populations with stricter age ranges (e.g., $\geq$ 70 years old). Finally, it is important to note that, in order to guarantee a homogeneous sample, the inclusion criteria were very selective, and only people who met the required health conditions could participate in this study. Thus, the study findings cannot be generalized to older people with age-related diseases and medication use.

In summary, to the best of our knowledge, the current study is the first to investigate, in healthy older men and women, both their DHEA and cortisol response to an acute stressor and their verbal and arithmetic performance during the stressor. Our results revealed that older people did not show a significant DHEA response, but they displayed a consistent cortisol response to an acute psychosocial stressor. In addition, DHEA reactivity predicted verbal performance after controlling for cortisol reactivity. These findings support the idea that DHEA could help individuals to cope successfully with a stressor, at least when this stressor involves a verbal task.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopsycho.2019.107786.

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