Research report

Acute stress affects free recall and recognition of pictures differently depending on age and sex

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HIGHLIGHTS

- Acute stress has effects on memory retrieval in mixed-sex samples of different ages.
- Older people showed a lower stress-induced cortisol response than young people.
- Pictures from the IAPS were used to study the stress effects on memory retrieval.
- Stress impaired free recall of emotional and neutral pictures only in young men.
- Stress impaired recognition memory for positive pictures in all participants.

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ABSTRACT

Little is known about age differences in the effects of stress on memory retrieval. Our aim was to perform an in-depth examination of acute psychosocial stress effects on memory retrieval, depending on age and sex. For this purpose, data from 52 older subjects (27 men and 25 women) were reanalyzed along with data from a novel group of 50 young subjects (26 men and 24 women). Participants were exposed to an acute psychosocial stress task (Trier Social Stress Test) or a control task. After the experimental manipulation, the retrieval of positive, negative and neutral pictures learned the previous day was tested. As expected, there was a significant response to the exposure to the stress task, but the older participants had a lower cortisol response to TSST than the younger ones. Stress impaired free recall of emotional (positive and negative) and neutral pictures only in the group of young men. Also in this group, correlation analyses showed a marginally significant association between cortisol and free recall. However, exploratory analyses revealed only a negative relationship between the stress-induced cortisol response and free recall of negative pictures. Moreover, stress impaired recognition memory of positive pictures in all participants, although this effect was not related to the cortisol or alpha-amylase response. These results indicate that both age and sex are critical factors in acute stress effects on specific aspects of long-term memory retrieval of emotional and neutral material. They also point out that more research is needed to better understand their specific role.

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1. Introduction

A large body of research in animals and humans shows that stress affects memory. Stress involves the release of glucocorticoids (corticosterone in rodents, cortisol in humans) and catecholamines due to the activation of the hypothalamus–pituitary–adrenal axis (HPA-axis) and the sympathetic nervous system (SNS), respectively. While glucocorticoids can cross the blood–brain barrier and bind to receptors (i.e., mineralcorticoid and glucocorticoid receptors) located in the hippocampus, prefrontal cortex and amygdala, brain areas related to memory processes [1–3], the catecholamines...
do not have this property. Thus, the latter exert their action on memory by activating the β-adrenergic receptors on vagal afferents projecting to the nucleus of the solitary tract in the brainstem [4], and these noradrenergic projections influence the neuronal activity of the amygdala [5]. Nevertheless, memory can be enhanced, impaired or even unaffected by stress because factors such as the memory phase tested (i.e., learning, consolidation or retrieval), the emotional valence of the material to be remembered (i.e., emotional or neutral), or the age and sex of the individuals can modulate this relationship.

As found in animal studies, a pharmacologically-induced [6–9] or stress-induced [10–16] cortisol increase impairs retrieval performance in young people. The effect of stress on long-term memory (24 h at least) retrieval seems to be rather consistent because impairing effects have been observed when stress triggers high [11,13,14] and moderate [10,15,16] cortisol responses. In these studies, different types of memory tasks with different levels of difficulty have been employed, such as lists of words (with 30 words in Kuhlmann et al. [13] and Smeets [16], 80 words in Buchanan et al. [10] and 100 words in Smeets et al. [15]), pictures (20 in Buchanan and Tranel [11]) and paragraphs [14]. A few studies have shown a lack of a stress effect on long-term memory retrieval in young women in the luteal phase of the menstrual cycle [17] and when the memory retrieval was performed two or more days after learning [18,19].

One modulatory factor in the relationship between cortisol and memory seems to be the emotional valence of the material to be remembered (i.e., emotional or neutral). Emotional material induces a greater noradrenergic activation of the amygdala than neutral material, and, as has been described, the interactions between the amygdala and hippocampus are crucial in finding cortisol effects on hippocampus-dependent memory performance [3]. Thus, the majority of studies carried out in young people showed a stronger impact of cortisol or stress on memory for emotionally arousing material than for neutral material (for a review see: [20]).

Most of the studies on the effects of cortisol administration or stress-induced cortisol increases on memory have been conducted in young people. However, some age-related changes may affect the relationship between stress-induced cortisol response and memory performance in the older population. Previous studies have suggested that older people show (compared to young people) changes in the functional connectivity between the amygdala and hippocampus and decreases in amygdala activation for negative stimuli [21–24]. Thus, given that interactions between the amygdala and hippocampus seem to be essential to observe cortisol effects on hippocampus-dependent memory performance [3], this age-related change may influence the effects of stress and cortisol on long-term memory retrieval in older people. Another change that can be observed in the aging brain is a loss and/or dysfunction of mineralcorticoid and glucocorticoid receptors [25–27], which could make older people’s memory less sensitive to being affected by cortisol increases [28,29].

In spite of evidence suggesting an age-related change in stress and cortisol effects on memory performance, only a few studies have been reported in older people. Previous studies investigating the effects of stress on memory in older people have mainly shown that cortisol increases before learning (i.e., without differentiating stress effects on the learning, consolidation or retrieval phases) impair memory performance [30–33]; but see [34], an effect that seems to be due to the detrimental effect of cortisol on retroactive interference in older people, but not in young adults [31]. By contrast, studies in animals and humans have shown a lack of stress and cortisol effects on working memory, spatial memory and declarative and non-declarative memory [33,35–38]. To our knowledge, only one study investigated the effects of acute stress on long-term memory retrieval in a sample of older people, finding no effects of stress [39]. However, although some previous studies have used both older and young samples to investigate the effects of cortisol increases on learning [31], and a short-time after learning [33], there are no studies that have directly compared the effects of a stress-induced cortisol increase on long-term memory retrieval in young and older people.

In order to further examine the lack of cortisol effects on long-term memory retrieval in older people found in our previous study [39], we aimed to compare them to effects in young people. To do so, we investigated the stress effects on long-term memory retrieval performance for pictures in the older sample and in a novel sample of young people. Thus, in the present study we have compared, for the first time, the effects of a stress-induced cortisol increase on long-term memory retrieval of pictures in older and young people. To this end, two age groups of participants (older and young) were exposed to the Trier Social Stress Test (TSST) or a control task. After the stress or control task, free recall and recognition of pictures learned one day before were assessed. Moreover, in order to investigate whether the emotional arousal of the memory material plays a crucial role in the acute stress effects on memory retrieval, we used positive, negative and neutral pictures. Finally, we also tested whether the participants’ sex influenced the stress effects on retrieval, due to the existence of sex differences in the stress response and their effects on this type of memory. Based on the literature, we expected stress to impair long-term memory retrieval in young people [10,11,13–16], but not in older people [39]. In addition, because sex-related differences in young people have been reported [40–42], we hypothesized that there would be a stronger impairing effect in young men, due to their expected higher cortisol response to the stressor [43–45] and the protective effects of estrogen in women [46].

2. Methods

2.1. Participants

The current study is part of an extensive on-going project (Mneme Project) aimed to investigate the effects of psychosocial stress on memory performance, taking into account different moderating factors (including age and sex) through separate and consecutive studies in healthy people. Here, we studied a sample composed of 102 subjects divided into a group of older people (from 56 to 76 years of age) and a group of young people (from 18 to 27 years of age). Participants were submitted to one of two different conditions (stress or control). The older group (N = 52) was composed of 27 men (stress = 12, control = 15) and 25 women (stress = 13, control = 12). The young group (N = 50) consisted of 26 men (stress = 14, control = 12) and 24 women (stress = 12, control = 12), all undergraduate students. The older group belonged to a study program at the University for people over 55 years of age, and they had an educational level beyond high school. There were no significant differences between the two (stress vs. control) conditions on age, educational level or body mass index (BMI) (p > 0.286). Partial results from the older subsample have been previously reported [39]. In the current study, we added a group of young participants to the previous study in order to test whether the same experimental design would show stress effects on long-term memory retrieval of pictures in young adults. All the participants completed a general questionnaire to check whether they met the study prerequisites. In order to obtain an optimal comparison of the two age cohorts and eliminate a number of possible confounding factors that could interfere with the aim of the study, we applied very restrictive criteria. The exclusion criteria were: smoking more than 10 cigarettes a day; alcohol or other drug abuse; dental, visual or hearing problems; presence of cardiovas-
cular, endocrine, neurological, or psychiatric disease; having been under general anesthesia once or more than once in the past year; and the presence of a stressful life event during the past year (volunteers were asked whether they had experienced any situation that would affect them negatively). The presence of a stressful life event was considered an exclusion criterion because of its effects on both cognitive performance and HPA-axis functioning [2,47–49]. The 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 salivary alpha-amylose (sAA) levels, such as glucocorticoids, β-blockers, antidepressants, benzodiazepines, asthma medication, thyroid therapies or psychotropic substances. All the older women were postmenopausal, having had their last menstrual period more than 3 years before the testing time, and none of them were receiving estrogen replacement therapy. All the young women were regular, free-cycling and nulliparous, and none of them had taken oral contraceptives. All the participants in the older group scored more than twenty-eight on the MEC (Spanish version of the Mini-Mental Status Examination: [50]), indicating the absence of cognitive impairment, and none of them met the criteria for dementia, as defined by the NINCDS–ADRDA criteria for Alzheimer’s disease, or the criteria for Mild Cognitive Impairment, as defined by the European Consortium on Alzheimer’s Disease [51].

The participants who met the criteria were contacted by telephone and asked to attend two sessions that took place in a laboratory at the University. Previously, 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 two hours prior to the session, and not brush their teeth at least one hour prior to the session. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Research Ethics 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 consisted of two individual sessions: acquisition and retrieval. The first session (acquisition session) was similar for all subjects and was held between 10:00 and 12:00 h. In this session, the experimenter first checked whether they had followed the instructions given previously, and he/she noted their weight and height. After a period of habituation to the laboratory, participants were shown 30 color pictures consisting of 10 unpleasant (e.g., mutilated bodies), 10 pleasant (e.g., baby smiling) and 10 neutral (e.g., glass of water on a table) pictures extracted from the Spanish version [52] of the International Affective Picture System (IAPS; [53]). Pictures were presented individually for 5 s on a screen, followed by a black screen for 15 s, during which participants rated the pictures using the Self-Assessment Manikin (SAM) scales [54]. No mention of a memory test was made in order to ensure incidental encoding of stimuli. Participants were asked to return the next day, and they were not informed about the procedure. The second session (retrieval session) was carried out the next day between 16:00 and 18:00 h. In it, half the participants were randomly assigned to the stress condition, and the other half were assigned to the control condition.

Stress condition: To produce stress we used the Trier Social Stress Test (TSST, [55]). 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 and were filmed throughout both tasks. Before the TSST, participants completed the Positive and Negative Affect Schedule (pre-task PANAS) to obtain the baseline measure for mood. Immediately after the TSST, subjects filled out a questionnaire about some aspects of the task (Situational Appraisal) and the PANAS again (post-task PANAS). Finally, 15 min after the end of the TSST, participants performed the free recall and recognition memory tasks with the pictures they had seen the previous day. We collected four saliva samples to measure sAA and cortisol. Specifically, 15 min before the TSST (habituation phase), the first saliva sample was taken (−15 min pre-stress). The second saliva sample was collected immediately after the TSST (+10 min post-stress) at the onset of the recovery phase. Before the free recall, participants contributed the third saliva sample (+25 min post-stress). Finally, the last saliva sample was taken after the recognition memory test (+45 min post-stress).

Control condition: Both the stress and control conditions had the same schedules, but participants in this condition performed the control task instead of the stressful task. This control task consisted of 5 min of talking aloud about a recent non-emotional experience (i.e., a film or book) and 5 min of counting by 5 aloud, as in previous studies [41,56]. During this task, the participants remained standing, as in the stress condition. This task was designed to be similar to the stress task in mental workload and overall physical activity. However, to avoid evaluative threat and uncontrollability, the main components capable of provoking stress [57], during the control task participants were left alone in the room, and there was no video or committee present, unlike in the stress task. The two conditions were identical (same timing of the saliva samples, phase durations and questionnaires applied), and only the task differed (TSST vs. Control).

2.3. Questionnaires

Situational appraisal: Immediately after the task (stress or control), participants completed a questionnaire consisting of four questions about the following aspects of the task: stress, difficulty, frustration and effort. These questions were created based on previous studies on this topic [58,59]. Participants responded to each question on a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely).

Mood: It was evaluated by the Spanish version [60] of the PANAS [61]. This questionnaire is composed of 20 items distributed in 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. Subjects completed this questionnaire twice, before (pre-task PANAS) and immediately after the stress or control task (post-task PANAS), during the habituation and recovery phases, respectively. They were instructed to give their answers based on how they felt at that particular moment. Participants responded using a 5-point Likert scale ranging from 1 (not at all) to 5 (extremely). Sandín et al. [60] 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. Memory

Free recall task: To assess free recall, in the second session (retrieval session) participants were instructed to recall as many pictures as possible from the set they had seen in the first session (acquisition session). To do so, participants wrote a brief description of the pictures for 10 min. Free recall was scored by two independent judges who were blind to the group to which each participant belonged, and who determined which picture (if any) was being described. Agreement between judges was 91.5%, and discrepancies were discussed until a consensus was reached.
Recognition task: Participants viewed 60 pictures (30 new and 30 previously-viewed pictures) individually on a screen for 5 min. Each of the two sets of pictures was composed of 10 negative, 10 positive and 10 neutral pictures. Participants had to recognize the pictures they had seen before (in session 1). Thus, they verbally responded “yes” or “no” after seeing each picture on the recognition test. Recognition received two different scores: Hits, the number of pictures correctly recognized as being in the target presentation; and False alarms, the number of pictures incorrectly recognized as being in the target presentation. The difference between the percentage of hits and the percentage of false alarms was calculated to analyze the effects on recognition [62].

2.5. Biochemical analyses

Saliva samples were collected using salivettes (Sarstedt, Nümbrecht, Germany) for cortisol and sAA. Participants were instructed to keep the cotton swab in their mouths for exactly 2 min, not chew the cotton, and move the swab around in a circular pattern to collect saliva from all salivary glands. The samples were centrifuged at 3000 rpm for 15 min, resulting in a clear supernatant with low viscosity that was stored at −80°C until the analyses were performed in the Central Research Unit (Unidad Central de Investigación) of the Faculty of Medicine, University of Valencia (Spain). Salivary cortisol and sAA levels were measured in duplicate, and each participant’s sample was analyzed in the same trial.

Cortisol: The samples were analyzed by a competitive solid phase radioimmunoassay (tube coated), using the commercial kit Spectria Cortisol RIA from Orion Diagnostica (Espoo, Finland). Assay sensitivity was 0.8 nmol/L, and the within- and inter-assay variation coefficients were all below 8%.

Alpha-amylase (sAA): The concentration of sAA was measured by using an enzyme kinetic method with the commercial salivary α-amylase assay kit from Salimetrics (USA). Assay sensitivity was 0.4 U/mL. Inter- and intra-assay variation coefficients were all below 10%. Analyses of sAA failed to detect the sAA concentrations in the samples of three participants in the stress condition (one young man and two young women) and one in the control condition (one young woman). Therefore, these participants were removed from the statistical analyses for sAA.

2.6. Statistical analyses

Cortisol and sAA values were logarithmic transformed because they did not have a normal distribution after Kolmogorov–Smirnov and Levene’s tests were applied.

Student’s t-tests were conducted to evaluate differences in the demographic variables by condition (stress vs. control). Three-way ANOVAs were used to study condition, age (older vs. young) and sex (men vs. women) differences in situational appraisal. ANOVAs for repeated measures were performed to investigate the mood, the physiological response, ratings of picture material and memory performance. Finally, bivariate Pearson’s correlations were conducted between the free recall or recognition outcomes and cortisol or sAA responses to stress, calculated as the percentage increase from baseline to peak [62].

One outlier in the cortisol data (one older woman in the control condition) and two outliers in the sAA data (one older man and one young man in the stress condition) were removed from the analyses because their concentrations differed by more than 3 S.D. from the total sample mean. Four outliers in the recognition data (one older woman and one young woman in the stress condition, and one older man and one older women in the control condition) were removed from the recognition analysis because their scores differed by more than 3 S.D.

We used Greenhouse–Geisser when the requirement of sphericity in the ANOVA for repeated measures was violated. Post-hoc planned comparisons were performed using Bonferroni adjustments for the p values. The level of significance was taken as <0.05. When not otherwise specified, the results shown are means ± SEM. We used SPSS 19.0 to perform the statistical analyses. In order to provide an easy interpretation of the figures, the values in the figures represent raw values and not logarithmic-transformed values.

3. Results

3.1. Psychological response

Situational appraisal: Participants in the stress condition perceived the stress task as more stressful ($F(1, 93) = 43.399, p < 0.001$), difficult ($F(1, 93) = 42.577, p < 0.001$), frustrating ($F(1, 93) = 25.882, p < 0.001$) and requiring more effort ($F(1, 93) = 40.430, p < 0.001$) than participants in the control condition. Older participants ($25.1 ± 1.68$) perceived the stress task as less frustrating than younger participants ($27.2 ± 1.60$); however, no age differences were found for stress, difficulty or effort (for all $p > 0.226$). No sex differences were found on any of the variables evaluated (for all $p > 0.392$).

Mood: The repeated-measures ANOVA with Time (pre vs. post) as a within-subject factor and Condition, Age and Sex as between-subject factors showed that there were no baseline differences between the stress and control conditions on positive and negative mood (both $p > 0.437$). The Time × Condition interaction was significant for both positive ($F(1, 93) = 10.127, p = 0.002$) and negative ($F(1, 93) = 34.374, p < 0.001$) mood. Participants in the stress condition decreased their positive mood and increased their negative mood after the TSST task (both $p < 0.001$), while participants in the control condition did not (both $p > 0.391$). The Age factor was significant for both positive ($F(1, 93) = 19.125, p < 0.001$) and negative mood ($F(1, 93) = 5.507, p = 0.021$); overall, older participants had higher positive mood and lower negative mood than younger participants. For both affects, no significant effects of Sex (both $p > 0.145$) or the Condition × Sex × Age interaction (both $p > 0.349$) were found.

3.2. Physiological response

Salivary cortisol: The repeated-measures ANOVA with Time ($−15$, $+10$, $+25$, $+45$ min) as a within-subject factor and Condition, Age and Sex as between-subject factors showed main effects for Condition ($F(1, 93) = 32.960, p < 0.001$), Time ($F(1, 942, 180.652) = 21.580, p < 0.001$) and the Condition × Time interaction ($F(1, 942, 180.652) = 50.381, p < 0.001$). Baseline cortisol concentrations were similar in both conditions ($p = 0.773$). In the stress condition, cortisol levels increased immediately after the TSST ($p < 0.001$), reaching their peak 25 min after the onset of the stress task ($p = 0.001$). Although cortisol concentrations decreased in the last saliva sample, participants did not recover their baseline levels ($p < 0.001$). In the control condition, there were no differences in the cortisol concentrations between the $−15$, $+10$ and $+25$ min saliva samples (both $p > 0.99$), reflecting a lack of cortisol response to the control task. In addition, in the last saliva sample ($+45$ min), the cortisol concentrations decreased ($p < 0.001$), reaching lower levels than in the first sample ($−15$ min) ($p < 0.001$), in accordance with the cortisol circadian rhythm.

The Age factor was significant ($F(1, 93) = 18.487, p < 0.001$), as was the Condition × Time × Age interaction ($F(1, 942, 180.652) = 8.214, p < 0.001$). In both age groups, baseline cortisol did not differ between conditions (both $p > 0.126$). Higher cortisol concentrations were found in the stress condition than in the control condition in the rest of the salivary samples in both age
groups (all \( p < 0.008 \)). In the stress condition, older participants had significantly lower cortisol concentrations than young participants in the +10, +25 and +45 min saliva samples (all \( p < 0.007 \)) and, as a trend, in their baseline levels (\( p = 0.068 \)). However, in the control condition, older participants had lower baseline cortisol levels (\( p < 0.001 \)) and, as a trend, in the +10 min saliva sample (\( p = 0.058 \)), with similar levels found in the rest of the samples (both \( p = 0.128 \)) (see Fig. 1).

Finally, the Sex factor was significant (\( F(1, 93) = 8.790, p = 0.004 \)), with men showing higher cortisol concentrations than women. None of the interactions between Sex and the other factors were significant (all \( p > 0.187 \)).

**Salivary alpha-amylase (sAA):** The repeated-measures ANOVA with Time as a within-subject factor and Condition, Age and Sex as between-subject factors indicated that the factor Condition was not significant (\( F(1, 87) = 0.011, p = 0.918 \)), but the factor Time (\( F(2.653, 230.827) = 14.649, p < 0.001 \)) and the Time \( \times \) Condition interaction (\( F(2.653, 230.827) = 4.375, p = 0.007 \)) were significant. There were no baseline sAA concentration differences between the stress and control conditions (\( p = 0.328 \)). In the stress condition, the sAA concentrations increased immediately after the TSST (\( p = 0.015 \)), decreasing 25 min after the onset of the stress task (\( p = 0.002 \)), and recovering baseline concentrations in the last saliva sample (\( p > 0.99 \)). In the control condition, the sAA concentrations were similar to baseline after the control task (\( p > 0.99 \)), and they decreased over time (all \( p < 0.033 \)). There were no differences between the conditions in sAA concentrations in any sample (all \( p > 0.111 \)).

The Age factor was significant (\( F(1, 87) = 7.160, p = 0.009 \)), as the older participants had higher sAA concentrations. However, the Sex factor was not significant (\( F(1, 87) = 1.339, p = 0.250 \)). Nor were its interactions with other factors (all \( p > 0.99 \)) (see Fig. 2).

### 3.3. Ratings of picture material

A repeated-measures ANOVA with Valence (positive, negative, neutral) as a within-subject factor and Condition, Age and Sex as between-subject factors was used to analyze the classification of the valence and arousal of the pictures to be remembered.

**Valence:** Results confirmed the a priori classification, so that the negative pictures (\( M = 1.709, \text{SEM} = 0.089 \)) were rated lower than the neutral (\( M = 5.117, \text{SEM} = 0.081 \)) and positive pictures (\( M = 7.045, \text{SEM} = 0.082 \)) (for all \( p < 0.001 \)), and neutral pictures were rated lower than positive pictures (\( p < 0.001 \)). There were no significant differences based on condition, age or sex (all \( p < 0.217 \)).

**Arousal:** Results revealed that the neutral pictures (\( M = 3.711, \text{SEM} = 0.122 \)) were significantly scored as less arousing than the negative pictures (\( M = 7.605, \text{SEM} = 0.104 \)) (\( p < 0.001 \)) and, as a trend, less than the positive pictures (\( M = 4.007, \text{SEM} = 0.147 \) (\( p = 0.064 \)). Older participants (\( M = 5.5833, \text{SEM} = 0.136 \)) scored all the pictures as more arousing than the younger participants (\( M = 4.633, \text{SEM} = 0.137 \) (\( p < 0.001 \)). There were no significant differences based on condition or sex (both \( p > 0.203 \)).

### 3.4. Memory Performance

**Free Recall:** A repeated-measures ANOVA with Valence as a within-subject factor and Condition, Age and Sex as between-subject factors was used to measure the effect of stress on free recall of pictures. The results showed the main effects for Valence (\( F(2, 174) = 62.032, p < 0.001 \)), Age (\( F(1, 87) = 99.698, p < 0.001 \)), and the Valence \( \times \) Age interaction (\( F(2, 174) = 4.762, p < 0.010 \)). Post-hoc analyses revealed that all the participants recalled the negative pictures more than the positive and neutral pictures (both \( p < 0.001 \)), and the positive pictures more than the neutral pictures (both \( p < 0.001 \)). Regarding Age, older participants recalled fewer positive, negative and neutral pictures than young participants (all \( p < 0.001 \)). In addition, in both age groups, negative pictures were recalled more than positive (both \( p < 0.007 \)) and neutral pictures (both \( p < 0.001 \)), and positive pictures were recalled significantly more than neutral pictures by both older (\( p < 0.001 \)) and, as a trend, young participants (\( p = 0.063 \)).

The factors Condition (\( F(1, 87) = 0.154, p = 0.696 \)) and Sex (\( F(1, 87) = 0.122, p = 0.727 \)) were not significant, but the Condition \( \times \) Sex \( \times \) Age interaction (\( F(1, 87) = 6.219, p = 0.015 \)) was significant. Older participants showed significantly worse free recall performance than young participants in both conditions and both sex groups (all \( p < 0.005 \)). Among older people, there were no condition differences in men or women (both \( p > 0.476 \)). By contrast, among young people, condition differences were found, so that the young men in the stress condition recalled fewer pictures than the young men in the control condition (\( p = 0.025 \)). This result was not found in young women (\( p = 0.185 \)). Moreover, in the stress condition young men recalled fewer pictures than young women (\( p = 0.012 \)). This significant difference was not observed in young people in the control condition (\( p = 0.316 \)), or in older people in either of the two conditions (both \( p > 0.260 \)) (see Fig. 3).

**Recognition:** A repeated-measures ANOVA with Valence as a within-subject factor and Condition, Age and Sex as between-subject factors was used to measure the effect of stress on the

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1. Because older participants rated all pictures as more arousing than young participants, the effect of the arousal rating on the relationship between stress and memory performance was assessed. However, the inclusion of the arousal rating as a covariate in the ANOVAs does not substantially change the statistical conclusion of the memory performance analyses, except the recognition analysis, in which the Valence factor loses its significance, Valence (\( F(1.724, 141.382) = 0.875, p = 0.405 \)).
recognition task. Results revealed a main effect for Valence ($F(1, 179, 142.711) = 7.008$, $p = 0.002$), but not for Condition ($F(1, 83) = 1.591$, $p = 0.211$). The Valence $\times$ Condition interaction was significant ($F(1, 179, 142.711) = 4.007$, $p = 0.043$), but not the Valence $\times$ Age interaction ($F(1, 179, 142.711) = 0.025$, $p = 0.962$). Post-hoc analyses revealed that the positive pictures were recognized less in the stress condition than in the control condition ($p = 0.004$). No condition differences were found in negative and neutral picture recognition (both $p > 0.435$). Age and Sex were not significant (both $p > 0.455$); nor were their interactions with other factors (all $p > 0.1$) (see Fig. 4).

3.5. The relationship between the stress response and retrieval performance

To minimize Type I error rates, the correlations between the physiological response to the TSST and memory performance were analyzed only in young men in the stress condition for free recall data, and in participants in the stress condition for recognition of positive pictures data, based on the significant effects found.

Free Recall: Results showed a high association between cortisol and free recall performance, but this result was only marginally significant ($r = -0.434$, one-tail $p = 0.079$). If we explore the relationship between cortisol and free recall for negative, neutral and positive pictures separately, we observe that young men who reacted to the stressor with large cortisol responses recalled fewer negative pictures ($r = -0.584$, $p = 0.046$). However, this relationship was not significant for positive ($r = -0.155$, $p = 0.630$) or neutral ($r = 0.286$, $p = 0.367$) pictures. Moreover, sAA response did not show any significant correlations (all $p > 0.360$).

Recognition: Neither cortisol nor the sAA responses to stress were associated with recognition performance on positive pictures when correlations were performed with young and older participants together (both $p < 0.410$).

4. Discussion

The present study intended to parse the effects of an acute psychosocial stressor on long-term memory retrieval in different age groups in order to better understand the importance of age-related changes. To do so, we reanalyzed data from a group of older people...
along with new data from a novel group of young participants, in order to compare their performance on a long-term memory retrieval task for pictures after a stress or control task. No significant stress effects were found on memory retrieval for positive, negative and neutral pictures in the older group. Conversely, in young people the stressor diminished memory retrieval, but only in men. Additionally, this impairment was negatively associated with the cortisol response to stress in young men and, although in a very tentative way, especially with negative pictures. Regardless of age and sex, stress impaired recognition memory for positive pictures, but this effect was not correlated with the cortisol or sAA response.

The task used as the stressor, the TSST, was able to induce stress at both psychological and physiological levels. At the psychological level, the stress task was perceived as more stressful, difficult, frustrating and requiring more effort than the control task. Moreover, the TSST increased negative mood at the same time that it decreased positive mood similarly in both age groups. At the physiological level, the stress task provoked greater cortisol and sAA responses than the control task and, coinciding with previous studies, no age or sex differences were found in the sAA response to stress [56,63–65]. Regarding the cortisol response to stress, our results also agree with previous findings showing a higher cortisol response in men than in women (for a review see: [45]). Age had a modulating effect on the stress-induced cortisol response; thus, older participants had a lower cortisol response to the stressor than young participants, particularly older women, who showed a small cortisol response, although the age, sex and condition interaction was not significant. Other studies have reported no age differences in the cortisol response to stress [31,66–68], and even a higher cortisol response in older people than in young people [56,69]. In general, the TSST was able to provoke a psychological, sympathetic adrenal medullary (i.e., sAA) and cortisol response; however, the cortisol response in older participants was lower than in previous studies.

As expected, older people performed worse on free recall than young people. When we compared the stress effects on the performance of older and young participants, these effects were only observed in young men. Young men in the stress condition had lower free recall performance when compared to: (i) young men in the control condition and (ii) young women in the stress condition. This impairment was marginally related to the cortisol response to stress. However, when we considered each type of picture separately, the relationship was only observed for cortisol and free recall of negative pictures. Our results seem to agree with previous studies that have shown a detrimental effect of stress on memory retrieval in young men [13,14,33]. Therefore, because the stress task impairs memory retrieval in young men, but not in older people, this finding might confirm our suggestions about the role of age in explaining the results obtained previously [39]. This result would support the idea that, as shown for working memory, spatial memory, declarative and non-declarative memory in older animals and humans [33,35–38], older people might be less sensitive to stress effects on long-term memory retrieval than young people.

One possible explanation for the lack of stress effects on memory performance in older people would be an age-related reduction in the sensitivity and density of the glucocorticoid receptors (GRs or Type II) in the aging brain [26,70], which might decrease cortisol’s direct effects on the hippocampus. Furthermore, a decrease in the functional interconnectivity between the amygdala and hippocampus has been observed [22–24]. This age-related change might also reduce the effect of the noradrenergic activation of the amygdala, which has been shown to be necessary in order to observe stress effects on memory [3,71]. Taken together, these factors may contribute to reducing stress effects on memory retrieval in older people.

It is also conceivable that the lack of stress effects on free recall reported in the older group is due to the lower cortisol response observed in the older participants in our study. However, another study showed that an approximately 10-fold cortisol increase due to hydrocortisone administration in older people did not affect their performance on various cognitive tests (including memory tasks) [36]. Additionally, previous studies carried out with young individuals have shown impairing stress effects on memory retrieval performance with similar cortisol response magnitudes to those of the older men in our study [15,16]. Together, our findings are in accordance with previous research in older people, but they differ from studies in young people. At the same time, we cannot rule out the possibility that our results are explained by the lower cortisol response to the TSST in the older group. This is especially applicable to the older women because they did not show a considerable cortisol response to the TSST. This result points out the clear need for further studies to investigate these possible age-related differences more in depth.

Interestingly, while the present study found that stress affects free recall in young men, but not in older people, some previous studies directly comparing older and young individuals have shown a different pattern of results. In a previous study, we showed that stress impaired a very specific aspect of declarative memory, immediate recall after interference (i.e., retroactive interference), in older but not young individuals [31]. Similarly, Wolf et al. [33] showed impairing effects of a hydrocortisone injection on memory retrieval of a word list learned 75 min before cortisol administration in both young and older men. In this study, the word–list recall was measured after other memory tasks were performed, and so it is possible that the effect observed was also due to the effect of cortisol on retroactive interference. One explanation for these contradictory effects could be that the pattern of sensitivity to the effects of acute stress on memory in older people differs depending on the type of memory, with retroactive interference being more affected by stress and cortisol than other memory processes (e.g., working memory, declarative and non-declarative memory, long-term memory retrieval). This difference may be due to age-related changes in the sensitivity to cortisol’s effects on memory performance. Roozenendaal [72] proposed that stress blocks long-term memory retrieval in order to facilitate the consolidation of new information in young people. It has been suggested that this mechanism would diminish retroactive interference, allowing the brain to learn important new information to be used in the future [72,73]. Thus, the increase in retroactive interference after stress observed in previous studies may be due to the fact that stress and cortisol do not block the memory retrieval of previously learned material in older people, as observed in our results.

Sex differences were only found in young people. In fact, the stressor only impaired young men’s retrieval performance, while this effect was not found in young women. This result found in men coincides with previous studies performed solely in men after both pharmacological treatment [7] and acute stress [13,14]. At the same time, the lack of a stressor effect in women agrees with findings reported in a study conducted to investigate the effects of stress in luteal women [17]. It is important to note that most of the studies with both sexes did not report sex-related differences, and they did not control the phase of the menstrual cycle of the women [6,10,11,15,18]. In our opinion, an explanation for this discrepancy between these studies and the present study might be that, as we did not register the menstrual cycle phase of the young women, it is impossible to know whether the null effect of the stressor on free recall performance in young women is related to the sex factor or, on the contrary, to sex hormone levels. Taking into account the results found by Schoofs and Wolf [17], it is possible that most of the young women in our sample were in the luteal phase of their menstrual cycle, which would explain the lack of effects on them.
However, other studies have reported no differences between men and women in the luteal or follicular phases [16], or between men and women in the luteal phase of their menstrual cycle [19]. Therefore, further research is needed to examine the role of sex and sex hormone levels, as well as the use of oral contraceptives, in the relationship between acute stress and memory retrieval in young people.

Unlike on the free recall task, we found similar recognition performance in older and young individuals. It is important to note that in our previous study with the older group, we failed to find stress effects on recognition performance [39]. However, in the current study, which combines data from older and young participants, the stress impaired the recognition memory of positive pictures, regardless of age and sex. It is worth noting that this cross-study difference can be explained by the fact that we are analyzing different data, as well as the fact that the current study has a larger sample size and, possibly, higher statistical power. This result coincides with Domes et al. [12], who found an impairing stress effect on the recognition of positive words, but only in young men. Interestingly, in the present study stress selectively impaired free recall in young men, but it impaired recognition in the entire sample (i.e., older and young men and women). Numerous studies have shown that recognition memory consists of two components, reollection (i.e., remembering details about previously learned material) and familiarity (i.e., knowing whether the material has been previously presented or not) [74,75], which seem to be dependent on the hippocampus and the adjacent perirhinal cortex, respectively [76]; but see [75]. Along these lines, we can only speculate that the effect observed on recognition may be related to the adjacent perirhinal cortex, as subjects were asked to answer whether they had seen the pictures before by saying “yes” or “no”. Therefore, the task could be a recognition task with a stronger familiarity component. Given that recognition is a cognitive function that does not seem to suffer an age-related decline [77], it is possible that both young and older people are sensitive to the detrimental effects of stress on this type of memory task. However, it should be noted that correlation analyses did not show a significant relationship between the stress-induced cortisol or sAA response and memory recognition, suggesting that cortisol and sAA are not the main contributors to this effect. Along these lines, and as proposed by Domes et al. [12], the effect on the recognition of positive pictures might reflect a state dependent effect. Thus, in a negative mood, participants perform worse on recognizing positive pictures. Moreover, other factors not addressed in this study might account for the results observed; therefore, further research is clearly needed to investigate this hypothesis.

A limitation of the current study is that, in order to avoid introducing confounding factors and obtain the best comparison of old and young people, we made an effort to obtain a very healthy sample by applying restrictive exclusion criteria. This strategy allowed us to obtain two cognitively and physically homogeneous age groups. However, at the same time, it makes it difficult to generalize our results to the general older population, which frequently has age-related diseases (e.g., diabetes or hypertension). Future studies with a more general population should be carried out. Another limitation of our study is the sample size. Despite having a large number of participants (i.e., 102 participants), dividing the sample according to the condition, age and sex factors caused the sample size of each subgroup to be reduced. Finally, our study coincides with previous studies that have observed stress effects on long-term memory retrieval when testing 10 or fewer items for each emotional category in young people [11,13,71]. However, future studies could explore whether stress effects are observed in older people when more items to-be-recalled and/or more difficult memory tasks are used.

5. Conclusion

In conclusion, our study is unique in examining, for the first time, age differences in acute stress effects on memory retrieval in men and women. However, future research should consider more age ranges in order to better understand the role of age in stress effects on memory retrieval across the lifespan. Moreover, this study adds evidence to the issue of sex differences in stress effects on memory retrieval among young people. Finally, this study points out that age-related differences might be observed in the stress effects on long-term memory retrieval, and it highlights the lack of studies performed to investigate the effect of acute stress-induced cortisol response on memory in older people compared to young people.

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