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Contribution of stress and sex hormones to memory encoding

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ABSTRACT

Distinct stages of the menstrual cycle and the intake of oral contraceptives (OC) affect sex hormone levels, stress responses, and memory processes critically involved in the pathogenesis of mental disorders. To characterize the interaction of sex and stress hormones on memory encoding, 30 men, 30 women in the early follicular phase of the menstrual cycle (FO), 30 women in the luteal phase (LU), and 30 OC women were exposed to either a stress (socially evaluated cold-pressor test) or a control condition prior to memory encoding and immediate recall of neutral, positive, and negative words. On the next day, delayed free and cued recall was tested. Sex hormone levels verified distinct estradiol, progesterone, and testosterone levels between groups. Stress increased blood pressure, cortisol concentrations, and ratings of stress appraisal in all four groups as well as cued recall performance of negative words in men. Stress exposure in OC women led to a blunted cortisol response and rather enhanced cued recall of neutral words. Thus, pre-encoding stress facilitated emotional cued recall performance in men only, but not women with different sex hormone statuses pointing to the pivotal role of circulating sex hormones in modulation of learning and memory processes.

1. Introduction

Stress and stress hormones exert tremendous effects on emotional learning and memory processes playing a crucial role in the pathogenesis of various mental disorders such as posttraumatic stress disorder (PTSD) or anxiety disorders (De Quervain et al., 2017; Merz et al., 2016). Importantly, these effects depend on the exact timing between stress and the respective memory phase. Sex hormones also critically modulate these relationships and contribute to the development, maintenance and treatment of mental disorders (Cover et al., 2014; Lebron-Milad and Milad, 2012; Merz and Wolf, 2017). Thus, the effects of stress and sex hormones need to be considered together when investigating learning and memory processes to understand their potential clinical relevance.

Stress triggers the activation of two systems: on the one hand, stress initializes the sympathetic nervous system (SNS) to release catecholamines such as norepinephrine and epinephrine. On the other hand, stress triggers activation of the hypothalamus-pituitary-adrenocortical (HPA) axis leading to a hormonal cascade ending in the secretion of glucocorticoids (GCs; mainly cortisol in humans). GCs and norepinephrine jointly modulate (emotional) learning and memory processes by acting on respective receptors especially located in the amygdala and hippocampus (De Kloet et al., 2005; Roozendaal et al., 2009).

While it has been consistently reported that stress hormones impair memory recall, but enhance memory consolidation (Schwabe and Wolf, 2013; Wolf, 2009), there is still no consensus how exactly stress hormones influence memory encoding. Several theories have been proposed to explain these discrepant findings (cf. Merz and Wolf, 2015b) including temporal proximity of stress and encoding (Akirav and Richter-Levin, 1999, 2002; Diamond et al., 2007; Joëls et al., 2006, 2011; Schwabe et al., 2012), time of day (Het et al., 2005), and emotionality of the learning material (e.g. Buchanan and Lovallo, 2001; Payne et al., 2007; Rimmele et al., 2003). Importantly, it has also been assumed that stress effects on memory encoding might critically depend on sex hormone status (Merz and Wolf, 2017).

The release of sex hormones (such as estradiol, progesterone, or testosterone) is under the control of the hypothalamus-pituitary-gonadal (HPG) axis and varies substantially in women over the course of the menstrual cycle. It is typically reduced during intake of oral contraceptives (OCs; Fleischman et al., 2010; Montoya and Bos, 2017), but can be modulated by different factors such as OC type and brand (D'Arpe et al., 2016; Elliott-Sale et al., 2013; London and Jensen, 2016). Likewise, sex hormones influence different brain structures such as the amygdala and the hippocampus by targeting their respective receptors (McEwen and Milner, 2017). Sex hormones also modulate the salivary cortisol response to a psychosocial stressor with a similar pattern in men and women in the luteal phase of the menstrual cycle (LU; high estradiol and progesterone levels) but reduced or blunted responses in

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women in the follicular phase (FO; low estradiol and progesterone levels) or taking OCs (Kirschbaum et al., 1999; see also Childs et al., 2010; Cornelisse et al., 2011; Espin et al., 2013; Rohleder et al., 2003). In the present report, this sex hormone status effect on the stress response will be investigated using a combination of a psychosocial and a physical stressor and its impact on memory encoding will be characterized.

Previous studies focusing on interactive effects of sex and stress hormones on memory encoding found partly discrepant results: While men remembered emotional pictures better in a recognition test when encoding took place after a psychosocial stress induction, this effect was absent for neutral pictures and in women (Cornelisse et al., 2011). More specifically, pre-encoding stress also led to a better immediate free recall of neutral material in men, but not in FO, LU, or OC women (Espin et al., 2013). Another study reported free recall of neutral words to be enhanced in men and OC women alike when free recall was tested one hour or one day after encoding (Schwabe et al., 2008a). Taken together with studies including men only (e.g. Nater et al., 2007; Quaedflieg et al., 2013; Tops et al., 2003; Wolf, 2012) or both sexes without explicitly testing the impact of different sex hormone status in women (e.g. Rimmele et al., 2003; Zoladz et al., 2011), pre-encoding stress effects seem to be more stable in men compared with women. However, available results need to be expanded to a detailed investigation of the relevance of sex hormone milieu concerning the impact of pre-encoding stress on delayed memory recall. Therefore, the current report contrasts men with FO, LU, and OC women in their delayed recall performance after being exposed to pre-encoding stress.

2. Material and methods

2.1. Participants

All participants were recruited through email announcements at the University of Trier, Germany, or by personal address. Most of them were students (117; 56 students of psychology), the remaining three participants were working at the university. To assess different sex hormone statuses, 30 men, 60 free-cycling women, and 30 OC taking women were included. Free-cycling women did not take any kind of contraceptives and reported to have a regular menstrual cycle. One half of them was invited in the early follicular phase (FO; 3rd–9th day after the onset of their last menstruation) and the other half in the luteal phase (LU; 3rd–9th day before the onset of their next menstruation) of the individual menstrual cycle. OC women were required to have been taking their birth control pill (only monophasic preparations with a 0.02–0.035 mg ethinylestradiol and a gestagenic component) for at least the last three months. Preparations included gestagenic components with androgenic (desogestrel, levonegestrel) and anti-androgenic properties (chlormadinone acetate, cyproterone acetate, dienogest, drospirenone). They were tested during the pill intake phase on both experimental days.

None of the participants was taking regular medication except OCs or reported a history of psychiatric or neurological treatment. Exclusion criteria covered somatic diseases (e.g., high blood pressure, Raynaud's disease or allergies), in particular endocrine diseases known to influence endogenous hormone levels (e.g., hyper-/hypothyroidism), and smoking more than five cigarettes/month. Inclusion criteria comprised an age between 18 and 35 years and a body mass index (BMI) between 18 and 28 kg/m^2. The final sample had a mean age of 23.22 (SD = 2.92) years and a mean BMI of 21.96 (SD = 2.49) kg/m^2.

The study was approved by the local ethics committee of the University of Trier.

2.2. Procedure

On two consecutive days, experimental sessions were run between 1 and 6 p.m. and participants had to be awake for at least three hours before testing in order to control for circadian fluctuations in salivary cortisol (Kudielka et al., 2009). They were instructed to refrain from intense physical exercise, smoking, eating, and drinking anything but water for at least ninety minutes before the start of the experiment.

After arrival on day one, participants received a detailed explanation of the general procedure and gave written informed consent. They were then instructed to provide a first saliva sample (S1) and demographical details as well as a second saliva sample (S2) after a resting phase of ten minutes; both saliva samples served the determination of sex hormone status (see 2.3). After that, a third saliva sample (C1) for the determination of baseline cortisol concentrations was required (see 2.4). Then, blood pressure was measured and participants from each sex hormone status group were randomly assigned to one of two experimental conditions comprising 15 persons each (stress condition: socially evaluated cold-pressor test (SECPT)) vs. warm water control condition; (Schwabe et al., 2008b). Both conditions required participants to immerse their dominant hand up to the elbow into water, with a temperature between 0 and 3 °C in the stress condition and between 36 and 37 °C in the control condition. A neutral female experimenter (only present for the duration of the SECPT) videotaped and observed participants during the SECPT while blood pressure was recorded simultaneously. In the control condition, neither videotaping nor observation took place. In both conditions, participants were instructed to remove their arm from the water after three minutes. If they did not manage to keep their arms in the ice water in the SECPT for this duration, they were instructed to hold their hands above the water for the remaining time. After cessation, participants answered three questions concerning their subjective appraisal of the task (see 2.4). The fourth saliva sample (C2) was provided and blood pressure was measured eight minutes after onset of the experimental condition. Twenty minutes after stress onset, participants were asked for a fifth saliva sample (C3), followed by memory encoding and immediate recall (see 2.5). Thirty minutes after stress onset, participants provided the sixth saliva sample (C4).

At the beginning of day two, participants provided two saliva samples (S3, C5) before free and cued memory recall (see 2.5) was tested in a different room than on day one. Ten minutes after the first saliva sample and after free memory recall, the next saliva sample (S4) was collected. After cued recall, participants gave the last saliva sample (C6) and finally received either partial course credits or 20€ as a monetary compensation for their attendance.

2.3. Measurement and analysis of sex hormones

Eppendorf tubes were used for the collection of saliva samples required for the determination of the sex hormones estradiol, progesterone, and testosterone (samples S1-S4). These four samples were pooled before analyses, thus generating one concentration for each sex hormone subserving to check for expected differences between men, FO, LU, and OC women (cf. Merz et al., 2012). All saliva samples were stored at −20 °C until assayed. Commercially available enzyme-linked immunosorbent assays (for estradiol and testosterone: Demeditec, Kiel, Germany) and enzyme imunoassays (for progesterone: Salimetrics, Newmarket, Suffolk, UK) were used to measure free hormone concentrations. Intra-assay coefficients of variations (CV) for all analyses were below 8% with inter-assay CV below 11%. Data of one OC woman in the control condition could not be analyzed for progesterone and testosterone concentrations, since hormonal levels were outside the measurable range of the assay. Except for analyses of progesterone and testosterone levels, the respective data were included.

All data were analyzed using the SPSS 20.0 software (SPSS Inc., Chicago, USA) with the significance level set to $\alpha = .05$. Estradiol, progesterone, and testosterone were subjected to separate analyses of variance (ANOVA) with the between-subjects factors stress (stress vs. control condition) and sex hormone status (men vs. FO vs. LU vs. OC women). Where appropriate, Greenhouse-Geisser degrees of freedom

adjustment in case of a violation of sphericity was applied and effect sizes (η_p^2) are reported accordingly.

2.4. Measurement and analysis of the stress response

Stress-induced activation of the SNS was verified by measurements of systolic and diastolic blood pressure using an automatic upper arm blood pressure monitor (Bosch + Sohn, Jungingen, Germany). The cuff was placed 2 cm above the elbow of the non-dominant arm. In order to reduce measurement errors, participants were instructed to avoid speaking and moving during the procedure. Measurements took place seven minutes before stress onset (baseline), during the three minutes of the stressor (peak) and eight minutes after stress onset (post). Assessments were carried out three times in a row at each time point in order to calculate mean values of blood pressure within a time window of three minutes.

Stress-induced activation of the HPA axis was confirmed by collection of saliva samples (C1-4) using Salivette collection devices (Sarstedt, Nuembrecht, Germany) ten minutes before (baseline) as well as immediately (+4 min), 20 and 30 min after onset of the experimental procedure. Additional two saliva samples (C5, C6) were obtained at the beginning of day two and after the cued recall test. All samples were stored at −20 °C until analyses. The fraction of free unbound salivary cortisol was determined by use of a Dissociation-Enhanced Lanthanide Fluorescent Immunoassay as described previously by Dressendörfer et al. (1992). Intra- and inter-assay coefficients of variance were below 6.7% and 9.0%, respectively.

Ratings of stressfulness, painfulness, and unpleasantness of the respective procedure were obtained immediately after cessation of the stress or control condition on a scale ranging from 0 ('not at all') to 100 ('very much'; ratings adopted from Schwabe et al., 2008b).

Repeated measures ANOVA were applied to investigate changes in physiological parameters due to the experimental manipulation with the repeated measurement factor time (baseline, during, and post hand immersion for blood pressure; baseline, +4, +20, and +30 min for changes in cortisol concentrations) as well as the between-subjects factors stress and sex hormone status. In addition, a repeated measures ANOVA was calculated for cortisol concentrations on day two with the repeated measurement factor time (baseline, post) and the between-subjects factors stress and sex hormone status. Ratings of stress appraisal were analyzed using ANOVA with the between-subjects factors stress and sex hormone status.

2.5. Assessment and analyses of memory performance

Before encoding on day one, participants were instructed to learn the following 30 words by heart and that immediately afterwards, they should write down as many words as they can remember. A word list containing ten neutral (e.g., object, symbol), ten positive (e.g., angel, warmth), and ten negative (e.g., horror, terror) German nouns (adapted from Kuhlmann et al., 2005) was presented to the participants on a piece of paper. There were no differences among the neutral, positive, and negative words regarding word frequency, word length, or semantic cohesion (cf. Kuhlmann et al., 2005). After two minutes of encoding time, immediate free recall was tested during which participants had to write down as many words as they could remember with no time limit. This procedure (encoding + immediate free recall) was directly repeated resulting in two learning trials for all participants.

On day two, delayed free and cued recall was tested with no timeout. Cued recall was tested immediately after free recall with a random presentation of the first two letters of each learned word on a piece of paper. Participants were instructed to complete the word stems with the previously learned words.

Possible within- and between-subject variances in initial learning were accounted for by using the percentage of words remembered in relation to the second learning trial of day one (cf. Kuhlmann et al., 2005). Data concerning neutral words of one man in the control condition had to be excluded for the percentage free and cued recall scores, since he did not recall any of the neutral words (thus, calculating the percentage score with 0/0 was not possible).

Memory performance was analyzed using repeated measures ANOVA separately for the percentage free and cued recall scores (and additionally for the first immediate recall trial) with the between-subjects factors stress and sex hormone status as well as the within-subjects factor emotion (neutral vs. positive vs. negative). Pearson product-moment correlations were conducted to test whether stress-induced cortisol increases are associated with memory performance. Therefore, a post-encoding increase in cortisol (Δ cort) was calculated by subtracting the +30 value from the baseline value of day one. Additionally, the area under the curve with respect to increase (AUC_i; Prüssner et al., 2003) was computed including the four cortisol values of day one.

3. Results

3.1. Sex hormones

Estradiol and progesterone levels were significantly different among the four sex hormone status groups with highest levels observed in LU women compared to all other groups (estradiol: main effect sex hormone status, $F_{(3;112)} = 4.59$, $p = .005$, $\eta_p^2 = .11$; progesterone: main effect sex hormone status, $F_{(3;111)} = 4.59$, $p < .001$, $\eta_p^2 = .27$; all post hoc tests comparing LU women with each of the other three groups: $p < .05$; cf. Table 1). Testosterone levels also significantly differed among the four sex hormone status groups with highest levels observed in men compared to all other groups (main effect sex hormone status: $F_{(3;111)} = 4.59$, $p < .001$, $\eta_p^2 = .52$; all post hoc tests comparing men with each of the other three groups: $p < .001$; cf. Table 1). Additionally, post hoc tests indicated testosterone levels to be slightly reduced in OC women compared to LU women ($p = .012$). No significant main or interaction effects with stress occurred. More detailed information about minimum, maximum, and standard deviations of estradiol, progesterone, and testosterone levels for all groups can be found in Supplementary Table 1.

Table 1
Mean (± SEM) estradiol, progesterone, and testosterone levels (in pg/ml) are separately shown for the stress and control condition as well as for men, women tested in the follicular (FO) or luteal (LU) phase of their respective menstrual cycle, and women taking oral contraceptives (OC).

	men		FO women		LU women		OC women	
	control	stress	control	stress	control	stress	control	stress
estradiol (pg/ml)	6.62 ± 0.91	5.76 ± 1.04	7.64 ± 0.95	6.88 ± 0.67	9.71 ± 1.39[a]	11.05 ± 1.81[a]	7.54 ± 1.11	7.23 ± 1.26
progesterone (pg/ml)	55.29 ± 22.79	35.85 ± 8.70	55.61 ± 6.94	45.57 ± 7.19	146.42 ± 33.18[a]	159.05 ± 38.54[a]	31.99 ± 7.01	40.41 ± 12.42
testosterone (pg/ml)	144.43 ± 11.92[b]	122.96 ± 8.83[b]	73.22 ± 6.09	67.44 ± 4.21	78.09 ± 6.58[c]	82.42 ± 5.46[c]	57.12 ± 5.16	66.87 ± 5.77

[a] Control + stress condition: higher levels compared to all other sex hormone status groups ($p < .05$).
[b] Control + stress condition: higher levels compared to all other sex hormone status groups ($p < .001$).
[c] Control + stress condition: higher levels compared to OC women ($p = .012$).

Table 2
Mean (± SEM) systolic and diastolic blood pressure data and stress ratings are separately shown for the stress and control condition and for men, women tested in the follicular (FO) or luteal (LU) phase of their respective menstrual cycle, and women taking oral contraceptives (OC).

	men[b]		FO women		LU women		OC women[c,d]	
	control	stress	control	stress	control	stress	control	stress
systolic blood pressure (mmHg)								
baseline	121.68 ± 2.04	126.22 ± 4.77	108.42 ± 2.87	106.09 ± 2.36	111.56 ± 2.00	108.73 ± 2.33	116.53 ± 3.90	113.24 ± 3.10
during hand immersion	124.11 ± 1.91	149.37 ± 4.06[a]	109.02 ± 2.45	130.61 ± 3.99[a]	112.47 ± 1.80	132.36 ± 3.76[a]	118.38 ± 2.59	136.68 ± 3.81[a]
5 min after stress/control	117.34 ± 2.10	124.31 ± 2.94	104.71 ± 2.56	105.18 ± 1.98	109.18 ± 1.23	105.73 ± 2.78	112.42 ± 2.61	111.16 ± 2.31
diastolic blood pressure (mmHg)								
baseline	70.31 ± 2.14	73.00 ± 3.16	69.71 ± 1.91	71.11 ± 1.72	76.33 ± 1.64	70.22 ± 1.68	77.91 ± 2.78	74.89 ± 2.21
during hand immersion	74.40 ± 2.68	97.54 ± 3.12[a]	71.71 ± 1.98	91.89 ± 2.71[a]	74.76 ± 1.96	93.04 ± 3.15[a]	80.19 ± 2.41	98.56 ± 2.65[a]
5 min after stress/control	68.93 ± 2.26	79.09 ± 3.15	68.51 ± 1.76	71.42 ± 1.23	74.42 ± 1.87	69.62 ± 2.42	77.27 ± 2.75	74.67 ± 1.95
stress ratings after experimental manipulation								
stressful	3.33 ± 2.11	50.67 ± 4.31[a]	10.00 ± 4.88	51.33 ± 8.83[a]	2.67 ± 1.18	49.33 ± 7.53[a]	2.00 ± 1.45	59.33 ± 7.96[a]
painful	0.67 ± 0.67	56.00 ± 6.00[a]	1.33 ± 0.91	68.67 ± 7.61[a]	0.67 ± 0.67	68.00 ± 5.18[a]	1.33 ± 0.91	78.00 ± 5.36[a]
unpleasant	4.67 ± 2.36	58.67 ± 6.61[a]	9.33 ± 3.16	67.33 ± 8.75[a]	8.67 ± 5.93	65.33 ± 7.55[a]	0.67 ± 0.67	72.00 ± 7.25[a]

[a] Stress condition: higher values compared to the control group ($p < .001$).
[b] In general higher systolic blood pressure compared to all women groups ($p \leq .001$).
[c] In general higher systolic and diastolic blood pressure compared to FO women ($p \leq .005$).
[d] In general higher systolic blood pressure compared to LU women ($p = .047$).

3.2. Stress responses

Analyses of blood pressure measures showed that stress induction was successful (systolic blood pressure: time x stress interaction, $F_{(1.7,193.3)} = 128.06$, $p < .001$, $\eta_p^2 = 0.53$, main effect stress, $F_{(1,112)} = 14.44$, $p < .001$, $\eta_p^2 = 0.11$, main effect time, $F_{(1.7,193.3)} = 223.67$, $p < .001$, $\eta_p^2 = 0.67$; diastolic blood pressure: time × stress interaction, $F_{(2.0,221.7)} = 138.53$, $p < .001$, $\eta_p^2 = 0.55$, main effect stress, $F_{(1,112)} = 20.93$, $p < .001$, $\eta_p^2 = 0.16$, main effect time, $F_{(2.0,221.7)} = 208.42$, $p < .001$, $\eta_p^2 = 0.65$). Follow-up analyses revealed significantly higher blood pressure during hand immersion (systolic blood pressure: $F_{(1,112)} = 89.59$, $p < .001$, $\eta_p^2 = 0.44$; diastolic blood pressure: $F_{(1,112)} = 116.72$, $p < .001$, $\eta_p^2 = 0.51$) in the stress compared to the control condition, but not at baseline or post hand immersion (all $p > .37$; cf. Table 2). Additionally, the main effect sex hormone status (systolic blood pressure: $F_{(3,112)} = 15.54$, $p < .001$, $\eta_p^2 = 0.29$; diastolic blood pressure: $F_{(3,112)} = 3.38$, $p = .021$, $\eta_p^2 = 0.83$) showed that men had higher systolic blood pressure compared to all women groups (all $p \leq .001$). Besides, OC women had higher systolic and diastolic blood pressure compared to FO women ($p \leq .005$) and higher diastolic blood pressure compared to LU women ($p = .047$).

Higher cortisol concentrations were found in the stress compared to the control condition over the course of time (time × stress interaction, $F_{(1.3,149.6)} = 32.94$, $p < .001$, $\eta_p^2 = 0.23$; main effect time, $F_{(1.3,149.6)} = 11.24$, $p < .001$, $\eta_p^2 = 0.09$; main effect stress, $F_{(1,112)} = 16.04$, $p < .001$, $\eta_p^2 = 0.13$). Follow-up analyses confirmed significant between-group differences 20 min ($F_{(1,112)} = 32.71$, $p < .001$, $\eta_p^2 = 0.23$) and 30 min ($F_{(1,112)} = 22.23$, $p < .001$, $\eta_p^2 = 0.17$), but neither 4 min after stress onset nor at baseline (both $p > .67$; cf. Fig. 1). No significant differences were obtained for main and interaction effects with sex hormone status. As observed before (Cornelisse et al., 2011; Kirschbaum et al., 1999; Merz et al., 2013), planned comparisons revealed that men showed higher increases in cortisol concentrations after stress induction when only compared to OC women (time × stress interaction, $F_{(1.3,35.7)} = 5.68$, $p = .016$, $\eta_p^2 = 0.17$), which was evident 20 min after stress onset ($F_{(1,28)} = 9.52$, $p = .005$, $\eta_p^2 = 0.25$). On day two, a significant decline in cortisol concentrations was observed from baseline to post recall ($F_{(1,112)} = 11.63$, $p = .001$, $\eta_p^2 = 0.09$) without any further significant effects.

Finally, participants in the stress condition rated the hand immersion into water as significantly more stressful (main effect stress: $F_{(1,112)} = 149.56$, $p < .001$, $\eta_p^2 = 0.57$), painful ($F_{(1,112)} = 467.45$, $p < .001$, $\eta_p^2 = 0.81$) and unpleasant ($F_{(1,112)} = 204.97$, $p < .001$, $\eta_p^2 = 0.65$; cf. Table 2) compared to participants in the control condition. No significant main or interaction effects with sex hormone status occurred.

3.3. Stress effects on immediate and delayed memory recall

Analyses of immediate free recall performance revealed a main effect of emotion only ($F_{(2.0;222.4)} = 7.14$, $p = .001$, $\eta_p^2 = .06$), which was driven by worse recall of neutral compared to positive and negative words (both $p < .003$), whereas no difference between recall of positive and negative words emerged ($p > .95$).

Delayed free recall performance was different between the four sex hormone status groups (main effect sex hormone status, $F_{(3;111)} = 3.48$, $p = .018$, $\eta_p^2 = .09$; cf. Fig. 2). Post hoc tests revealed that men remembered less words compared to FO and LU women (both $p \leq .028$). Additionally, exploratory analyses could show that this effect was only found for positive words (main effect sex hormone status, $F_{(3;112)} = 4.45$, $p = .005$, $\eta_p^2 = .11$), which men could recall worse compared to all three women groups (all $p \leq .011$). No other main or interaction effects were found for free recall performance.

Delayed cued recall performance was subject to a trend-level modulation by emotion, stress and sex hormone status (main effect emotion, $F_{(1.6,180.9)} = 3.62$, $p = .038$, $\eta_p^2 = 0.03$; emotion × stress × - sex hormone status interaction, $F_{(4.9,180.9)} = 1.97$, $p = .087$, $\eta_p^2 = 0.05$; cf. Fig. 3). Separate and planned analyses of the four sex hormone status groups could localize this interaction in men (emotion × stress interaction, $F_{(1.9,51.9)} = 3.60$, $p = .036$, $\eta_p^2 = 0.12$) and OC women (emotion × stress interaction, $F_{(1.5,41.4)} = 4.26$, $p = .031$, $\eta_p^2 = 0.13$). Whereas pre-encoding stress enhanced cued recall of negative words in men ($p = .023$), it tended to increase cued recall of neutral words in OC women ($p = .063$). This stress effect in men was confirmed with correlational analyses revealing positive associations between cortisol increases and cued recall performance of negative words (Δ cort: $r = .45$, $p = .013$; AUC_i: $r = .38$, $p = .041$). No correlation was found for the result obtained in OC women.

4. Discussion

The current study characterized interactive effects of stress and sex hormones on memory encoding. Main results revealed pre-encoding stress to enhance cued recall of negative words in men, but tended to

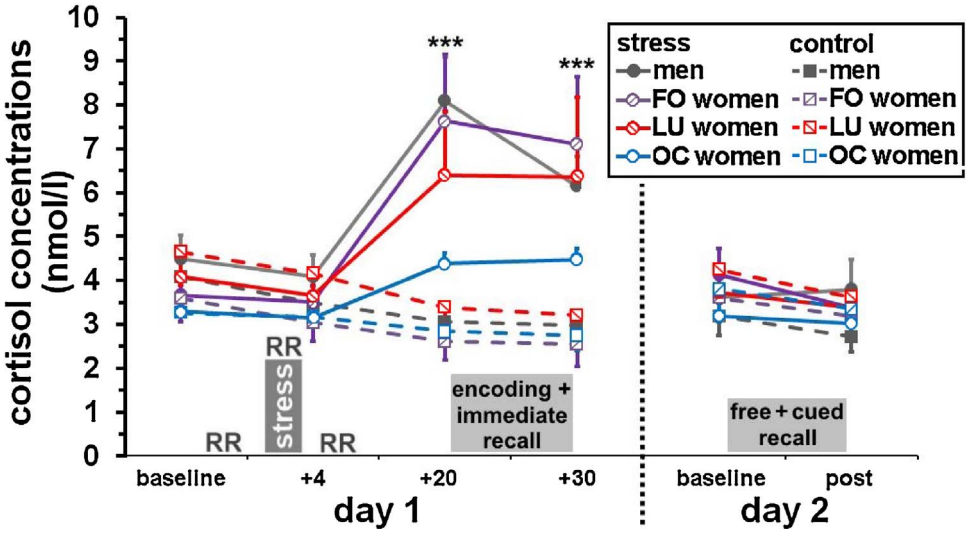

Fig. 1. Mean (± SEM) salivary cortisol responses in the stress (solid lines) and the control condition (dashed lines) are depicted over the course of the experimental timeline (baseline, 4, 20, and 30 min after stress onset on day 1; baseline and post recall on day 2) and separated for men, women tested in the follicular (FO) or luteal (LU) phase of their respective menstrual cycle or women taking oral contraceptives (OC).Stress induction led to significantly increased cortisol concentrations in the stress compared to the control condition, evident 20 and 30 min after stress onset (*** $p < .001$). Memory encoding and immediate free recall took place between these two time points.Delayed free and cued recall was tested on day 2, where no significant group differences for salivary cortisol concentrations were found.RR: blood pressure measurement.

increase cued recall of neutral words in OC women, while FO and LU women were not subject to this memory modulation.

In general, the present study replicates and expands prior work and suggestions on the differential impact of stress on memory encoding by underlining the consideration of sex hormone status. Firstly, it has been proposed that temporal proximity matters: pre-encoding stress facilitated memory processes when stress took place within the learning context (e.g. stress occurring shortly before or during encoding), but stress reduced memory performance when experienced outside of the learning context (e.g. with a longer time lag between stress and encoding), suggested to rely on stress-induced amygdala activation modulating hippocampal plasticity (Akirav and Richter-Levin, 1999, 2002; Diamond et al., 2007; Joëls et al., 2006, 2011; Schwabe et al., 2012). The present results obtained in men regarding negative words fit very well to this suggestion, since stress was induced temporally close to memory encoding (20 min; for encoding taking place immediately vs. 30 min after the SECPT, cf. Zoladz et al., 2011, 2017). Additionally, exposure to stress took place in the same room as encoding, probably leading participants to experience the stressor as part of the learning context. Extending the period between stress and encoding to 30 min can change the picture as previously observed in free recall and recognition performance in men (Zoladz et al., 2011, 2013, 2017). Probably, a shift between facilitating and impairing effects of stress on subsequent encoding occurs within this time frame (between 20 and 30 min) and/or depends on the realization of the memory test procedure (free recall vs. cued recall vs. recognition), which needs to be systematically investigated in future studies.

Secondly, time of day is supposed to play a pivotal role: meta-analytically, Het et al. (2005) showed that cortisol administration impairs memory when given in the morning, but enhances memory

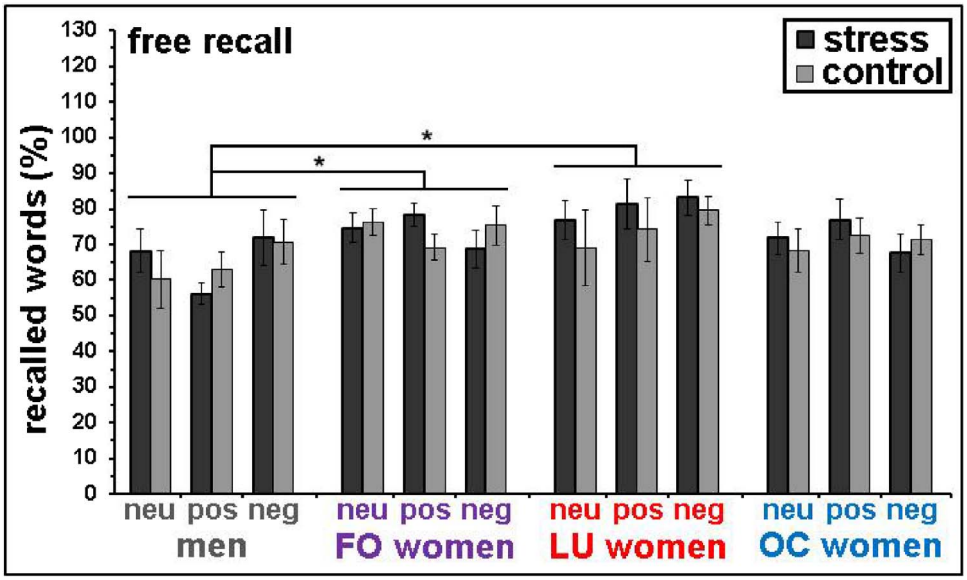

Fig. 2. Effects of pre-encoding stress (vs. a control condition) on delayed free recall of neutral (neu), positive (pos), and negative (neg) words in men, women in the follicular phase (FO), women in the luteal phase (LU) of their respective menstrual cycle, and in women taking oral contraceptives (OC). Free recall performance is presented as percentage of words remembered from the second immediate recall of the previous day.Recall was reduced in men compared to FO and LU women (* $p \leq .028$). In particular, reduced recall was observed for positive words in men compared to all women groups ($p \leq .011$, not marked).

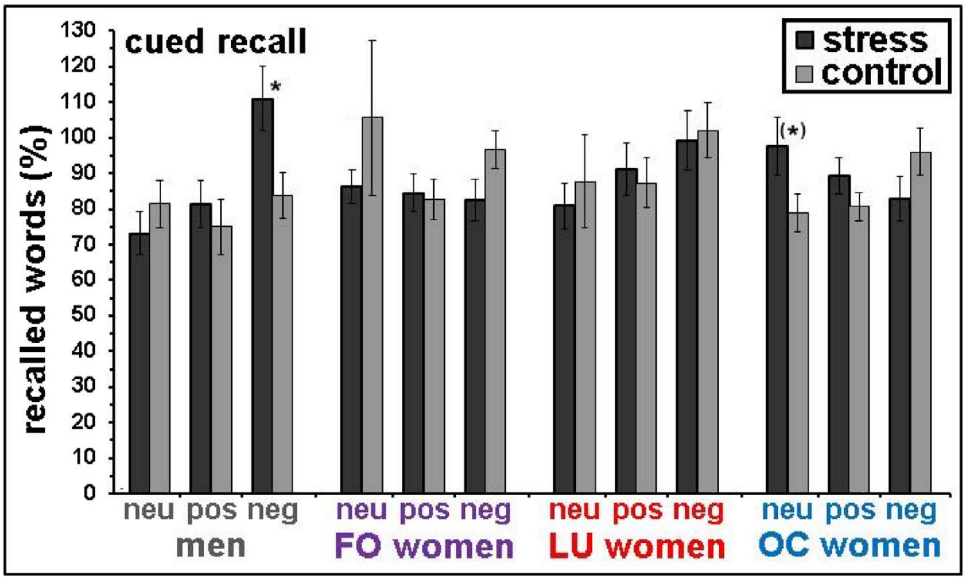

Fig. 3. Effects of pre-encoding stress (vs. a control condition) on delayed cued recall of neutral (neu), positive (pos), and negative (neg) words in men, women in the follicular phase (FO), women in the luteal phase (LU) of their respective menstrual cycle, and in women taking oral contraceptives (OC). Cued recall performance is presented as percentage of words remembered from the second immediate recall of the previous day.Pre-encoding stress enhanced recall of negative words in men (* p = .023), but rather increased recall of neutral words in OC women ($^{(*)}$ p = 0.063).

when given in the afternoon. Again, the current findings in men confirm a part of this suggestion in revealing enhancing effects of pre-encoding stress for negative words in the afternoon. Thirdly, emotionality matters: high stress hormone concentrations promote encoding of emotionally arousing material at the cost of nonarousing material (Buchanan and Lovallo, 2001; Kuhlmann and Wolf, 2006; Payne et al., 2007; Rimmele et al., 2003; Smeets et al., 2006; Wolf, 2012). Once more, the present results in the male sample correspond to this suggestion, at least partly, since pre-encoding stress enhanced cued recall of negative material, but did not have an impact on positive material as also reported before (Schwabe et al., 2008a). Most likely, positive words were less arousing relative to negative words making them less susceptible to stress effects (cf. Schwabe et al., 2008a).

Altogether, the current results confirm all of these prior suggestions, but only for the results obtained in men for negative words. As a trend, cued recall of neutral words was enhanced in OC women when exposed to stress prior to encoding, which once more fits with the time of day hypothesis. In addition, both negative and positive words were better remembered compared to neutral words during immediate free recall without stress modulating this effect as observed before (e.g., Zoladz et al., 2017). Similarly, stress did not influence memory performance of free-cycling women, no matter if tested in the FO or LU phase. These results in women question the generalizability of the mentioned three suggestions and highlight the pivotal role of the sex hormone status when considering stress effects on memory (cf. Merz and Wolf, 2017).

Opposing stress hormone effects in men and women have also been reported in a variety of emotional and cognitive processes, for example in studies using fear conditioning (e.g. Merz et al., 2012, 2013; Stark et al., 2006), reward anticipation (Kinner et al., 2016b), or a predictive learning task (Kinner et al., 2016a). GCs and norepinephrine (De Kloet et al., 2005; Roozendaal et al., 2009) as well as sex hormones (McEwen and Milner, 2017) occupying receptors in the amygdala and hippocampal areas seem to be jointly involved in these effects (cf. Merz and Wolf, 2017). Thus, the present findings add to an existing literature highlighting the importance of stress and sex hormones acting together in the modulation of emotional learning and memory processes and more generally to a variety of other emotional and cognitive processes such as executive functions or motivation (for recent reviews, see Laman-Maharg and Trainor, 2017; McEwen et al., 2015; Shields et al., 2016; Shors, 2004).

The physiological stress response was also subjected to a modulation by sex hormone status. Men showed higher systolic blood pressure compared to all women groups, which is well in line with previous work (e.g. Carroll et al., 2000; Childs et al., 2010; Lepore et al., 1993; Steptoe et al., 1996). In general, higher blood pressure was also found in OC women compared to free-cycling women previously (Boldo and White, 2011; for a review including potential mechanisms see Issa et al., 2015). Sex hormones also modulated the salivary cortisol response to the SECPT in a comparable manner as a pure psychosocial stressor such as the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), in which particularly blunted cortisol increases were found in OC women (Kirschbaum et al., 1999; see also Childs et al., 2010; Cornelisse et al., 2011; Espin et al., 2013; Rohleder et al., 2003). In addition to these laboratory stressors, blunted cortisol concentrations in women under hormonal contraception have also been observed during oral presentations at the university (Merz and Wolf, 2015a). These findings can be explained by corticosteroid binding globulin (CBG) capturing the major part of circulating cortisol in blood, leaving less cortisol to enter saliva in women taking contraceptives, who exhibit higher CBG levels compared to men and free-cycling women (Kirschbaum et al., 1999; Kudielka et al., 2009; Kumsta et al., 2007). Taken together with the present results, hormonal contraception could be proposed to lead to blunted salivary cortisol increases in situations including psychosocial, physical, and real-life stress speaking in favor of the generalizability of the observed effects. Lastly, free recall performance was generally poorer in men compared to free-cycling women replicating previous work (for a detailed review see Andreano and Cahill, 2009).

In summary and as outlook, the present results together with others can inspire translational attempts deploying stress hormone effects on memory processes involved in mental disorders such as PTSD or anxiety disorders (cf. De Quervain et al., 2017; Wolf et al., 2016). For example, therapeutic strategies might benefit from individual adjustment depending on sex and sex hormone status. There is a clear need for future studies tackling this issue in depth, since research in females is largely underrepresented despite the existence of clear contributions of sex hormones for a variety of mental disorders (Cover et al., 2014; Lebron-Milad and Milad, 2012).

5. Conclusions

Pre-encoding stress enhanced memory performance regarding negative material in men, but rather tended to increase cued recall of neutral material in OC women, while free-cycling women were unaffected. In addition, the cortisol stress response towards the psychosocial-physical stressor was blunted in OC women. These findings emphasize the importance of considering sex hormone availability in stress and memory studies possibly explaining previous divergent findings. A translation of the mentioned aspects to applied questions (e.g. investigating stress hormone effects in clinical populations) seems needed in order to improve existing treatment options for mental disorders using individualized approaches.

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Conflict of interest

The author declares no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2017.05.002.

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