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Memory impairment induced by different types of prolonged stress is dependent on the phase of the estrous cycle in female rats

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ABSTRACT

A growing body of evidence demonstrates that estrogen and corticosterone (CORT) impact on cognition and emotion. On the one hand, ovarian hormones may have beneficial effects on several neurophysiological processes, including memory. On the other hand, chronic exposure to stressful conditions has negative effects on brain structures related to learning and memory. In the present study, we used the plus-maze discriminative avoidance task (PMDAT) to evaluate the influence of endogenous variations of sex hormones and exposure to different types of prolonged stressors on learning, memory, anxiety-like behavior and locomotion. Female *Wistar* rats were submitted to seven consecutive days of restraint stress (4 h/day), overcrowding (18 h/day) or social isolation (18 h/day) and tested in different phases of the estrous cycle. The main results showed that: (1) neither stress conditions nor estrous cycle modified PMDAT acquisition; (2) restraint stress and social isolation induced memory impairments; (3) this impairment was observed particularly in females in metestrus/diestrus; (4) stressed females in estrus displayed less risk assessment behavior, suggesting reduced anxiety-like behavior; (5) restraint stress and social isolation, but not overcrowding, elevated corticosterone levels. Taken together, our findings suggest that the phase of the estrous cycle is an important modulatory factor of the cognitive processing disrupted by stress in female rats. Negative effects were observed in metestrus/diestrus, indicating that the peak of sex hormones may protect females against stress-induced memory impairment.

1. Introduction

In the last decades, numerous studies have shown that exposure to different types of stressors leads to changes in cognitive processes such as learning, memory and emotion (Roozendaal et al., 2009; Ulrich-Lai and Herman, 2009; Herman et al., 2003), with the hippocampal formation playing an integrative role on these processes (Izquierdo and Medina, 1997; O'Reilly and McClelland, 1994). High expression of glucocorticoid receptors (GR) in this structure suggests that adrenal hormones act on the hippocampal formation, hence this area is susceptible to stress effects (Luine, 2014). Indeed, some studies report that chronic stress alters hippocampal morphology and functionality by producing atrophy of dendritic branching, thus decreasing synaptic plasticity (Kim and Diamond, 2002), which is accompanied by deficits in different behavioral tasks (Sauro et al., 2003). Conversely, the effects of stressors on memory performance may vary, so that enhancing or impairing effects may be observed depending on the nature of the stressor (i.e. type, intensity, duration), the type of memory being tested, the animal's age, gender and status of gonadal hormones (Toufexis et al., 2014; Rincón-Cortés et al., 2019).

In addition to the well-known role of gonadal hormones on reproductive function, in females, these hormones also influence neuronal activity and cognitive functions (Luine, 2014; Tabatadze et al., 2015). The modulatory role of estrogen have been observed in brain areas such as the prefrontal cortex, hippocampus and amygdala (Zeidan et al., 2011; Mukai et al., 2010), such that either estradiol replacement in ovariectomized rats or endogenous variation during the estrous cycle in intact rats modulates growth of dendritic spines, synaptogenesis and neurogenesis in the hippocampus (Scharfman et al., 2003; Li et al., 2011; Arevalo et al., 2015; Tabatadze et al., 2015). Furthermore, there

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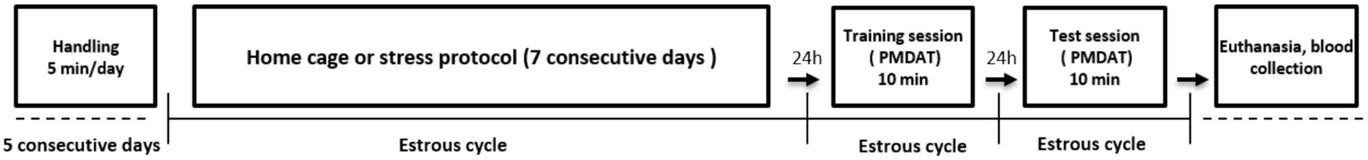

Fig. 1. Schematic representation of the experimental design.

is a crosstalk between the hypothalamic-pituitary-adrenal axis (HPA) and the hypothalamic-pituitary-gonadal (HPG) axis, insofar as activation of the former can be modulated by the latter (Toufexis et al., 2014). Oscillations in the levels of sex hormones across the estrous cycle may also buffer deficits triggered by chronic stress exposure in female rats (Pisu et al., 2016) inasmuch as stressful conditions fail to alter learning and memory processes during proestrus and estrus phases (Conrad et al., 2004). Besides their influence on cognitive processes, glucocorticoids and estrogens also affect anxiety-like behavior (see Maeng and Milad, 2015 for review) and the animal's emotional state influences its performance in memory tasks (Silva and Frussa-Filho, 2000). Thus, we hypothesized that distinct stressors will differently influence the performance of female rats in emotional context-dependent tasks and that variation of physiological levels of gonadal hormones throughout the estrous cycle will also influence their performance. The present study aimed to investigate the effects of different types of prolonged stressors (restraint, social isolation or overcrowding) on memory and anxiety-like behaviors of female rats tested in different phases of the estrous cycle. Learning and memory performance, anxiety-like behavior and locomotor activity were simultaneously assessed in the plus-maze discriminative avoidance task (PMDAT - Silva and Frussa-Filho, 2000).

2. Material and methods

2.1. Animals

Adult female *Wistar* rats (200–250 g) were kept in groups (4–5 animals per cage) under conditions of acoustic isolation, controlled airflow and temperature (25 ± 1 °C) and a 12 h light/12 h dark cycle (lights on 06:30 a.m.). Food and water were provided ad libitum. The animals were handled according to the Brazilian law for the use of animals in scientific research (Law Number 11.794) and all procedures were approved by the local ethical committee (CEUA/UFRN Protocol #055/2011). All efforts were made to minimize the animals' pain, suffering or discomfort and to reduce the number of animals. Before the onset of any experimental protocol, all animals were gently handled for 5 min/day during 3 days.

2.2. Estrous cycle

The estrous cycle was monitored by daily collection of vaginal smears during the whole experimental protocol. Briefly, the tip of a plastic pipette filled with 100 μL of distilled water was gently introduced into the rat's vagina, the bulb of the pipette was slightly pressured and the vaginal fluid collected. This material was placed on glass slides, dyed with methylene blue and examined under light microscope. The rat's estrous cycle was identified according to cytological features: metestrus (or early diestrus) - similar proportion of nucleated cells, leucocytes and cornified cells; diestrus - predominance of leucocytes; proestrus - predominance of epithelial nucleated cells; and estrus - predominance of cornified cells. To ensure that all females were cycling regularly, the estrous cycle was determined for, at least, 10 days before the beginning of the experimental procedures. Metestrus and diestrus phases were pooled together for analysis, based on evidence of similar estradiol profile (Spornitz et al., 1999; Paccola et al., 2013).

2.3. General procedures and stress conditions

All animals were randomly assigned to one of four groups: control ($n = 27$), restraint ($n = 22$), social isolation ($n = 21$) and overcrowding ($n = 23$).

Control rats remained in their home cage (42 cm length, 34 cm width and 18 cm height; $n = 4–5$ animals per cage). Rats submitted to the different stress conditions were transferred to a separate room and exposed to one of the stressors for seven consecutive days.

In the restraint stress condition, animals were exposed to a 4 h period of restraint (2:00 to 6:00 p.m.); each animal was placed in a transparent plastic cylinder (18 cm length and 9 cm width), with a 0.3 cm hole at one extremity of the tube where the animal's head was positioned for breathing. After each restraint session, rats were returned to the home cages with their cage-mates.

In the social isolation stress condition, the animals were housed in individual cages (42 cm length, 34 cm width and 18 cm height) during 18 h (8:00 p.m. to 12:00 p.m.) for seven consecutive days. The individual cages were placed in isolated environments (different rooms) to prevent communication among animals.

Animals in the overcrowding condition were kept in groups of five animals in a small cage (31 cm in length × 20 cm wide × 22 cm high) during 18 h (from 8:00 p.m. to 14:00 a.m.) for seven consecutive days. In this group, two animals were excluded from the analysis due to technical problems in the video recording. Accommodation of unfamiliar subjects in a small space directly affects their social organization within the group and resembles the stress condition of high population density (Bartolomucci et al., 2005; Uarquin et al., 2016).

The experimental timeline is presented in Fig. 1.

2.4. Plus-maze discriminative avoidance task (PMDAT)

Twenty-four hours after the last day of stress exposure the animals were submitted to the training session in the PMDAT. The wood-made apparatus used is a modified version of the conventional elevated plus-maze. The maze contains two enclosed arms (50 cm in length × 15 cm wide × 40 cm high): one aversive enclosed arm (AV) and one non-aversive enclosed arm (NAV) opposite to two open arms (OA; 50 cm in length × 15 cm wide). The PMDAT was conducted in two sessions: training and test, each session lasting 10 min. In both sessions, the animals were individually placed in the center of apparatus facing one of the open arms. In the training session, the aversive stimuli were turned on each time the animal entered with the whole body in the aversive enclosed arm, and turned off when the animal left the arm. The aversive stimuli were an 80 dB noise and a 100 W light produced by speakers and lamp, respectively, placed over the AV arm. Memory acquisition was measured during the training session. In the test session (24 h later), the animals were placed again in the central segment and allowed to explored the apparatus without presentation of the aversive stimulation (see Fig. 1) for evaluation of memory retrieval. Learning and memory were evaluated by the percentage of time spent in the aversive arm [$\%TAV = \text{time in } AV / (\text{time in } NAV + AV) \times 100$] throughout the sessions (in three blocks of 200 s each), and comparing the total time spent in the AV in relation to the NAV (during 300 s) in the training and test sessions, respectively.

All behavioral experiments were performed between 1:00 p.m. and 5:00 p.m. The sessions were recorded by a digital camera placed above the apparatus and the behavioral parameters were registered by a video-tracking software (Anymaze, Stoelting, USA). Anxiety-like behavior was evaluated by the percentage of time spent in the open arms [%TOA = time in OA / (time in NAV + AV + OA) × 100], so that the lesser time spent in the open arms reflected higher levels of anxiety-like behavior, and by the time spent in risk assessment behaviors (stretched-attend posture – SAP and head dipping), so that the higher the SAP the greater the anxiety-like profile. SAP was recorded when the animal stretched forward and then retracted to the original position without doing any forward locomotion (Rodgers and Cole, 1993). Head dipping behavior was recorded when the animal oriented the head over the side of the maze and was classified in two categories: protected head dipping (PHD) – when the animals displayed this behavior being in the central area (relatively secure area) and unprotected head dipping (UPHD) – when animals displayed it in the open arms (relatively unsafe area). We only considered the training session for evaluation of anxiety-like behavior because exploration of open arms is absent in a second exposure to the plus maze apparatus (Pereira et al., 1999). Locomotion was measured by the distance travelled in the apparatus. At the end of each behavioral session, the apparatus was cleaned with a 5% alcohol solution.

2.5. Determination of the corticosterone levels

Immediately after the PMDAT test session, animals were decapitated and trunk blood samples were collected in EDTA-containing tubes. Samples were centrifuged at 3000 rpm for 10 min. Plasma was collected and stored at −20 °C until the assays. Plasma corticosterone concentrations were determined by radioimmunoassay with a commercial kit (ImmuChem Double Antibody Corticosterone RIA, MP Biomedicals, Orangeburg, NY, USA) and run in standard duplicates. The detailed procedure was conducted according to the manufacturer's instructions.

2.6. Statistical analysis

Analysis was carried out by effects of the different stressors considering the estrous cycle phases. All data were checked for normality with Kolmogorov-Smirnov test. Three-way ANOVA with repeated measures was run for the %TAV in time blocks across training and test sessions for the different stress conditions and estrous cycle phases. Pairwise comparisons between time spent in AV and NAV arms were performed using paired-samples Student's *t*-test. Two-way ANOVA (stress condition and cycle phase) was performed for %TAV in the first time block, %TOA, corticosterone levels and risk assessment parameters, as well as distance travelled in the apparatus. Analysis of covariance (ANCOVA) was performed to determine the independence of changes observed in the apparatus during test session, comparing anxiety and retrieval memory. Tukey post-hoc test for unequal group was applied (if necessary) to all parameters. Lastly, effect sizes were calculated for ANOVA and paired-samples Student's *t*-tests using eta squared (η2) and *d* Cohen's (Cohen, 1988), respectively, in site *http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-SMD28.php*. According to Cohen (1988), an effect size of 0.20 can be classified as small, 0.50 can be classified as moderate, and 0.80 can be classified as large. All results were considered significant at $p < 0.05$.

3. Results

3.1. Learning and memory

In the training session, three-way ANOVA with repeated measures for %TAV showed time effect [$F_{(2,162)} = 46.47$; $p < 0.05$, eta squared = 0.39]. All animals spent less time in the AV arm in the third than in the first block, regardless of stress and estrous phase, indicating that they learned the task (Fig. 2). Furthermore, total %TAV was not affected by stress condition [F(3,81) = 0,43; $p > 0.05$], phase [F(2,81) = 1,62; p > 0.05] and condition × phase interaction [F(6,81) = 1,68; p > 0.05], indicating that neither phase nor type of stress prevented the acquisition of the task. In addition, all groups spent less time in AV than in NAV arm, and a large effect (*d*) also observed: **Control** [MET/DIE t(13) = 8,93; $p < 0.05$, Cohen's $d = 6.31$, *effect size* = 0.98; PRO t(7) = 8,46; $p < 0.05$, Cohen's $d = 5{,}98$. *effect size* = 0.94; EST t(8) = 2.33; $p < 0.05$, Cohen's $d = 2.37$, *effect size* = 0.76]; **Restraint** [MET/DIE t(9) = 6.74; $p < 0.05$, Cohen's $d = 4.76$, *effect size* = 0.92; PRO t(7) = 10.10; $p < 0.05$, Cohen's $d = 7.14$, *effect size* = 0.96; EST t(6) = 3.33; $p < 0.05$, Cohen's $d = 3.84$, *effect size* = 0.88]; **Social Isolation** [MET/DIE t(7) = 4.34; $p < 0.05$, Cohen's $d = 5.02$, *effect size* = 0.92; PRO t(7) = 6.83; $p < 0.05$, Cohen's $d = 7.89$, *effect size* = 0.96; EST t(6) = 3.23; $p < 0.05$, Cohen's $d = 3.73$, *effect size* = 0.88]; **Overcrowding** [MET/DIE t(8) = 6.74; $p < 0.05$, Cohen's $d = 7.93$, *effect size* = 0.96; PRO t(8) = 7.26; $p < 0.05$, Cohen's $d = 8.38$, *effect size* = 0.97; EST t(7) = 5.17, $p < 0.05$, Cohen's $d = 5.97$, *effect size* = 0.94], suggesting acquisition of the task (Table 1).

For %TAV in the test session, there was a time × stress condition × cycle phase interaction [$F_{(12,160)} = 2.05$; $p < 0.05$, *eta squared* = 0.36]. Restraint and social isolation stressed-females in metestrus/diestrus explored more the aversive arm than the respective control group during the first time block (Fig. 3B and C). Moreover, control females in proestrus explored more the aversive arm in the last time block ($p < 0.05$), indicating extinction of the aversive memory (Fig. 3A). In addition, the two-way ANOVA showed an effect of phase [F(2,81) = 5,85; $p < 0.05$, *eta squared* = 0.71] and condition × phase interaction [F(6,81) = 2,71; p < 0,05, *eta squared* = 0.66], but not of stress condition [F(3,81) = 0,526; $p > 0.05$], and restraint reduced %TAV in PRO and EST ($p < 0.05$) compared with MET/DIE rats. Overcrowding reduced this parameter only in EST ($p < 0.05$), whereas social isolation had the same effect in PRO ($p < 0.05$) compared with MET/DIE rats. The pairwise comparison showed that in the test session, only control and overcrowding rats in all phases preferred to stay in the NAV than in the AV arm. For restraint and social isolation conditions preference for NAV was observed only in PRO and EST phases, with large effects (*d*); **Control** [MET/DIE t(13) = 4.01, $p < 0.05$, Cohen's $d = 2.53$, *effect size* = 0.75; PRO t(7) = 3.65, $p < 0.05$, Cohen's $d = 2.98$, *effect size* = 0.83; EST t(8) = 2.56, $p < 0.05$, Cohen's $d = 1.93$, *effect size* = 0.69]; **Restraint** [MET/DIE t(9) = 0.02, $p > 0.05$, Cohen's $d = 0.02$, *effect size* = 0.1; PRO t(7) = 5.44, $p < 0.05$, Cohen's $d = 4.44$, *effect size* = 0.91; EST t(6) = 3.05, $p < 0.05$, Cohen's $d = 2.73$, *effect size* = 0.80]; **Social isolation** [MET/DIE t(7) = 0,49, $p > 0.05$, Cohen's $d = 0.40$, *effect size* = 0.19; PRO t(7) = 2.60, $p < 0.05$, Cohen's $d = 2.12$, *effect size* = 0.72; EST t(6) = 3.94, $p < 0.05$, Cohen's $d = 3.53$, *effect size* = 0.87]; and **Overcrowding** [MET/DIE t(8) = 2.22, $p < 0.05$, Cohen's $d = 1.68$, *effect size* = 0.64; PRO t(8) = 2.78, $p < 0.05$, Cohen's $d = 2.78$, *effect size* = 0.72; EST t(7) = 4.44, $p < 0.05$, Cohen's $d = 3.356$, *effect size* = 0.87]. Further, ANCOVA showed that %TAV during the test session was not influenced by %TOA, suggesting that the differences found in memory retrieval were not related to the level of anxiety-like behavior [Control: $F_{(1,27)} = 0.006$; $p > 0.05$; Restraint: $F_{(1,22)} = 3.52$, $p > 0.05$; Social isolation: $F_{(1,23)} = 0.64$, $p > 0.05$; and Overcrowding = $F_{(1,23)} = 2.66$, $p > 0.05$].

3.2. Anxiety-like behavior and locomotor activity

During the training session, %TOA was not influenced by cycle or stress condition, but the time in SAP was influenced by an interaction between the factors [$F_{(6,81)} = 4.526$; $p < 0.05$, *eta squared* = 0.33]. Restraint and social isolation stressed-females in EST displayed less SAP than their in PRO counterparts and than control-females in EST (Table 1). Furthermore, a stress condition × estrous cycle phase interaction was revealed [$F_{(6,81)} = 3.66$; p < 0.05, *eta squared* = 0.29] for

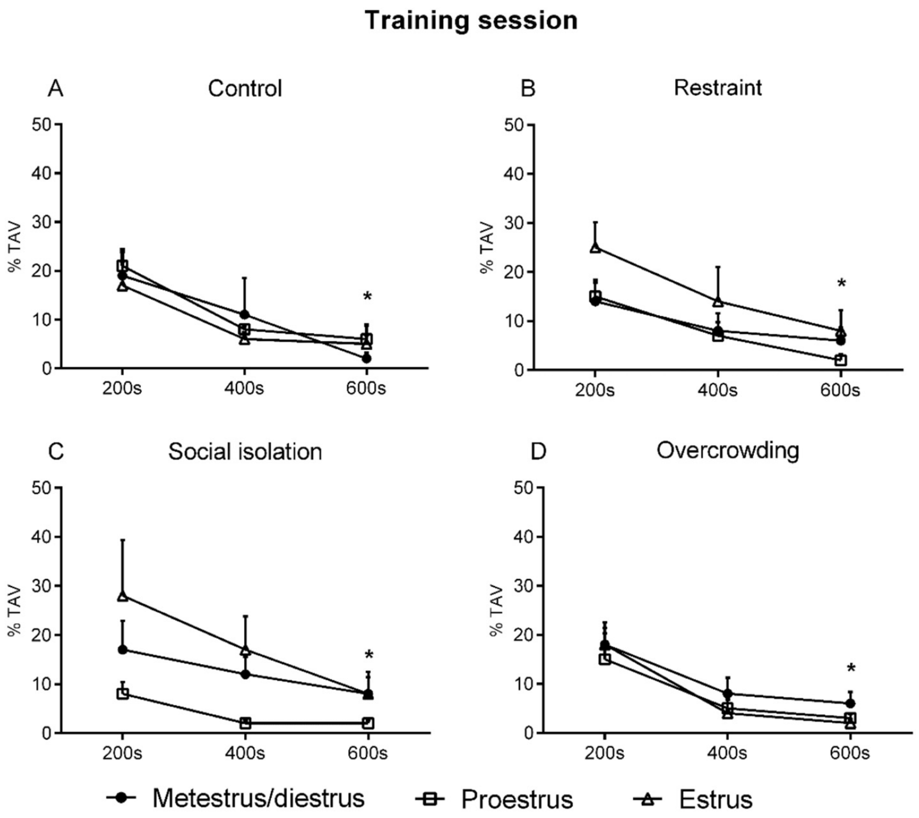

Fig. 2. Effects of stress conditions (A–D: control, restraint, social isolation and overcrowding) on learning of female rats submitted to the plus-maze discriminative avoidance task (PMDAT) in different estrous cycle phases (control: metestrus/diestrus, $n = 13$; proestrus $n = 6$; and estrus, $n = 8$; restraint: metestrus/diestrus, n = 8; proestrus, $n = 9$; and estrus, $n = 5$; Social isolation: metestrus/diestrus, $n = 8$; proestrus, $n = 7$; and estrus, $n = 6$; Overcrowding: metestrus/diestrus, $n = 8$; proestrus, $n = 8$; and estrus, $n = 7$). Mean ± SE for the percentage of time spent in aversive enclosed arm (%TAV) in 200 s time blocks during the training session. *$p < 0.05$ compared to the first block (three-way ANOVA with repeated measures followed by Tukey's post hoc test).

Table 1
Effects of the estrous cycle [metestrus/diestrus (M/D), proestrus (P) and estrus (E)] and stress conditions on the aversive memory, anxiety-like behavior and locomotion in rats submitted to plus-maze discriminative avoidance task (PMDAT). Mean ± SD for the time spend in the aversive enclosed arm (AV) and non-aversive enclosed arm (NAV), percentage of time spent in open arms (% TOA), stretched-attend posture (SAP), protected head dipping (PHD), unprotected head dipping (UPHD) and distance travelled (m) in the training session.

Groups	Phase	N	Learning		Memory		Anxiety-like behaviors				Locomotion
			AV (s)	NAV (s)	AV (s)	NAV (s)	%TOA	SAP (s)	PHD (s)	UPHD (s)	Distance (m)
Control	M/D	13	21.3 ± 14.9[c]	428.7 ± 158.3	85.7 ± 107.2[c]	195.2 ± 173.3	4.4 ± 2.9	5.9 ± 1.8	8.3 ± 3.6	8.1 ± 2.4	5.1 ± 0.9
	P	6	25.7 ± 6.9[c]	266.2 ± 65.1	82.7 ± 75.6[c]	167.8 ± 60.9	8.9 ± 1.8	8.7 ± 3.3	9.7 ± 3.2	12.2 ± 2.5	2.0 ± 0.1
	E	8	22.7 ± 13.8[c]	371.8 ± 100.2	93.6 ± 77.1[c]	191.7 ± 129.5	6.6 ± 4.6	11.2 ± 2.1	8.9 ± 2.6	6.5 ± 1.4	5.7 ± 0.8
Restraint	M/D	8	30.3 ± 29.3[c]	361.8 ± 129.1	110.2 ± 184.6	167.0 ± 125.8	4.4 ± 9.3	6.8 ± 4.3	9.3 ± 3.2	10.5 ± 7.0	3.9 ± 0.5
	P	9	28.3 ± 31.2[c]	394.5 ± 75.9	52.5 ± 68.3[c]	224.8 ± 87.0	6.6 ± 6.7	11.4 ± 2.8	8.6 ± 3.5	7.8 ± 3.2	10.9 ± 0.1
	E	5	34.8 ± 25.2[c]	222.2 ± 122.3	41.4 ± 57.2[c]	152.0 ± 270.2	14.5 ± 10.3	1.1 ± 0.3[ab]	8.4 ± 3.9	14.0 ± 1.6[a]	3.1 ± 0.3
Social Isolation	M/D	8	33.0 ± 21.4[c]	334.6 ± 123.4	154.6 ± 115.2	110.6 ± 95.1	12.4 ± 11.3	9.5 ± 3.4	11.7 ± 0.7	11.5 ± 2.9	9.1 ± 1.9
	P	7	11.3 ± 31.1[c]	411.9 ± 152.4	73.4 ± 96.3[c]	208.2 ± 148.9	2.5 ± 1.2	12.0 ± 0.8	10.5 ± 4.6	9.0 ± 4.0	5.9 ± 1.4
	E	6	30.3 ± 31.1[c]	360.2 ± 223.6	86.6 ± 49.2[c]	188.4 ± 109.5	15.5 ± 16.1	1.5 ± 0.9[a,b]	6.9 ± 3.2	14.3 ± 3.3[a]	6.7 ± 1.5
Overcrowding	M/D	8	37.9 ± 28.1[c]	378.6 ± 130.9	72.6 ± 43.3[c]	205.7 ± 82.1	9.8 ± 8.6	6.7 ± 1.1	4.4 ± 1.6	8.7 ± 0.9	8.2 ± 1.4
	P	8	17,8 ± 12.6[c]	407.1 ± 146	79.1 ± 85.7[c]	193.2 ± 112.7	5.4 ± 5.1	8.9 ± 1.7	9.9 ± 3.6	8.6 ± 4.7	5.4 ± 1.1
	E	7	16.2 ± 5.3[c]	343.0 ± 170.1	19.4 ± 49.9[c]	223.9 ± 118.5	9.8 ± 1.2	4.1 ± 0.8	6.0 ± 1.8	10.9 ± 3.9	7.4 ± 1.5

[a] $p < 0.05$ compared to estrus control.
[b] $p < 0.05$ compared to stress-matched in proestrus phase (two-way ANOVA followed by Tukey's post hoc test).
[c] $p < 0.05$ AV vs NAV (paired samples *t*-student test).

UPHD and restraint and social isolation stressed-females in EST spent more time in UPHD than the control group (Table 1). No significant effects for PHD behavior or distance travelled were found (Table 1).

3.3. Corticosterone levels

There was only an effect of stress condition [$F_{(2,81)} = 12.40$, $p < 0.05$, *eta squared* = 0.39], with increased corticosterone levels found in restraint and social isolation groups compared to control females, regardless of the phase of the cycle (Fig. 4).

4. Discussion

The main results showed that exposure to chronic restraint and social isolation, but not overcrowding, impaired retrieval of the aversive memory, despite the lack of effect on task acquisition. In addition, the phase of the estrous cycle had a modulatory effect on stress-induced memory impairment, inasmuch as only stressed female rats in metestrus/diestrus were susceptible to the memory deficits induced by prolonged stress, even though corticosterone levels were elevated in all restraint and isolated females. Regarding anxiety-like behavior, the stressors induced an anxiolytic profile specifically in EST females, whereas none of the groups displayed locomotor changes. Interestingly,

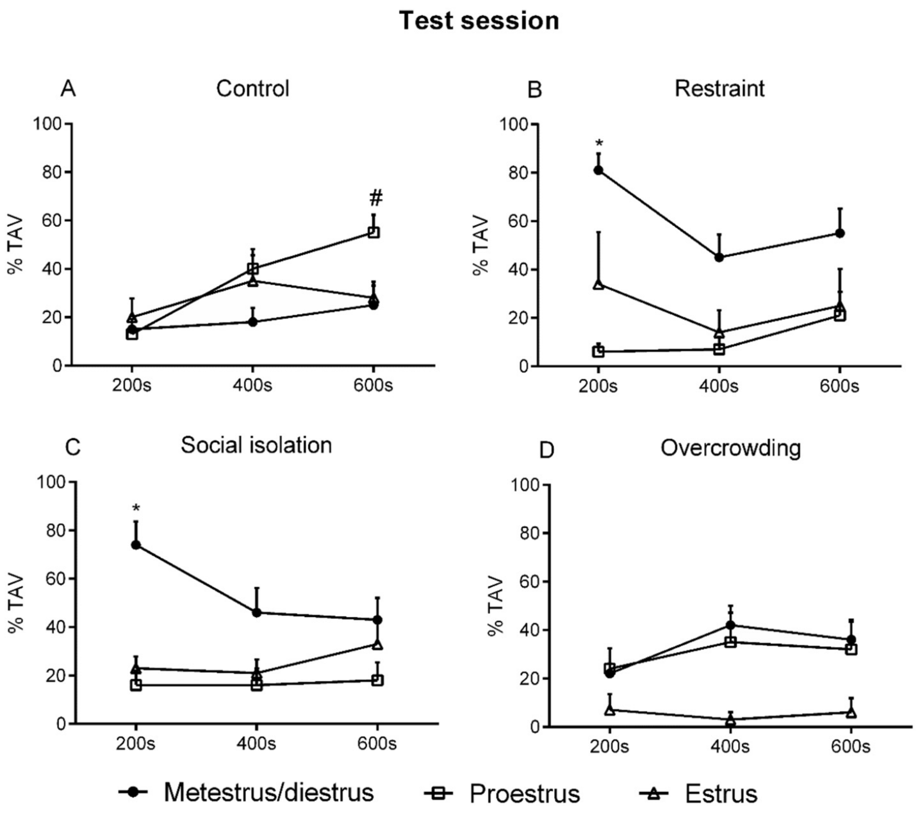

Fig. 3. Effects of stress conditions (A–D: control, restraint, social isolation and overcrowding) on memory of female rats submitted to the plus-maze discriminative avoidance task (PMDAT) in different estrous cycle phases (Control: metestrus/diestrus, $n = 13$; proestrus $n = 6$; and estrus, $n = 8$; Restraint: metestrus/diestrus, $n = 8$; proestrus, $n = 9$; and estrus, $n = 5$; Social isolation: metestrus/diestrus, $n = 8$; proestrus, $n = 7$; and estrus, $n = 6$; Overcrowding: metestrus/diestrus, $n = 8$; proestrus, $n = 8$; and estrus, $n = 7$). Mean ± SE for the percentage of time spent in aversive enclosed arm (%TAV) in time 200 s time blocks and in the first block of the test session (E). $^{\#}p < 0.05$ compared to the first block. $^{*}p < 0.05$ compared to proestrus (three-way ANOVA with repeated measures followed by Tukey's post hoc test).

there was a dissociation between the stress effects on memory performance and emotional response, suggesting that prolonged stress-induced changes on memory processing in the PMDAT may be independent of alterations in emotional states.

The reduction in %TAV throughout the training session, suggests that the stressors did not disrupt learning. Previous studies using repeated restraint stress (6 h/21 days) show that acquisition of spatial memory in the radial arm maze is spared in female rats (Bowman et al., 2001). Similarly, social isolation does not alter acquisition of spatial memory tasks in male (Wongwitdecha and Marsden, 1996) and female rats (Sandstrom, 2005). The inverted-U shape hypothesis of stress-induced learning deficits states that either low or high arousal levels result in poor behavioral performance. Thus, optimal stress and arousal levels are necessary for adequate attention, working memory and other cognitive mechanisms required for learning (Akirav et al., 2004; Salehi et al., 2010). In response to the PMDAT stressed females secreted almost twice as much corticosterone as control ones, which is expected in response to the elevated plus maze (Barbosa et al., 2012).

It is worth mentioning that the use of overcrowding stress to study learning is scarce. However, progesterone-treated male rats submitted to overcrowding display a deficit in the acquisition of the Morris water maze (MWM, Diaz-Burke et al., 2010). Young male rats (24–26 days old) reared in overcrowding conditions were also unable to learn either appetitive or aversive tasks (Goeckner et al., 1973), whereas adults showed facilitated acquisition of complex tasks (Wood and Greenough, 1974). To the best of our knowledge, this is the first report in the literature about unaltered learning process after overcrowding stress in female rats. Taken together, these findings suggest that the influence of different types of stressors on learning process depends on the type of the task, the period when the stressor is applied and the sex of the animal.

In the PMDAT training session, the animals showed the same pattern of avoidance of the aversive arm and exhibited similar acquisition slopes, regardless of the phase of the cycle and stress condition. In line with our results, Sava and Markus (2005) reported that the estrous cycle did not affect learning in the MWM task. In contrast, restraint-stressed females submitted to Y-maze task displayed a phase-dependent acquisition performance (Conrad et al., 2004). However, unstressed females in EST (Frye and Sturgis, 1995) or PRO (Rubinow et al., 2004) take longer to reach the platform in the MWM, suggesting that this outcome is due to the stressful experience of swimming. It is worth mentioning that the results of control females in the PMDAT training session replicated a previous report (Ribeiro et al., 2010). Most studies showing the beneficial effects of estrogen on acquisition are conducted

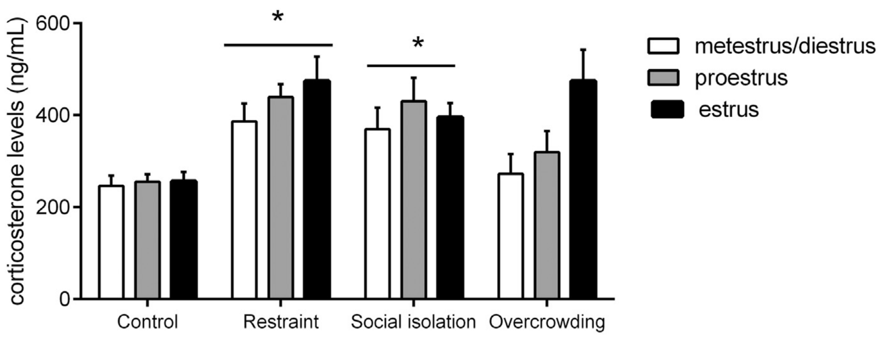

Fig. 4. Effects of stress conditions (control, restraint, social isolation, and overcrowding) on the corticosterone levels of female rats in different estrous cycle phases (control: metestrus/diestrus, $n = 13$; proestrus $n = 6$; and estrus, $n = 8$; restraint: metestrus/diestrus, $n = 8$; proestrus, $n = 9$; and estrus, $n = 5$; social isolation: metestrus/diestrus, $n = 8$; proestrus, $n = 7$; and estrus, $n = 6$; overcrowding: metestrus/diestrus, $n = 8$; proestrus, $n = 8$; and estrus, $n = 7$). Mean ± SE for the corticosterone levels [ng/ml]. $^{*}p < 0.05$ compared with control group analyzed by two-way ANOVA followed by Tukey's post hoc test.

in ovariectomized-estrogen replaced animals. In this respect, the use of supraphysiological levels may not be representative of the endogenous hormonal milieu in rodents (Beach et al., 1983). The present study contributes to the literature by showing that different prolonged stressors do not impair task acquisition during the normal fluctuation of the hormonal levels throughout the estrous cycle.

The PMDAT is based on aversive association and is dependent on amygdala function (Ribeiro et al., 2010) and hippocampal integrity (Leão et al., 2016). The hippocampal formation is an important target of glucocorticoid action and interaction between the stress response and cognition has been demonstrated (Conrad, 2008). From this standpoint, the literature on chronic stress and memory has extensively considered hippocampal glucocorticoid receptors (GRs) to be a core feature of stress-induced memory impairment (Frick et al., 2015). Activation of these receptors reduces glutamate transmission and release (Popoli et al., 2012), long-term potentiation (LTP) at CA3 and CA1 fields (Pavlides et al., 1993; Conrad et al., 2007; Chen et al., 2008; Kumar, 2011; Popoli et al., 2012) and dendritic spine branching (McEwen et al., 2016). Furthermore, it is well established that exposure to chronic stress decreases expression of neurotrophins in the hippocampus (Duman et al., 1999; Pittenger and Duman, 2008; Calabrese et al., 2009). Therefore, restraint- and social isolation-induced corticosterone levels may explain the observed deficits and may be related to functional abnormalities in the hippocampus. Many species, including nonhuman primates, cope with stress using social support, an essential mechanism for maintaining physical and psychological health (Meyer and Hamel, 2014). Female rodents are more sensitive to social stressors than males (for review, see Beery and Kaufer, 2015) and, therefore, the absence of social support during restraint and social isolation could be powerful stressors for females, whereas overcrowding does not seem to be detrimental.

Stress-induced memory deficits in females have been reported (for review, see ter Horst et al., 2012). This was also observed in the present study, but only when stressed females were in metestrus/diestrus. Because high levels of endogenous sex hormones are seen in both PRO and EST, but not in metestrus/diestrus, we propose that the hormonal milieu during these phases prevented the detrimental impact of chronic stressful conditions on memory. Accordingly, studies demonstrate the protective role of estradiol in stressed rats during the transition between PRO and EST (Mohammadkhani et al., 2015) or in estrogen-replaced ovariectomized rats (Mclaughlin et al., 2010; Takuma et al., 2007). A possible explanation is that estradiol would act as a natural buffer of the negative effects of chronic stress, that could be related to the long lasting genomic effect of estradiol on LTP-mediated neuroplasticity, dendritic sprouting and hippocampal neuronal growth (Woolley et al., 1990; Scharfman et al., 2003; Finocchi and Ferrari, 2011; Smith and McMahon, 2006; Gould et al., 1990; Smejkalova and Woolley, 2010; Frick et al., 2015; Mclaughlin et al., 2010) and enhancement of neurotrophic factors, such as BDNF (see Luine and Frankfurt, 2013).

The present results also indicated that memory extinction was related to the estrous cycle, insofar as higher %TAV in the last time block, compared to the first, was observed only control females in proestrus, in agreement with Milad et al. (2009). Interestingly, in our study this effect was absent in all other estrous phases and stress conditions, suggesting that stress interfered with extinction of the aversive memory in a phase-independent manner.

The animal's emotional state influences fear and motivation to explore the plus-maze in the PMDAT task (Silva and Frussa-Filho, 2000). Anxiety levels were not influenced by the phase of the estrous cycle in control rats, but restrained and socially isolated females in EST displayed less SAP and more UPHD, suggesting lower anxiety-like profile. No changes in anxiety-like behavior were observed in mete/diestrus phase, indicating phase-specific influence of the estrous cycle on emotional processing in stressed female rats. These effects could be related to progesterone levels, insofar as secretion of this hormone reaches the highest levels in the first hours of the estrus phase (Lovick, 2012). Previous studies report a potent neuromodulatory action of activated progesterone on GABAergic neurotransmission (Auger and Forbes-Lorman, 2008; Maguire and Mody, 2007) including the non-genomic effects mediated by its metabolite allopregnanolone (Bitran et al., 1995; Darbra and Pallares, 2012). Allopregnanolone is a positive allosteric modulator of $GABA_A$ receptors, with an inverted-U shaped anxiolytic effects in different animal models (Schule et al., 2014). Notwithstanding, withdrawal from progesterone (and hence allopregnanolone), which occurs during the late diestrus phase, triggers alterations in $GABA_A$ receptors subunit expression (Griffiths and Lovick, 2005; Lovick et al., 2005) producing an anxiogenic effect (Lovick, 2012). Interestingly, the effects of stress on anxiety-like behavior were dissociated from those on memory, because these effects occurred in rats that were in different phases, thus reinforcing the specific stress effects on memory processes independent of the animals' emotional state.

In conclusion, the results suggested a modulatory role of the estrous cycle on stress-induced memory impairment in PMDAT. Specifically, restraint and social isolation stressed female rats displayed memory impairment when tested in metestrus/diestrus. Although corticosterone levels endorsed stress-induced behavioral alterations, it was not altered by the estrous cycle. Moreover, the alterations produced by stress were memory-specific and dissociated from any interference of the emotional status or locomotion. Our data support the hypothesis that elevation of sex hormones in proestrus and estrus could counteract the cognitive deficits produced by chronic stress. Further investigations should clarify the mechanisms by which sex hormones prevented memory deficits.

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References

Akirav, I., Kozenicky, M., Tal, D., Sandi, C., Venero, C., 2004. A facilitative role for corticosterone in the acquisition of a spatial task under moderate stress. Learn. Mem. 11, 188–195. https://doi.org/10.1101/lm.61704.

Arevalo, M.A., Azcoitia, I., Garcia-Segura, L.M., 2015. The neuroprotective actions of oestradiol and estrogen receptors. Nat. Rev. Neurosci. 16, 17–29. https://doi.org/10.1038/nrn3856.

Auger, C.J., Forbes-Lorman, R.M., 2008. Progestin receptor-mediated reduction of anxiety-like behavior in male rats. PLoS One 3 (11), e 3606. https://doi.org/10.1371/journal.pone.0003606.

Barbosa, J.B., Tiba, P.A., Faturi, C.B., Castro-Neto, E.F., Naffah-Mazacoratti, M.G., MAri, J.J., Mello, M.F., Suchecki, D., 2012. Stress during development alters anxiety-like behavior and hippocampal neurotransmission in male and female rats. Neuropharmacology 62, 518–526. https://doi.org/10.1016/j.neuropharm.2011.09.011.

Bartolomucci, A., Palanza, P., Sacerdote, P., Panerai, A.E., Sgoifo, A., Dantzer, R., Parmigiani, S., 2005. Social factors and individual vulnerability to chronic stress exposure. Neurosci. Biobehav. Rev. 29, 67–81. https://doi.org/10.1016/j.neubiorev.2004.06.009.

Beach, J.E., Tyrey, L., Schomberg, D.W., Everett, J.W., 1983. Nocturnal and diurnal levels of prolactin, LH, FSH, estrogens, and progesterone in middle-aged, spontaneously persistent estrous rats. Age 6, 82–85. https://doi.org/10.1007/BF02432508.

Beery, A.K., Kaufer, D., 2015. Stress, social behavior, and resilience: insights from rodents. Neurobiol. Stress 1, 116–127. https://doi.org/10.1016/j.ynstr.2014.10.004.

Bitran, D., Shiekh, M., McLeod, M., 1995. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABAA receptors. J. Neuroendocrinol. 7, 171–177. https://doi.org/10.1111/j.1365-2826.1995.tb00744.

Bowman, R.E., Zrull, M.C., Luine, V.N., 2001. Chronic restraint stress enhances radial arm maze performance in female rats. Brain Res. 904, 279–289. https://doi.org/10.1016/S0006-8993(01)02474-X.

Calabrese, F., Molteni, R., Racagni, G., Riva, M.A., 2009. Neuronal plasticity: a link between stress and mood disorders. Psychoneuroendocrinology 34, 208–216. https://doi.org/10.1016/j.psyneuen.2009.05.014.

Chen, Y., Dubé, C.M., Rice, C.J., Baram, T.Z., 2008. Rapid loss of dendritic spines after stress involves derangement of spine dynamics by corticotropin-releasing hormone. J. Neurosci. 28, 2903–2911. https://doi.org/10.1523/JNEUROSCI.0225-08.2008.

Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences, 2nd ed. Lawrence Erlbaum Associates, Inc, Hillsdale, NJ, pp. 19–27.

Conrad, C.D., 2008. Chronic stress-induced hippocampal vulnerability: the glucocorticoid vulnerability hypothesis. Rev. Neurosci. 19, 395–411. https://doi.org/10.1515/REVNEURO.2008.19.6.395.

Conrad, C.D., Jackson, J.L., Wieczorek, L., Baran, S.E., Harman, J.S., Wright, R.L., Korol, D.L., 2004. Acute stress impairs spatial memory in male but not female rats: influence of estrous cycle. Pharm. Biochem. Behav. 78, 569–579. https://doi.org/10.1016/j.pbb.2004.04.025.

Conrad, C.D., Mclaughlin, K.J., Harman, J.S., Foltz, C., Wieczorek, L., Lightner, E., Wright, R.L., 2007. Chronic glucocorticoids increase hippocampal vulnerability to neurotoxicity under conditions that produce CA3 dendritic retraction but fail to impair spatial recognition memory. J. Neurosci. 27, 8278–8285. https://doi.org/10.1523/JNEUROSCI.2121-07.2007.

Darbra, S., Pallares, M., 2012. Effects of early postnatal allopregnanolone administration on elevated plus maze anxiety scores in adult male Wistar rats. Neuropsychobiology 65, 20–27. https://doi.org/10.1159/000328161.

Diaz-Burke, Y., Gonzalez-Sandoval, C.E., Valencia-Alfonso, C.E., Huerta, M., Trujillo, X., Diaz, L., García-Estrada, J., Luquín, S., 2010. Progesterone regulates corticosterone elevation and alterations in spatial memory and exploratory behavior induced by stress in Wistar rats. Univ. Psychol. 9, 627–640. http://doi.org/10.11144/Javeriana.upsy9-3.prce.

Duman, R.S., Malberg, J., Thome, J., 1999. Neural plasticity to stress and antidepressant treatment. Biol. Psychiatry 46, 1181–1191. https://doi.org/10.1016/S0006-3223(99)00177-8.

Finocchi, C., Ferrari, M., 2011. Female reproductive steroids and neuronal excitability. Neurol. Sci. 32, 31–35. https://doi.org/10.1007/s10072-011-0532-5.

Frick, K.M., Kim, J., Tuscher, J.J., Fortress, A.M., 2015. Sex steroid hormones matter for learning and memory: estrogenic regulation of hippocampal function in male and female rodents. Learn. Mem. 22, 472–493. https://doi.org/10.1101/lm.037267.114.

Frye, C.A., Sturgis, J.D., 1995. Neurosteroids affect spatial/reference, working, and long-term memory of female rats. Neurobiol. Learn. Mem. 64, 83–96. https://doi.org/10.1006/nlme.1995.1046.

Goeckner, D.J., Greenough, W.T., Mead, W.R., 1973. Deficits in learning tasks following chronic overcrowding in rats. J. Pers. Soc. Psychol. (2), 256–261. https://doi.org/10.1037/h0035783.

Gould, E., Woolley, C.S., Frankfurt, M., McEwen, B.S., 1990. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. J. Neurosci. 10, 1286–1291. https://doi.org/10.1523/JNEUROSCI.10-04-01286.1990.

Griffiths, J.L., Lovick, T.A., 2005. Withdrawal from progesterone increases expression of alpha 4, beta1, and delta GABA(A) receptor subunits in neurons in the periaqueductal gray matter in female Wistar rats. J. Comp. Neurol. 486, 89–97. https://doi.org/10.1002/cne.20540.

Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y.M., Ostrander, M.M., Choi, D.C., Cullinan, W.E., 2003. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front. Neuroendocrinol. 24, 151–180. https://doi.org/10.1016/j.yfrne.2003.07.001.

ter Horst, J.P., de Kloet, E., Schächinger, H., Oitzl, M., 2012. Relevance of stress and female sex hormones for emotion and cognition. Cell. Mol. Neurobiol. 32 (5), 725–735. https://doi.org/10.1007/s10571-011-9774-2.

Izquierdo, I., Medina, J.H., 1997. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol. Learn. Mem. 68, 285–316. https://doi.org/10.1006/nlme.1997.3799.

Kim, J.J., Diamond, D.M., 2002. The stressed hippocampus, synaptic plasticity and lost memories. Nat. Rev. Neurosci. 3, 453–462. https://doi.org/10.1038/nrn849.

Kumar, A., 2011. Long-term potentiation at CA3-CA1 hippocampal synapses with special emphasis on aging, disease, and stress. Front. Aging Neurosci. 20, 1–20. https://doi.org/10.3389/fnagi.2011.00007.

Leão, A.H., Cabral, A., Izídio, G.S., Ribeiro, A.M., Silva, R.H., 2016. Diazepam effects on aversive memory retrieval and extinction: role of anxiety levels. Pharm. Biochem. Behav. 141, 42–49. https://doi.org/10.1016/j.pbb.2015.11.012.

Li, J., Siegel, M., Yuan, M., Zeng, Z., Finnucan, L., Persky, R., Durn, P.D., McCullough, D., 2011. Estrogen enhances neurogenesis and behavioral recovery after stroke. J. Cereb. Blood Flow Metab. 31, 413–425. https://doi.org/10.1038/jcbfm.2010.181.

Lovick, T.A., 2012. Estrous cycle and stress: influence of progesterone on the female brain. Braz. J. Med. Biol. Res. 45 (4), 291–375. https://doi.org/10.1590/S0100-879X2012007500044.

Lovick, T.A., Griffiths, J.L., Dunn, S.M., Martin, I.L., 2005. Changes in GABA(A) receptor subunit expression in the midbrain during the oestrous cycle in Wistar rats. Neuroscience 131, 397–405.

Luine, V.N., 2014. Estradiol and cognitive function: past, present and future. Horm. Behav. 66, 602–618. https://doi.org/10.1016/j.yhbeh.2014.08.011.

Luine, V.N., Frankfurt, M., 2013. Interactions between estradiol, BDNF and dendritic spines in promoting memory. Neuroscience 239, 34–45. https://doi.org/10.1016/j.neuroscience.2012.10.019.

Maeng, L.Y., Milad, M., 2015. Sex differences in anxiety disorders: interactions between fear, stress, and gonadal hormones. Horm. Behav. 79, 106–117. https://doi.org/10.1016/j.yhbeh.2015.04.002.

Maguire, J., Mody, I., 2007. Neurosteroid synthesis-mediated regulation of GABAa receptors: relevance to the ovarian cycle and stress. J. Neurosci. 27 (9), 2155–2162. https://doi.org/10.1523/JNEUROSCI.4945-06.2007.

McEwen, B.S., Nasca, C., Gray, J.D., 2016. Stress effects on neuronal structure: hippocampus, amygdale, and prefrontal cortex. Neuropsychopharmacology 41, 3–23. http://doi.org/0.1038/npp.2015.171.

Mclaughlin, K., Wilson, J.O., Harman, J., Wright, J.H., Wieczorek, L.A., Gomez, J., Krol, D.L., Conrad, C.D., 2010. Chronic 17b-estradiol or cholesterol prevents stress-induced hippocampal CA3 dendritic retraction in ovariectomized female rats: possible correspondence between CA1 properties and spatial acquisition. Hippocampus 20, 768–786. https://doi.org/10.1002/hipo.20678.

Meyer, J.S., Hamel, A.F., 2014. Models of stress in nonhuman primates and their relevance for human psychopathology and endocrine dysfunction. ILAR J. 55, 347–360. https://doi.org/10.1093/ilar/ilu023.

Milad, M.R., Igoe, S.A., Lebron-Milad, K., Novales, J.E., 2009. Estrous cycle phase and gonadal hormones influence conditioned fear extinction. Neuroscience 164, 887–895. https://doi.org/10.1016/j.neuroscience.2009.09.011.

Mohammadkhani, R., Darbandi, N., Vafae, A., Ahmadalipour, A.R., 2015. Glucocorticoid-induced impairment of long-term memory retrieval in female rats: influences of estrous cycle and estrogen. Neurobiol. Learn. Mem. 118, 209–2015. https://doi.org/10.1016/j.nlm.2014.12.011.

Mukai, H., Kimoto, T., Hojo, Y., Kawato, S., Murakami, G., Higo, S., Hatanaka, Y., Ogiue-ikeda, M., 2010. Modulation of synaptic plasticity by brain estrogen in the hippocampus. Biochem. Biophys. Acta 1800, 1030–1044. https://doi.org/10.1016/j.bbagen.2009.11.002.

O'Reilly, R.C., McClelland, J.L., 1994. Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. Hippocampus (6), 661–682. https://doi.org/10.1002/hipo.450040605.

Paccola, C.C., Resende, C.G., Stumpp, T., Miraglia, S.M., Cipriano, I., 2013. The rat estrous cycle revisited: a quantitative and qualitative analysis. Anim. Reprod. 10, 677–683. https://doi.org/10.1002/0471142301.nsa04is48.

Pavlides, C., Watanabe, Y., McEwen, B.S., 1993. Effects of glucocorticoids on hippocampal long-term potentiation. Hippocampus 3, 183–192. https://doi.org/10.1002/hipo.450030210.

Pereira, J.K., Vieira, R.J., Konishi, C.T., Ribeiro, R.A., Frussa-Filho, R., 1999. The phenomenon of “one-trial tolerance” to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze is abolished by the introduction of a motivational conflict situation. Life Sci. 65, 101–107. https://doi.org/10.1016/S0024-3205(99)00339-2.

Pisu, M.G., Garau, A., Boero, G., Biggio, F., Pibiri, V., Dore, R., Locci, V., Paci, E., Porcu, P., Serra., 2016. Sex differences in the outcome of juvenile social isolation on HPA axis function in rats. Neuroscience 320, 172–182. doi:https://doi.org/10.1016/j.neuroscience.2016.02.009.

Pittenger, C., Duman, R.S., 2008. Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 33, 88–109. https://doi.org/10.1038/sj.npp.1301574.

Popoli, M., Yan, Z., McEwen, B.S., Sanacora, G., 2012. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat. Rev. Neurosci. 13, 22–37. https://doi.org/10.1038/nrn3138.

Ribeiro, A.M., Barbosa, F.F., Godinho, M.R., Fernandes, V.S., Munguba, H., Melo, T.G., Barbosa, M.T., Eufrasio, R.A., Izidio, G.S., Silva, R.H., 2010. Sex differences in aversive memory in rats: possible role of extinction and reactive emotional factors. Brain Cogn. 74, 145–151. https://doi.org/10.1016/j.bandc.2010.07.012.

Rincón-Cortés, M., Herman, J.P., Lupien, S., Maguire, J., Shansky, R.M., 2019. Stress: influence of sex, reproductive status and gender. Neurobiol. Stress 9 (10), 100155. https://doi.org/10.1016/j.ynstr.2019.100155.

Rodgers, R., Cole, J., 1993. Anxiety enhancement in the murine elevated plus maze by immediate prior exposure to social stressor. Physiol. Behav. 53, 383–388. https://doi.org/10.1016/0031-9384(93)90222-2.

Roozendaal, B., McEwen, B.S., Chattarji, S., 2009. Stress, memory and amygdala. Nat. Rev. Neurosci. 10, 423–433. https://doi.org/10.1038/nrn2651.

Rubinow, M.J., Arseneau, L.M., Beverly, J.L., Juraska, J.M., 2004. Effect of the estrous cycle on water maze acquisition depends on the temperature of the water. Behav. Neurosci. 118, 863–868. https://doi.org/10.1037/0735-7044.118.4.863.

Salehi, B., Cordero, M.B., Sandi, C., 2010. Learning under stress: the inverted-U-shape function revisited. Learn. Mem. 17, 522–530. https://doi.org/10.1101/lm.1914110.

Sandstrom, N.J., 2005. Sex differences in the long-term effect of preweanling isolation stress on memory retention. Horm. Behav. 47, 556–562. https://doi.org/10.1016/j.yhbeh.2004.12.008.

Sauro, M.D., Jorgensen, R.S., Pedlow, C.T., 2003. Stress, glucocorticoids and memory: a meta-analytic review. Stress 6, 235–245. https://doi.org/10.1080/1025389031000161648.

Sava, S., Markus, E.J., 2005. Intramaze cue utilization in the water maze: effects of sex and estrous cycle in rats. Horm. Behav. 48, 23–33. https://doi.org/10.1016/j.yhbeh.2005.01.011.

Scharfman, H.E., Mercurio, T.C., Goodman, J.H., Wilson, M.A., Maclusky, N.J., 2003. Hippocampal excitability increases during the estrous cycle in the rat: a potential role for brain-derived neurotrophic factor. J. Neurosci. 23, 11641–11652. https://doi.org/10.1523/JNEUROSCI.23-37-11641.2003.

Schule, C., Nothdurfter, C., Rupprecht, R., 2014. The role of allopregnanolone in depression and anxiety. Prog. Neurobiol. 113, 79–87. https://doi.org/10.1016/j.pneurobio.2013.09.003.

Silva, R.H., Frussa-Filho, R., 2000. The plus-maze discriminative avoidance task: a new model to study memory-anxiety interactions. Effects of chlordiazepoxide and caffeine. J. Neurosci. Methods 102, 117–125. https://doi.org/10.1016/S0165-0270(00)00289-2.

Smejkalova, T., Woolley, C.S., 2010. Estradiol acutely potentiates hippocampal excitatory synaptic transmission through a presynaptic mechanism. J. Neurosci. 30 (48), 16137–16148. https://doi.org/10.1523/JNEUROSCI.4161-10.2010.

Smith, C.C., McMahon, L.L., 2006. Estradiol-induced increase in the magnitude of long-term potentiation is prevented by blocking NR2b-containing receptors. J. Neurosci. 26, 8517–8522. https://doi.org/10.1523/JNEUROSCI.5279-05.2006.

Spornitz, U.M., Socin, C.D., Dravid, A.A., 1999. Estrous stage determination in rats by means of scanning electron microscopic images of uterine surface epithelium. Anat. Rec. 254, 116–126. https://doi.org/10.1002/(SICI)1097-0185(19990101)254:1<116::AID-AR15>3.0.CO;2-X.

Tabatadze, N., Huang, G., May, R.M., Jaint, A., Woolley, C.S., 2015. Sex differences in molecular signaling at inhibitory synapses in the hippocampus. J. Neurosci. (32), 11252–11265. https://doi.org/10.1523/JNEUROSCI.1067-15.2015.

Takuma, K., Matsuo, A., Himeno, Y., Hoshina, Y., Ohno, Y., Funatsu, Y., Kamel, H., Mizoguchi, H., Nagai, T., Koike, K., Noue, M., Yamada, K., 2007. 17b-Estradiol attenuates hippocampal neuronal loss and cognitive dysfunction induced by chronic restraint stress in ovariectomized rats. Neuroscience 146, 60–68. https://doi.org/10.1016/j.neuroscience.2007.01.017.

Toufexis, D., Rivarola, M.A., Lara, H., Viau, V., 2014. Stress and the reproductive axis. J. Neuroendocrinol. 26, 573–586. https://doi.org/10.1111/jne.12179.

Uarquin, D., Meyer, J.S., Cardenas, F.P., Rojas, M., 2016. Effect of overcrowding on hair corticosterone concentration in juvenile male Wistar rats. J. Am. Assoc. Lab. Anim. Sci. 55, 749–755. https://doi.org/10.1016/j.jchromb.2015.08.035.

Ulrich-Lai, Y.M., Herman, J.P., 2009. Neural regulation of endocrine and autonomic stress response. Nat. Rev. Neurosci. 10, 397–409. https://doi.org/10.1038/nrn2647.

Wongwitdecha, N., Marsden, C.A., 1996. Effects of social isolation rearing on learning in the Morris water maze. Brain Res. 715, 119–124. https://doi.org/10.1016/0006-8993(95)01578-7.

Wood, W.E., Greenough, W.T., 1974. Effects of grouping and crowding on learning in isolation-reared adult rats. Bull. Psychon. Soc. 3, 65–67. https://doi.org/10.3758/BF03333397.

Woolley, C.S., Gould, E., Frankfurt, M., McEwen, B.S., 1990. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. J. Neurosci. 10, 4035–4039. https://doi.org/10.1523/JNEUROSCI.10-12-04035.1990.

Zeidan, M.A., Igoe, S.A., Linnman, C., Vitalo, A., Levine, J.B., Klibanski, A., Goldstein, J.M., Milad, M.R., 2011. Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. Biol. Psychiatry 70, 920–927. https://doi.org/10.1016/j.biopsych.2011.05.016.