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Memory Retrieval After an Acute Academic Stressor: An Exploratory Analysis of Anticipatory Cortisol and DHEA Responses

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Abstract

The relationship between hormonal reactivity to acute stress and memory is well established, but the role of anticipatory cortisol and dehydroepiandrosterone (DHEA) levels remains underexplored. This study aimed to assess the psychobiological responses (anxiety, affect, cortisol and DHEA) to an academic examination, subsequent memory performance and associations between anticipatory hormonal response and memory retrieval. Seventy-nine undergraduates (10 males) completed an acquisition session involving picture encoding and immediate free recall. Forty-eight hours later, during the recall session, they sat a written examination followed by delayed free recall and recognition tasks. Results showed higher anticipatory anxiety, negative affect and cortisol levels in the recall session than in the acquisition session. Participants showed poorer delayed recall performance and reduced recognition of neutral pictures. In addition, after correction for multiple comparisons, exploratory hierarchical regression analyses indicated that anticipatory cortisol levels and the cortisol/DHEA ratio assessed prior to the recall session were negatively associated with total delayed free recall performance, with the cortisol/DHEA ratio also being negatively associated with delayed free recall of negative pictures. In the absence of a control group, these findings cannot be used to make causal inferences. However, they are consistent with theoretical accounts of DHEA's anti-glucocorticoid role and highlight associations between cortisol/DHEA balance and delayed free recall performance, particularly for negative emotional material.

Keywords: cortisol; DHEA; memory retrieval; academic examination; anticipatory response

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1. Introduction

The nature of stressors is thought to play a role in modulating the psychobiological response. Research employing laboratory-based stress paradigms has consistently demonstrated that both psychological and physiological responses are activated following the onset of stress (see meta-analyses by [1,2]). In contrast, ecological or naturalistic stressors—including sports competitions, academic examinations, or first-time parachute jumps—tend to elicit an anticipatory psychobiological response (e.g., [3–5]). This anticipatory response comprises physiological,

cognitive, and emotional changes that occur prior to the stressor, often resulting in elevated cortisol levels, heightened negative affect, and impaired cognitive performance [6], and also negatively affecting decision-making [7]. According to the Neurocognitive Framework for Regulation Expectation [8], such anticipatory stress may contribute to the development of depression and other stress-related psychopathologies. Developing a more nuanced understanding of how individuals anticipate and prepare for stressful situations is therefore essential to elucidating the mechanisms involved in stress regulation, as this anticipatory phase can influence both cognitive appraisals and subsequent physiological responses [9,10]. In light of this, university students, who are often exposed to high academic demands and evaluative pressures, may represent a particularly vulnerable population to the effects of anticipatory stress.

Acute stressors, such as academic examinations, activate the hypothalamic–pituitary–adrenal (HPA) axis, leading to the secretion of glucocorticoids, such as cortisol, from the zona fasciculata of the adrenal cortex [11] while dehydroepiandrosterone (DHEA) is secreted from the zona reticularis of the adrenal cortex [12]. Cortisol is a catabolic hormone, whereas DHEA is an anabolic hormone with protective and regenerative properties [13]. Accordingly, evaluating the cortisol-to-DHEA ratio provides a nuanced index of the balance between catabolic and anabolic processes [14].

In laboratory settings with acute stressors—where participants are typically unaware of the stressful event—peak levels of cortisol and DHEA usually occur approximately 20 min after the onset of the stressor (for meta-analyses, see [2] on cortisol and [15] on DHEA). In contrast, studies conducted in ecological settings such as academic examinations or oral presentations, in which participants can anticipate the stressor, have identified an anticipatory phase in which cortisol levels peak prior to the event (e.g., [3,16–18]). Hormonal responses to academic stress have primarily focused on cortisol, whereas DHEA has received comparatively less attention. Some studies such as Irshad [19] examined DHEA concentrations during examination and non-examination periods and found no significant differences. However, to our knowledge, only Garces-Arilla [17] analyzed salivary DHEA levels in response to a written academic examination, reporting an anticipatory increase. Given the limited research investigating DHEA alone and in conjunction with cortisol under conditions of anticipatory academic stress, further investigation of their joint hormonal dynamics in university students is warranted.

Several studies have shown that salivary cortisol and DHEA levels influence memory performance in response to an acute stressor (e.g., [17,20,21]). Memory is a cognitive function particularly sensitive to the effects of acute stress, largely due to interactions between glucocorticoids, such as cortisol, and mineralocorticoid receptors in brain regions critical for memory processing, including the prefrontal cortex, amygdala, and hippocampus (for a review, see [22]). In this context, acute stress is thought to impair memory retrieval via the action of glucocorticoids in these areas [23,24]. The effects of glucocorticoids may be mitigated by DHEA, as it exerts neuroprotective effects on hippocampal receptors, such as NMDA and sigma-1 receptors [25], both of which play a key role in memory processes [26]. DHEA also modulates neurocircuitry in limbic structures, including the hippocampus and amygdala [27], and protects against glucocorticoid-induced neurotoxicity in the hippocampus [28]. Indeed, an elevated cortisol/DHEA ratio has been associated with cognitive impairment [29], underscoring its value as a biomarker in research on the cognitive effects of stress.

Acute stress administered immediately before or during a retrieval task has been shown to impair memory retrieval performance (for a meta-analysis, see [30]). However, Shields et al. [30] also found no linear or non-linear relationship between the magnitude of cortisol reactivity to acute stress and this retrieval impairment. In line with this, studies in young adults using standard laboratory stress paradigms (e.g., the Trier Social Stress Test

or the Cold Pressor Test) have yielded mixed results regarding the relationship between cortisol levels and memory retrieval. Several studies have associated higher cortisol concentrations with poorer retrieval accuracy [31–35], while others have found no significant association [36,37]. It is important to note that these studies have primarily focused on cortisol reactivity in response to laboratory stressors. Therefore, a plausible explanation is that there is considerable variability in the extent to which laboratory stressors elicit cortisol responses, which may mask a relationship between cortisol reactivity and impaired memory retrieval (for meta-analysis, see [30]; for review, see [38]). Other methodological factors that influence cortisol responses (e.g., stress-retrieval interval and timing of induction), alongside biological moderators of cortisol responsivity (e.g., time of day, sex and menstrual-cycle phase) and inter-individual variability in cortisol reactivity, appear to account for the inconsistencies observed in the link between stress-induced cortisol and memory retrieval (for a review, see [38]). Moreover, Shields et al. [30] propose that stress-induced increases in cortisol may exert inverted U-shaped effects on memory performance. These authors also highlight the importance of including additional stress-responsive hormones alongside cortisol, such as DHEA, to achieve a more comprehensive understanding of how acute stress modulates episodic memory. Overall, research on the relationship between hormonal response and memory retrieval has predominantly focused on laboratory-induced stress. However, little attention has been paid to other relevant cortisol measures, such as anticipatory cortisol levels, which may also play a critical role in memory retrieval processes. Thus, investigating hormonal responses to more ecologically valid stressors, such as academic examinations, may provide greater insight into the stress–memory retrieval link in real-life contexts in young adults.

Studies of young adults often report that the relationship between cortisol reactivity to acute stress and memory retrieval is more robust for emotional material than for neutral material [32,35,39]. This heightened sensitivity to emotional content may be partly explained by the functional overlap of key brain regions involved in both stress and memory. Specifically, the amygdala, hippocampus, and prefrontal cortex are recruited during the retrieval of emotionally salient memories—particularly when these regions were active during encoding (see [40], for a review). Glucocorticoids released in response to stress may modulate memory retrieval by altering the functional connectivity among these regions [41], affecting retrieval, especially for emotional material [23]. Also, DHEA administration has been found to increase activation in prefrontal areas involved in emotion regulation and to enhance amygdala–hippocampal connectivity, thereby modulating the retrieval of emotional information [27]. Taken together, these findings highlight the need for study designs that are sensitive to the valence of material to be remembered. Accordingly, Shields et al. [30] recommend using mixed lists of emotional and neutral stimuli, but focusing analyses on the emotional items, as this maximizes sensitivity to stress-induced retrieval impairments.

To date, empirical evidence on the role of DHEA in modulating memory retrieval under acute stress in young adults remains sparse and yields mixed findings. For instance, Smith et al. [42] found no association between DHEA reactivity to a laboratory-based stressor and long-term memory retrieval performance in young adults. In contrast, Garces-Arilla [17] reported that higher anticipatory DHEA levels prior to an academic examination were associated with poorer academic performance in university students. However, neither Smith et al. [42] nor Garces-Arilla [17] found any relationship between task performance and the cortisol/DHEA or DHEA/cortisol ratios, respectively. Given the variability in findings regarding cortisol, the limited literature on DHEA, and the lack of studies examining both hormones simultaneously in ecologically valid settings, further research is needed to clarify how these hormonal responses may be associated with memory retrieval performance under anticipatory stress.

Therefore, the aims of this study were:

(i) To examine the psychobiological (state anxiety, negative and positive affect, cortisol and DHEA) responses to an ecological stressor (i.e., a real-life academic examination). State anxiety, as well as negative and positive affect, refer to temporary emotional states and were included specifically to validate the academic examination as an effective psychological stressor.
(ii) To investigate the visual memory performance for both emotional and neutral material after the stressor.
(iii) To explore whether the anticipatory hormonal responses were related to emotional and neutral visual memory performance.

Based on previous literature, the following hypotheses were formulated:

H1. *Participants were expected to show elevated anticipatory levels of state anxiety* [3,17,43], *negative affect* [3,17], *salivary cortisol* [3,16–18,44], *and salivary DHEA* [17] *in the recall session (examination session).*

H2. *Visual memory performance was expected to be poorer in the recall session than in the acquisition session* [31,39,45–48], *with emotional material being recalled more poorly than neutral material* [39,46–48]. *In addition, recognition of positive pictures was expected to be lower than recognition of negative or neutral pictures* [32,39,49].

H3. *Although no studies to date have examined anticipatory hormone levels during academic examinations in relation to visual memory, we hypothesized that higher anticipatory hormone levels would be associated with poorer performance on both emotional and neutral visual memory tasks. This hypothesis was informed by studies using acute laboratory stressors, which have reported similar associations with performance in free recall (e.g.,* [31–34,39,48]*) and recognition tasks* [32].

2. Materials and Methods

2.1. Participants

The sample consisted of 79 undergraduate psychology students (10 males, 12.7%) aged 18 to 25 years. The participants were recruited voluntarily from the Fundamentals of Biology I course at the University of Zaragoza, with the majority being in their first year (92.4%). The characteristics of the sample are summarized in Table 1.

Table 1. Characteristics of the sample.

Variables	
Sociodemographic	
Age—M (SD, range)	18.60 (1.055, 18–25)
SES—M (SD, range)	6.68 (0.468, 6–7)
Physiological	
BMI—*n* (%)	
Underweight	3 (3.8%)
Normal weight	67 (84.8%)
Overweight	9 (11.4%)
MCP—*n* (%)	
Menstruation	17 (30.9%)
Follicular	8 (14.5%)

Table 1. *Cont.*

Variables	
Ovulation	8 (14.5%)
Luteal	14 (25.5%)
Premenstrual	8 (14.5%)
Nulliparous—*n* (%)	69 (100%)
Oral contraceptives—*n* (%)	14 (20.3%)
Negative life events—*n* (%)	10 (12.7%)

Note: SES = subjective educational and socio-economic status (scale ranging from 1 to 10); BMI = Body Mass Index; MCP = Menstrual cycle phase (naturally cycling women only); Oral contraceptives = oral contraceptive users; Negative life events = negative life events over the past year.

Inclusion criteria required participants to be students enrolled in the Fundamentals of Biology I course at the University of Zaragoza, aged 18 to 30, with no visual impairments. Exclusion criteria included any history or current diagnosis of cardiovascular, endocrine, neurological, or psychiatric disorders; the use of medications that could affect endocrine, cognitive, or emotional function; having undergone one or more general anesthetic procedures in the past year; consuming more than ten alcoholic drinks per week; or smoking more than ten cigarettes per week. These exclusion criteria were applied due to their impact on the cortisol response [50].

2.2. Procedure

Participants attended two individual sessions—an acquisition session and a recall session. The acquisition session took place 48 h before the recall session, during which participants completed the actual final examination (stressor) of the Fundamentals of Biology I course. Both sessions took place in the same classroom between 11:00 and 13:10. For clarity, a schematic overview of the procedure is shown in Figure 1.

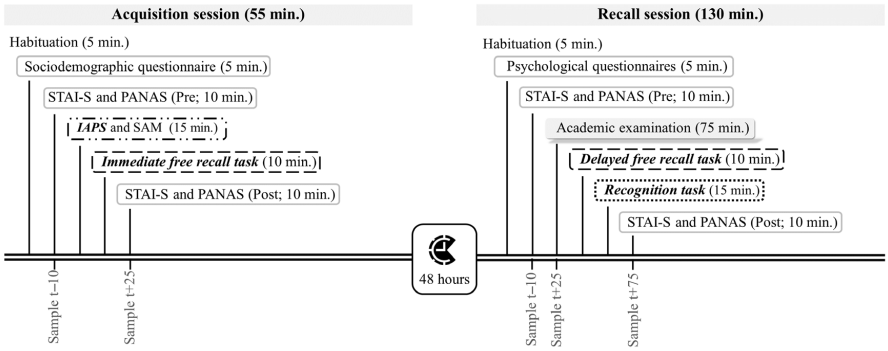

Figure 1. Schematic overview of study session procedures. The study comprised two sessions, separated by 48 h. In the acquisition session, following a habituation period, participants completed a sociodemographic questionnaire, followed by the State-Trait Anxiety Inventory (STAI-S) and Positive and Negative Affect Schedule (PANAS) (pre), while providing a saliva sample (t − 10). Participants then viewed pictures from the International Affective Picture System (IAPS) and simultaneously rated them using the Self-Assessment Manikin (SAM) scale. After this, they performed an immediate free recall task. Subsequently, participants completed the STAI-S and PANAS questionnaires again (post) and provided another saliva sample (t + 25). In the recall session, which followed a similar structure to the acquisition session, participants first completed the STAI-S and PANAS questionnaires (pre) and provided a saliva sample (t − 10). The real academic written examination then commenced, and 25 min after the stressor began, a saliva sample was collected (t + 25). Immediately after the examination, participants performed a delayed free recall and recognition task. Lastly, they completed the STAI-S and PANAS questionnaires (post) while providing a final saliva sample (t + 75).

Participants were asked to follow their usual habits the day before each session, including maintaining their regular sleep patterns, abstaining from alcohol, and avoiding strenuous physical activity. They were also instructed not to smoke or use stimulants, to drink only water for two hours before each session, and not to brush their teeth for at least one hour before each session. Compliance with these instructions was verified through a pre-session questionnaire.

2.2.1. Acquisition Session

The acquisition session began with a habituation period. Following this, participants completed a questionnaire that collected sociodemographic information (sex, age, subjective socioeconomic status [SES; [51]) as well as physiological data (body mass index [BMI] and menstrual cycle phase, assessed only in naturally cycling women [MCP]), among other variables). Participants also completed the State-Trait Anxiety Inventory (STAI-S, pre; see Section State Anxiety, for further details) and the Positive and Negative Affect Schedule (PANAS, pre; see Section Negative and Positive Affect).

After completing these questionnaires, they were shown 60 color pictures: 20 negative (e.g., an amputated arm), 20 positive (e.g., a group of friends on the beach), and 20 neutral (e.g., a middle-aged man staring into the camera). These pictures were selected from the Spanish version [52]) of the International Affective Picture System (IAPS) [53]. To ensure a standardized and validated assessment of memory performance, IAPS stimuli were used instead of content from the final examination—the stressor in this study—in order to minimize variability due to differences in participants' prior knowledge. Each picture was projected individually on a screen for 10 s, followed by a neutral black slide for 15 s, during which participants rated its valence (1 = very positive to 9 = very negative) and arousal (1 = high arousal to 9 = low arousal) using the Self-Assessment Manikin (SAM) scale [54].

Participants completed an immediate free recall task (see Section Free Recall), followed by the STAI-S and PANAS questionnaires (post). To ensure unintentional encoding of the stimuli, no memory task was announced beforehand. The study protocol, including the use of unintentional encoding, was approved by the ethics committee. All participants provided informed consent and were debriefed after completion of the experiment.

Two saliva samples were collected during this session to measure cortisol and DHEA levels, taken 35 min apart. The first sample ($t - 10$) was collected during the administration of the STAI-S and PANAS (pre), and the second sample ($t + 25$) during the administration of the STAI-S and PANAS (post), with a 35-min interval to capture hormonal fluctuations.

2.2.2. Recall Session

Similarly to the acquisition session, the recall session began with a habituation period. Participants then completed the STAI-S and PANAS (pre) before being exposed to the ecological stressor—the actual final examination for Fundamentals of Biology I, consisting of 50 multiple-choice questions and lasting 75 min. The actual time spent completing the examination was recorded (in minutes; $M = 73.29$, $SD = 8.83$) and used as an objective indicator of task duration and potential fatigue. Following the academic examination, they completed a delayed free recall task (see Section Free Recall), followed by a recognition task (see Section Recognition) involving the pictures from the acquisition session. Finally, participants completed the STAI-S and PANAS (post). Three saliva samples ($t - 10$, $t + 25$ and $t + 75$) were collected during this session. The first saliva sample ($t - 10$) was collected while participants completed the STAI-S and PANAS (pre), prior to the examination. The second sample ($t + 25$) was collected during the examination at a standardized time point (approximately 25 min after examination onset). Importantly, saliva collection did not require interrupting or pausing the examination; participants continued completing the examination while providing the sample, and no task-related feedback or interaction oc-

curred during this process. The collection procedure was brief, non-invasive, and designed to minimize any additional stress beyond that inherently associated with the academic examination. The third sample (t + 75) was collected at the end of the examination, while participants completed the STAI-S and PANAS (post).

2.3. Questionnaires and Measures

2.3.1. Psychological Response

State Anxiety

The Spanish version [55] of the STAI-S [56] was used to assess state anxiety. This 20-item questionnaire measures anxiety as an emotional state. Participants responded using a 4-point Likert scale, ranging from 0 (not at all) to 3 (extremely), based on how they felt at that moment. Total scores on the scale range from 0 to 60. Participants completed the STAI-S twice in each session—before (pre) and after (post) the session (see Section 2.2 Procedure, for more details). In the current study, Cronbach's alpha values ranged from 0.88 to 0.91.

Negative and Positive Affect

Participants' affect was evaluated by the Spanish version [57] of the PANAS [58]. This questionnaire consists of 20 items divided into two dimensions, negative affect and positive affect, with 10 items measuring each dimension. Participants were asked to rate how they felt at that moment. Similarly to state anxiety, the PANAS was administered twice in each session, pre- and post- session (see Section 2.2 Procedure). Responses were recorded on a 5-point Likert scale (1 = not at all to 5 = extremely). Each subscale has a minimum possible score of 10 and a maximum of 50. In the present study, Cronbach's alpha values ranged from 0.79 to 0.88 for the positive affect subscale and from 0.85 to 0.89 for the negative affect subscale.

2.3.2. Memory Assessment

Free Recall

Participants were instructed to recall as many pictures as possible and were given 10 min to write a brief description of each recalled picture. The free recall task was completed twice: immediately after the IAPS presentation in the acquisition session and as a delayed free recall after the examination in the recall session (see Section 2.2. Procedure). Free recall performance was assessed by two independent judges. Inter-rater agreement was high, with agreement rates of 92.9% for immediate recall and 92.5% for delayed recall. Cohen's kappa coefficients were k = 0.606 for immediate recall and k = 0.678 for delayed recall, indicating moderate to substantial inter-rater reliability. Disagreements were resolved through discussion until consensus was reached.

Recall performance was assessed using four outcomes for both immediate and delayed recall: the total percentage of pictures recalled and the percentage of positive, negative and neutral pictures recalled.

Recognition

A total of 120 pictures were individually projected onto a screen—60 previously seen and 60 new. Participants were instructed to indicate whether they had seen each picture during the acquisition session by marking 'yes' or 'no' after each picture. Both sets of pictures contained 20 negative, 20 positive and 20 neutral pictures. The pictures were drawn from the Spanish adaptation of the International Affective Picture System (IAPS; [52,53]).

Recognition performance was assessed following the procedure outlined by Stanislaw and Todorov [59]. The sensitivity index d′, derived from Signal Detection Theory, was calculated to quantify participants' ability to discriminate between target and non-

target stimuli in the recognition tasks. The d′ value was computed using the formula d′ = Z(Hit Rate) − Z(False Alarm Rate). Calculations were performed separately for total recognition, as well as for negative, positive, and neutral pictures. To address undefined Z-scores resulting from extreme proportions (i.e., hit or false-alarm rates of 0 or 1), the log-linear correction described by Stanislaw and Todorov [59] was applied, whereby 0.5 was added to the number of hits and false alarms and 1 was added to the number of signal and noise trials prior to computing proportions.

2.4. Biochemical Analyses

Participants provided five saliva samples through passive drooling, two samples in the acquisition session (t − 10 and t + 25: minutes relative to the onset of the IAPS) and three samples in the recall session (t − 10, t + 25 and t + 75: minutes relative to the onset of the academic examination) to assess cortisol and DHEA levels. For each sample, participants deposited approximately 3 mL of saliva in plastic vials. After collection, all saliva samples were frozen at −20 °C until the biochemical analyses were performed in the Laboratory of Social Cognitive Neuroscience at the University of Valencia.

All samples were analyzed in duplicate using Salimetrics cortisol enzyme-immunoassay and DHEA kits (Salimetrics, State College, PA, USA). Assay sensitivity was 0.007 μg/dL and 5 pg/mL for the cortisol and DHEA kits, respectively. For each participant, all the samples were analyzed in the same trial. Both inter- and intra-assay coefficients of variation were below 10%.

2.5. Statistical Analyses

Salivary cortisol and DHEA values were logarithmically transformed due to non-normal distribution, as indicated by the Kolmogorov–Smirnov test. However, for ease of interpretation, the raw data are presented in the figures. The distribution of individual cortisol and DHEA values across sessions and time points is shown in Supplementary Materials (Sections S1 and S2). Descriptive statistics for raw values (mean and standard deviation) are provided in Supplementary Materials (Section S3).

Psychological variables (i.e., state anxiety, negative and positive affect) were analyzed separately using repeated-measures ANOVAs, with Session (acquisition vs. recall) and Time (pre vs. post) as within-subject factors.

Preliminary analyses were conducted to examine whether salivary hormone levels varied as a function of sex, MCP [60], BMI [15,61] or negative life events in the past year [62], as these variables may influence hormonal responses. The variable SES was excluded from the analysis due to low variability in the data (Table 1). In the remaining analyses, none of the covariates showed significance for cortisol ($p > 0.100$) or DHEA ($p > 0.092$) levels.

Therefore, to compare hormone levels across sessions, separate repeated-measures ANOVAs were performed for each hormone, with Session and Time (t − 10 vs. t + 25) as within-subject factors. Hormone levels within the recall session were examined using repeated-measures ANOVAs with Time (t − 10, t + 25, vs. t + 75) as the within-subject factor.

In addition, repeated-measures ANOVA were conducted with Session and Valence (negative, positive, vs. neutral) as within-subject factors for free recall performance. Paired-samples *t*-tests were used to assess valence, arousal, and recognition performance (d′) across different picture valences.

Post hoc comparisons were performed using Bonferroni corrections, and the Greenhouse–Geisser correction was applied when the assumption of sphericity was violated

Finally, exploratory separate hierarchical regression analyses were conducted to examine whether pre-examination (t − 10) hormone levels from the recall session (cortisol, DHEA, and the cortisol/DHEA ratio) were associated with memory performance in delayed free recall and recognition tasks. Analyses were performed separately for each

outcome (total, negative, positive, and neutral). In Step 1, the corresponding pre-session (t − 10) hormone level (cortisol, DHEA, or cortisol/DHEA ratio) from the acquisition session was entered as a control variable. In Step 2, the pre-examination (t − 10) hormone level from the recall session was entered as the predictor. The rationale for this hierarchical approach is provided in the Supplementary Materials (Section S4). Multicollinearity was assessed using tolerance and variance inflation factors (VIF); no evidence of problematic multicollinearity was detected across Step 2 models (tolerance range = 0.456–0.514; VIF range = 1.946–2.192). Given the number of exploratory regression analyses, Bonferroni correction for multiple comparisons was applied separately within each family of analyses defined by memory task (delayed free recall and recognition outcomes), resulting in an adjusted significance threshold of $p \leq 0.004$. As a robustness check, examination duration (in minutes) was initially included as an additional control variable in the first step of the hierarchical regression models to account for potential fatigue effects. Examination duration was not significantly associated with memory performance in any model (all ps ≥ 0.213) and was therefore not retained in the final analyses.

A sensitivity power analysis was conducted using G*Power (version 3.1.9.7) to determine the minimum effect size detectable for the primary regression analyses. With a sample size of 79 participants, an alpha level of 0.05, a power of 0.80, and two predictors, the analysis indicated that the study was powered to detect effects of $f^2 \geq 0.13$, corresponding to small-to-medium effect sizes.

Statistical analyses were conducted using SPSS 25.0.

3. Results

3.1. Session Differences in Psychological Responses

3.1.1. State Anxiety

The repeated-measures ANOVA revealed significant main effects for Session [$F(1, 78) = 64.870$, $p < 0.001$, $\eta p^2 = 0.454$] and Time [$F(1, 78) = 77.650$, $p < 0.001$, $\eta p^2 = 0.499$] factors. State anxiety levels were significantly higher in the recall session compared to the acquisition session ($p < 0.001$) and in the pre-session than in post-session ($p < 0.001$). Additionally, the Session × Time interaction was significant [$F(1, 78) = 40.789$, $p < 0.001$, $\eta p^2 = 0.343$]. Post hoc analyses revealed that in the recall session, state anxiety scores were significantly higher in the pre-session than in the post-session ($p < 0.001$). In contrast, during the acquisition session, state anxiety levels did not differ significantly between the pre-session and the post-session ($p = 0.149$). When comparing sessions, state anxiety levels were significantly higher in the recall session than in the acquisition session at both pre-session and post-session ($p < 0.001$ and $p = 0.005$, respectively). See Figure 2A.

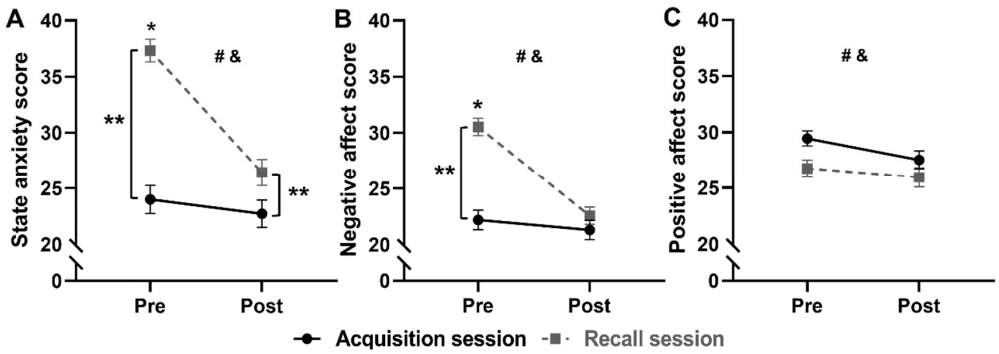

Figure 2. Scores for state anxiety (**A**), negative affect (**B**), and positive affect (**C**) during both the acquisition and recall sessions. Depicted values represent means, and error bars indicate the standard

error of the mean (SEM). State anxiety and negative affect scores were significantly higher in the recall session compared to the acquisition session ((**A**,**B**): both # $p < 0.001$), whereas positive affect scores were higher in the acquisition session ((**C**): # $p = 0.002$). State anxiety and negative and positive affect scores were higher in the pre-session than in the post-session ((**A**–**C**): all & ps < 0.001). Also, in the recall session, state anxiety and negative affect scores were higher in the pre-session than in the post-session ((**A**,**B**): both * $p < 0.001$), whereas in the acquisition session, state anxiety and negative affect levels were similar between the pre- and post-session assessments ($p = 0.149$ and $p = 0.165$, respectively). Furthermore, state anxiety scores were higher in the recall session compared to the acquisition session at both the pre-session ((**A**): ** $p < 0.001$), and the post-session ((**A**): ** $p = 0.005$). However, negative affect was higher in the pre-session of the recall session compared to the acquisition session ((**B**): ** $p < 0.001$), whereas in the post-session, negative affect scores were similar across both sessions ($p = 0.136$).

3.1.2. Negative and Positive Affect

The repeated-measures ANOVA revealed main effects of the Session factor for both negative [$F(1, 78) = 38.967$, $p < 0.001$, $\eta p^2 = 0.333$] and positive [$F(1, 78) = 10.827$, $p = 0.002$, $\eta p^2 = 0.122$] affect. The Time factor was also significant for both negative [$F(1, 78) = 78.618$, $p < 0.001$, $\eta p^2 = 0.502$] and positive [$F(1, 78) = 14.362$, $p < 0.001$, $\eta p^2 = 0.155$] affect. Participants had lower negative affect scores ($p < 0.001$) in the acquisition session compared to the recall session (Figure 2B). Conversely, positive affect scores were higher in the acquisition session ($p = 0.002$; Figure 2C). Also, both negative and positive affect scores were higher in the pre-session than in the post-session (both ps < 0.001; Figures 2B and 2C, respectively). The Session $\times$ Time interaction was significant for negative affect [$F(1, 78) = 50.236$, $p < 0.001$, $\eta p^2 = 0.392$] but not for positive affect [$F(1, 78) = 2.491$, $p = 0.119$, $\eta p^2 = 0.031$]. Post hoc analyses revealed that in the recall session, negative affect scores were significantly higher in the pre-session than in the post-session ($p < 0.001$, Figure 2B). In contrast, in the acquisition session, negative affect scores were similar in the pre-session and in the post-session ($p = 0.165$). Additionally, negative affect in the pre-session was significantly higher in the recall session compared to the acquisition session ($p < 0.001$), whereas negative affect scores at post-session were similar across sessions ($p = 0.136$) (see Figure 2B).

3.2. Hormonal Response

3.2.1. Salivary Cortisol

Comparison Between Sessions

The repeated-measures ANOVA revealed significant main effects for Session [$F(1, 76) = 48.232$, $p < 0.001$, $\eta p^2 = 0.388$], and Time [$F(1, 76) = 90.669$, $p < 0.001$, $\eta p^2 = 0.544$] factors. Thus, participants showed higher cortisol levels in the recall session compared to the acquisition session ($p < 0.001$), and at pre-session ($t - 10$) than at post-session ($t + 25$) ($p < 0.001$). In addition, the Session $\times$ Time interaction [$F(1, 76) = 10.314$, $p = 0.002$, $\eta p^2 = 0.119$] was significant, revealing that cortisol levels were significantly higher at $t - 10$ compared to $t + 25$ in both sessions (both ps < 0.001). Furthermore, cortisol levels were higher at both $t - 10$ and $t + 25$ in the recall session than in the acquisition session (both ps < 0.001) (see Figure 3A).

Recall Session

The repeated-measures ANOVA for salivary cortisol levels revealed a significant main effect of the Time factor, $F(1.645, 126.676) = 82.236$, $p < 0.001$, $\eta p^2 = 0.516$. Post hoc pairwise comparisons showed that cortisol levels were significantly higher at pre-session ($t - 10$) than during the examination ($t + 25$) and post-session ($t + 75$) (both ps < 0.001). In addition, cortisol levels were significantly higher at $t + 25$ than at $t + 75$ ($p < 0.001$) (see Figure 3B).

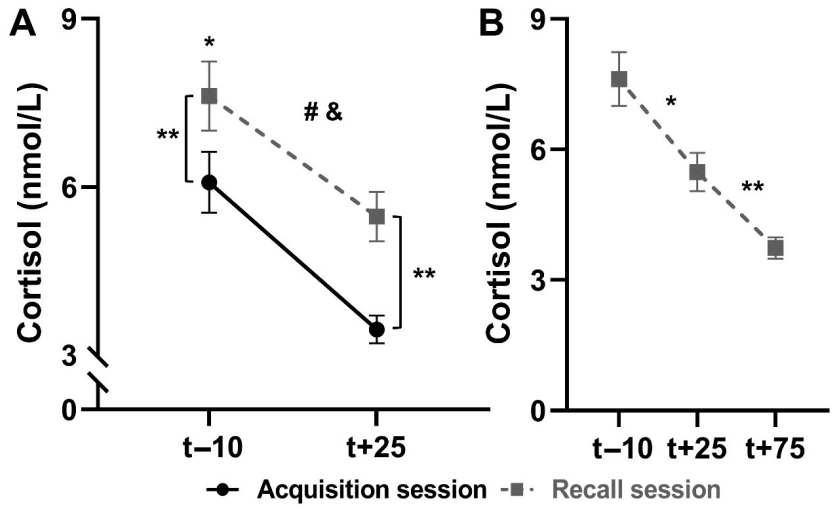

Figure 3. Comparison of salivary cortisol concentrations between sessions (**A**) and within the recall session (**B**). Depicted values are means, and error bars represent the SEM. Comparing cortisol levels between sessions, cortisol levels were higher during the recall session compared to the acquisition session ((**A**): # $p < 0.001$). Also, overall cortisol levels were higher at pre-session (t − 10) than post-session (t + 25) ((**A**): & $p < 0.001$). Participants exhibited higher cortisol levels at t − 10 than at t + 25 in both sessions ((**A**): both * ps < 0.001). Furthermore, participants had higher cortisol levels at both t − 10 and t + 25 during the recall session than during the acquisition session ((**A**): both ** ps < 0.001). In the recall session, cortisol levels were higher at pre-session (t − 10) compared to during the examination (t + 25) and post-session (t + 75) ((**B**): both * ps < 0.001). Also, participants had higher cortisol levels at t + 25 than at t + 75 ((**B**): ** $p < 0.001$).

3.2.2. Salivary DHEA

Comparison Between Sessions

The repeated-measures ANOVAs revealed significant main effects for both Session [F(1, 76) = 25.789, $p < 0.001$, $\eta p^2 = 0.251$] and Time [F(1, 76) = 21.473, $p < 0.001$, $\eta p^2 = 0.218$] factors. Consistent with the main effect of Session, participants showed higher salivary DHEA levels in the recall session than in the acquisition session ($p < 0.001$). In addition, consistent with the main effect of Time, DHEA levels were higher at t − 10 than at t + 25 ($p < 0.001$) (see Figure 4A). However, the Session × Time interaction [F(1, 76) = 0.065, $p = 0.799$, $\eta p^2 = 0.001$] was not significant, indicating that temporal changes in DHEA did not differ between sessions.

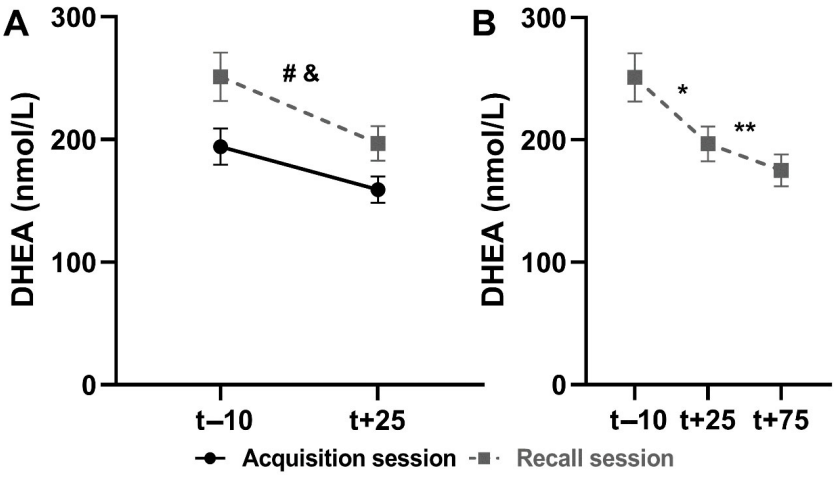

Figure 4. Comparison of salivary DHEA concentrations between sessions (**A**) and within the recall session **B**). Depicted values are means, and error bars represent the (SEM. Overall, salivary DHEA levels

were higher in the recall session compared to the acquisition session ((**A**): # $p < 0.001$) and higher at t − 10 than at t + 25 ((**A**): & $p < 0.001$). Similarly to cortisol levels, in the recall session, DHEA levels were higher at t − 10 compared to both during the examination (t + 25) and in the post-session (t + 75) ((**B**): both * ps < 0.001) and were higher at t + 25 than at t + 75 ((**B**): ** $p = 0.032$).

Recall Session

The repeated-measures ANOVA revealed a significant main effect for the Time factor [F(1.715, 132,033) = 19.634, $p < 0.001$, $\eta p^2 = 0.203$]. Participants had significantly higher DHEA levels in the pre-session (t − 10) compared to both during the examination (t + 25) and post-session (t + 75) (both ps < 0.001). Also, DHEA levels were higher at t + 25 than at t + 75 ($p = 0.032$) (see Figure 4B).

3.3. Emotional Ratings of Picture Stimuli

3.3.1. Valence Ratings

Results revealed that negative pictures (M = 7.28, SD = 0.61) were rated as significantly more negative than both positive (M = 2.90, SD = 0.75) and neutral (M = 4.61, SD = 0.53) pictures [t(78) = 34.773, $p < 0.001$, d = 3.912 and t(78) = 30.658, $p < 0.001$, d = 3.449, respectively]. Neutral pictures were also rated as more negative than positive pictures [t(78) = 21.553, $p < 0.001$, d = 2.425].

3.3.2. Arousal Ratings

As expected, negative pictures (M = 3.60, SD = 1.11) were perceived as significantly more arousing than both positive (M = 4.11, SD = 1.35) and neutral (M = 6.80, SD = 1.26) pictures [t(78) = 3.732, $p < 0.001$, d = 0.420 and t(78) = 20.888, $p < 0.001$, d = 2.350, respectively]. Additionally, positive pictures were rated as more arousing than neutral pictures [t(78) = 20.846, $p < 0.001$, d = 2.345].

3.4. Memory Performance

3.4.1. Free Recall Performance

The repeated-measures ANOVA revealed significant main effects for Session [F(1, 78) = 81.781, $p < 0.001$, $\eta p^2 = 0.512$] and Valence [F(2, 78) = 18.468, $p < 0.001$, $\eta p^2 = 0.191$] factors. However, the Session × Valence interaction was not significant [F(2, 78) = 1.128, $p = 0.326$, $\eta p^2 = 0.014$]. Participants recalled a higher percentage of pictures in the acquisition session compared to the recall session ($p < 0.001$; Figure 5A). Additionally, a main effect of valence showed that, irrespective of session, participants recalled a higher percentage of both positive ($p < 0.001$) and negative pictures ($p = 0.002$) compared to neutral pictures (see Figure 5A). The recall rates for positive and negative pictures were similar ($p = 0.061$; Figure 5A).

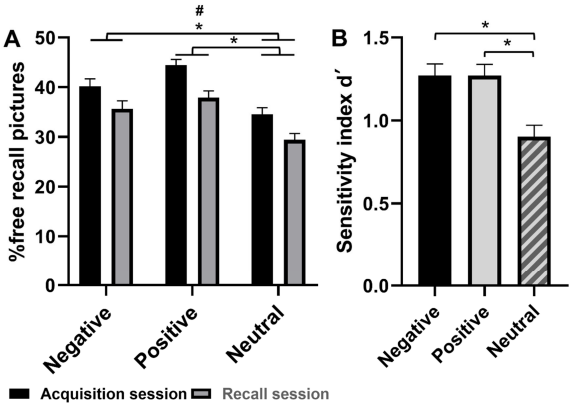

Figure 5. Immediate and delayed free recall performance for neutral and emotional (positive and negative) pictures (**A**) and recognition (**B**). Depicted values are means, and error bars represent the SEM.

A higher percentage of pictures were recalled in the acquisition session than in the recall session ((**A**): # $p < 0.001$). In addition, both negative and positive pictures were recalled at a higher rate than neutral pictures ((**A**): * $p = 0.002$ and * $p < 0.001$, respectively). Recall rates for positive and negative pictures were similar ($p = 0.061$). Recognition of neutral pictures was significantly worse than recognition of both negative ((**B**): * $p < 0.001$) and positive pictures ((**B**): * $p < 0.001$), with no difference observed between negative and positive pictures ($p = 0.498$).

3.4.2. Recognition Performance

The paired-samples *t*-test revealed that sensitivity (d′) for the recognition of neutral pictures was lower than that for both negative and positive pictures [t(78) = 4.501, $p < 0.001$, d = 0.510; t(78) = 4.574, $p < 0.001$, d = 0.510]. However, sensitivity in the recognition of negative pictures was similar to that of positive pictures [t(78) = 0.006, $p = 0.498$, d = 0.001] (see Figure 5B).

3.5. Exploratory Analysis of the Relationship Between Pre-Examination Hormonal Levels and Memory Performance

Exploratory hierarchical regression analyses were conducted to examine the relationship between pre-examination hormonal levels and memory performance. To control for multiple comparisons, Bonferroni correction was applied within each family of regression models (adjusted significance threshold $p \leq 0.004$). Associations meeting the Bonferroni-adjusted threshold ($p \leq 0.004$) are described in the main text, and the corresponding full regression models (Steps 1 and 2) are presented in Table 2. Results narrowly missing the adjusted threshold ($0.004 < p \leq 0.005$) are noted as near-threshold findings but are not interpreted as statistically significant. All remaining non-significant associations are reported in the Supplementary Materials (Sections S5 and S6).

Table 2. Exploratory regression analyses examining the relationship between pre-examination hormone levels and delayed free recall performance.

Predictors	**Criterion: % Total Delayed Free Recall Performance**	
Step 1	$R^2 = 0.058$, Adj $R^2 = -0.010$, $p = 0.617$	
	β (95% CI)	*p*
Cortisol (AS_t − 10)	−0.058 (−9.103, 5.436)	0.617
Step 2	$R^2 = 0.111$, Adj $R^2 = 0.087$, $\Delta R^2 = 0.108$, $p = 0.003$ *	
	β (95% CI)	*p*
Cortisol (AS_t − 10)	0.279 (−1.007, 18.788)	0.078
Cortisol (RS_t − 10)	−0.470 (−26.515, −5.420)	0.003 *
	Criterion: % Total delayed free recall performance	
Step 1	$R^2 = 0.003$, Adj $R^2 = -0.024$, $p = 0.887$	
	β (95% CI)	*p*
C/D ratio (AS_t − 10)	−0.012 (−18.726, 16.803)	0.914
Step 2	$R^2 = 0.119$; Adj. $R^2 = 0.095$; $\Delta R^2 = 0.118$; $p = 0.002$ *	
	β (95% CI)	*p*
C/D ratio (AS_t − 10)	0.322 (1.641, 48.489)	0.036
C/D ratio (RS_t − 10)	−0.480 (−69.085, −15.815)	0.002 *
	Criterion: % Negative pictures remembered in delayed free recall	

Table 2. *Cont.*

Predictors	Criterion: % Total Delayed Free Recall Performance	
Step 1	R^2 = 0.001, Adj R^2 = −0.012, p = 0.778	
	β (95% CI)	p
Cortisol (AS_t − 10)	0.032 (−9.679, 12.878)	0.778
Step 2	R^2 = 0.100, Adj R^2 = 0.076, ΔR^2 = 0.099, p = 0.005	
	β (95% CI)	p
Cortisol (AS_t − 10)	0.355 (2.106, 32.969)	0.026
Cortisol (RS_t − 10)	−0.451 (−40.174, −7.286)	0.005 †
Step 1	R^2 = 0.002; Adj. R^2 = −0.011; p = 0.665	
	β (95% CI)	p
C/D ratio (AS_t − 10)	0.050 (−21.492, 33.500)	0.665
Step 2	R^2 = 0.114; Adj. R^2 = 0.090; ΔR^2 = 0.111; p = 0.003 *	
	β (95% CI)	p
C/D ratio (AS_t − 10)	0.374 (8.717, 81.509)	0.016
C/D ratio (RS_t − 10)	−0.466 (−105.173, −22.402)	0.003 *

Note. β shows standardized values. Adj R^2 = Adjusted R-squared; ΔR^2 = R-squared change; 95% CI = Confidence interval; AS_t − 10 = pre-session hormone levels in the acquisition session; RS_t − 10 = pre-examination hormone levels in the recall session; C/D ratio = cortisol/DHEA ratio. Bonferroni corrections were applied, resulting in an adjusted significance threshold of $p \leq 0.004$. Significant effects after Bonferroni correction are indicated by an asterisk (*). A dagger (†) indicates near-threshold effects ($0.004 < p \leq 0.005$).

3.5.1. Delayed Free Recall

After Bonferroni correction ($p \leq 0.004$), pre-examination cortisol levels and the cortisol/DHEA ratio, assessed prior to the recall session (RS_t − 10), were negatively associated with the percentage of total delayed free recall performance [cortisol: β = −0.470, p = 0.003, 95% CI (−26.515, −5.420); cortisol/DHEA ratio: β = −0.480, p = 0.002, 95% CI (−69.085, −15.815), respectively]. For delayed free recall of negative pictures, only the cortisol/DHEA ratio assessed prior to the recall session remained significantly associated with poorer performance after correction for multiple comparisons [β = −0.466, p = 0.003, 95% CI (−105.173, −22.402)]. Also, a near-threshold association was observed between higher pre-examination cortisol levels and poorer delayed free recall of negative pictures (β = −0.451, p = 0.005, 95% CI [−40.174, −7.286]). However, this effect did not meet the corrected significance threshold and should therefore be interpreted with caution. No other associations survived correction for multiple comparisons (all ps $\geq$ 0.016).

3.5.2. Recognition

After Bonferroni correction ($p \leq 0.004$), no significant associations were observed between pre-examination hormonal levels and recognition performance ($p \geq 0.017$).

4. Discussion

The aims of this study were: (i) to examine the psychobiological (state anxiety, negative and positive affect, cortisol and DHEA) responses to an ecological stressor (i.e., a real-life academic examination), (ii) to investigate the visual memory performance for both emotional and neutral material after the stressor, and (iii) to determine whether the anticipatory hormonal responses were related to emotional and neutral visual memory performance.

Results indicated that the academic examination induced anticipatory distress, as state anxiety and negative affect were significantly higher in the pre-session of the recall

session compared to the acquisition session. Although no differences in positive affect were observed between the pre- and post-sessions, overall levels of positive affect were higher in the acquisition session. Similar studies involving stressors such as examinations or academic oral presentations have shown elevated anticipatory state anxiety compared to a control day [3,17,43] or control group [47,63]. However, Hulme et al. [64] observed no significant change in state anxiety between an examination day and a control day, possibly due to the participants' habituation to such stressors. In contrast to this study, 92.4% of our participants were facing a university examination for the first time, which may explain their pronounced psychological response. Other studies, such as those by Verschoor and Markus [44], reported elevated levels of both negative and positive affect before examinations, followed by a decrease afterwards. Conversely, our findings indicate that positive affect remained consistent between the pre- and post-examination assessments. It is plausible that while participants in Verschoor and Markus [44] saw the exam as a growth-oriented challenge, those in our study, facing their first assessment, perceived it as more threatening because of their unfamiliarity with the university environment and increased performance uncertainty.

Regarding hormonal levels, the examination elicited a significant increase in salivary cortisol and DHEA concentrations during the recall session compared to the acquisition session, supporting the effectiveness of a written academic examination in inducing both psychological and endocrine responses. Consistent with previous studies comparing examination and control sessions [3,16–18,44,65], cortisol levels were significantly higher at the pre-session (t − 10) and during the academic examination (t + 25) in the recall session compared to the acquisition session, indicative of an anticipatory cortisol response. This increased anticipatory response, together with higher baseline levels and a steeper decline during the recall session compared to the acquisition session, reinforces the ecological validity of the academic examination as a naturalistic stressor. The marked hormonal variation suggests that the examination elicited a pronounced endocrine response, which cannot be explained by circadian patterns alone. Interestingly, cortisol also decreased from t − 10 to t + 25 in the acquisition session, despite the absence of a stressor. This decline may reflect initial arousal due to task novelty or the testing environment, as well as the natural circadian rhythm of cortisol in late morning hours (for a meta-analysis, see [2]). However, the decrease was steeper in the recall session, which supports the interpretation of a specific anticipatory stress response beyond general session-related effects.

Moreover, during the recall session, both cortisol and DHEA levels were elevated at pre-session and then gradually decreased over time. Although overall DHEA levels were higher in the recall session compared to the acquisition session, no significant Session × Time interaction was observed, indicating that the temporal pattern of change across time points (t − 10 to t + 25) was comparable between sessions. In line with our findings, studies using ecological stressors have not consistently reported higher anticipatory DHEA levels. For example, Dehghan et al. [66] reported a response during a skateboarding competition in adolescent females, but Oberbeck et al. [67] reported a lack of anticipatory DHEA response in young males before a first-time parachute jump. Interestingly, in the study by Oberbeck et al. [67], DHEA levels increased immediately after the jump but fell below baseline levels within an hour. To our knowledge, only the study of [17] has reported elevated DHEA levels during examination sessions, along with anticipatory pre-examination elevations. Given the mixed results and limited research on DHEA responses to an academic written examination, more research is needed on this type of stressor.

As expected, the results showed a decrease in visual memory performance after the examination in the recall session compared to the acquisition session. However, without a control group, it is not possible to conclude whether this decrease was due to the stressor itself,

the time lapse between sessions, or proactive interference resulting from the written academic examination administered prior to the delayed task. Nevertheless, these findings are consistent with studies that used control groups and laboratory-induced stressors in young adults to assess the impact of stress on memory retrieval (e.g., [31,39,45–48]). This stress-induced memory impairment may be explained by the model proposed by Schwabe [23], which suggests that acute stress triggers the release of catecholamines and glucocorticoids, interacting in the basolateral amygdala to initiate a 'memory formation mode'. This mode enhances encoding and consolidation of the stressor, directing resources toward managing the event while inhibiting retrieval of past information to avoid interference. After the stressor, catecholamine levels decrease, and glucocorticoids activate a 'memory storage mode', reducing the encoding of new or unrelated material and limiting the retrieval of previously learned, unrelated information. This helps to consolidate the stressful event in the long-term memory by mitigating interference. The model also suggests that stress may affect emotional material more than neutral material. However, in our study, the change in free recall across sessions did not differ by valence (non-significant Session × Valence interaction). Conversely, other studies [39,46–48] found that acute stress impaired recall of negative material more than neutral material. This discrepancy may be due to the lack of a control group in our study. In contrast, recognition performance showed a different pattern, with participants performing worse when recognizing neutral pictures compared to negative and positive pictures. It is important to note that these results may be confounded by the preceding recall test and the passage of time. This pattern contrasts with findings from studies including a control group, which have typically reported poorer recognition of positive material relative to negative or neutral stimuli [32,39,49]. This discrepancy may be due to the fact that these studies did not use sensitivity indices, such as d′, to assess recognition performance. Further research is needed to examine retrieval performance for emotional and neutral material following a stressor, particularly using ecological stressors and including a control group.

In line with the notion that taking an academic examination prior to the delayed recall task may be associated with poorer memory performance, particularly for emotional stimuli [23], we found that higher pre-examination cortisol levels were associated with poorer delayed free recall overall and showed a near-threshold association with poorer recall of negative pictures. Similar patterns have been reported in research using laboratory stressors, linking elevated cortisol levels to poorer retrieval, particularly of negative emotional material, in young adults [31,33–35,48]. One possible interpretation is that these exploratory findings are consistent with accounts proposing that cortisol may prioritize consolidation of new, stressor-related information over retrieval processes, which could reduce access to previously learned material, particularly emotional material [23,24]. Similarly to other studies using laboratory stressors [42], we did not find evidence of an association between anticipatory DHEA levels prior to the examination and memory performance. Finally, the cortisol/DHEA ratio was negatively associated with performance in both the total and negative delayed free recall, indicating that a lower cortisol-to-DHEA balance (i.e., relatively lower cortisol in relation to DHEA) was associated with better performance. This pattern is consistent with theoretical accounts of DHEA's antiglucocorticoid properties and prior experimental work suggesting that DHEA may modulate glucocorticoid-related effects on memory retrieval [24]. As a tentative mechanistic pathway, DHEA has been proposed to antagonize the GABAa receptor and activate the sigma-1 receptor, thereby influencing NMDA function [68,69], mechanisms that may support memory enhancement. In young adults, the cortisol/DHEA ratio measured 25 min after the onset of acute laboratory stress showed no significant relationship with memory retrieval of an analogue story [42]. Similarly, the study by Garces-Arilla [17] found no association between anticipatory DHEA/cortisol levels before a written examination and examination performance (i.e.,

marks). This discrepancy may be due to the nature of the material recalled. Nevertheless, these associations should be interpreted with caution given the exploratory nature of the regression analyses. In addition, the relatively wide confidence intervals observed for some regression coefficients, particularly for the cortisol/DHEA ratio, indicate limited precision and potential instability of the effect size estimates. Moreover, it is important to note that, in the absence of a control group, alternative explanations cannot be ruled out. In particular, the observed decline in memory performance may reflect proactive interference arising from the cognitively demanding examination content, time-dependent decay across the interval between acquisition and recall, or cognitive depletion resulting from completing a prolonged academic examination prior to the memory tasks. Although examination duration was included as a robustness check, it showed no significant association with performance, which may partly reflect the restricted range of examination times in our sample (M = 73.29, SD = 8.83), with most participants taking broadly similar amounts of time.

This study has several limitations that should be taken into account. First, the absence of a control group represents the main limitation of the study and precludes causal inference. Consequently, it is not possible to determine whether the observed decline in memory performance after the examination was attributable to acute academic stress at retrieval or to alternative, non-mutually exclusive explanations, including (i) time-dependent retention or decay effects across the acquisition-recall interval, (ii) proactive interference arising from the cognitively demanding examination content administered prior to the memory tasks, (iii) examination-related cognitive depletion or mental fatigue, and (iv) more general anticipatory arousal associated with being evaluated. Second, although the use of a real-life academic examination enhances ecological validity, examinations may vary in difficulty and perceived academic consequences, which may limit the generalizability of the findings to other academic contexts. Third, to more accurately characterize hormonal responses, it would also be beneficial to collect hormone samples one hour after the stressor and again several weeks after the examination, when students are free from academic stress. Fourth, the sample was predominantly female. This imbalance limits the examination of potential sex-related differences in stress and hormonal responses, as the study was not sufficiently powered to test sex as a factor. Accordingly, the generalizability of the findings across sexes should be interpreted with caution. Finally, the precision of some regression estimates was limited, as reflected by wide confidence intervals, underscoring the need for replication in larger samples. Future studies should therefore include appropriate control conditions, such as non-examination recall sessions matched in timing and cognitive demands, to disentangle stress-related effects from retention, interference, and fatigue. In addition, recruiting more balanced samples with respect to sex and incorporating designs that manipulate examination stakes or stressor intensity would help to clarify the specificity and generalizability of endocrine–memory associations.

Despite these limitations, our findings suggest that exposure to an acute naturalistic stressor, such as an academic examination, may be associated with concurrent psychological and endocrine changes and an anticipatory emotional and hormonal response. Specifically, we observed a drop in cortisol levels after the examination, which may reflect post-stressor recovery, although pre-examination levels in the recall session remained higher than those observed in the acquisition session. Overall, these findings are consistent with the ecological validity of academic examinations as real-world stressors. In addition, the observed pattern for cortisol and DHEA is compatible with a potential buffering role of DHEA, in that a lower cortisol-to-DHEA balance (i.e., relatively lower cortisol in the context of higher DHEA) was associated with better performance, particularly for emotional pictures. Taken together, these results highlight the relevance of studying naturalistic stress responses in

everyday settings, such as universities, and provide a rationale for further research on the modulatory role of DHEA within the stress response. Although our observational design precludes causal inference, these exploratory patterns offer preliminary directions for future studies designed to test mechanisms more directly and to evaluate whether stress-reduction strategies during academic examinations could mitigate stress-related changes and their potential association with memory performance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app16031306/s1. Figure S1: Salivary cortisol distribution across sessions and time points; Figure S2: Salivary DHEA distribution across sessions and time points; Table S1: Mean and standard deviation for raw salivary cortisol and DHEA concentrations across sessions and time points; Table S2: Exploratory regression analyses examining the relationship between pre-examination hormone levels and delayed free recall performance; Table S3: Exploratory regression analyses examining the relationship between pre-examination hormone levels and recognition performance. Text S1: Hierarchical regression modelling strategy to account for baseline hormonal variability.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of the Aragon Community (protocol code: PI20/074; approval date: 28 February 2020) for studies involving humans.

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Abbreviations

The following abbreviations are used in this manuscript:

DHEA	Dehydroepiandrosterone
HPA	Hypothalamic–Pituitary–Adrenal (axis)
NMDA	N-methyl-D-aspartate (receptor)
SAM	Self-Assessment Manikin
STAI-S	State-Trait Anxiety Inventory—State version
PANAS	Positive and Negative Affect Schedule
IAPS	International Affective Picture System
SES	Subjective Educational and Socioeconomic Status

BMI	Body Mass Index
MCP	Menstrual Cycle Phase
SPSS	Statistical Package for the Social Sciences
ANOVA	Analysis of Variance
SEM	Standard Error of the Mean

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