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Research report

Social isolation and social support at adulthood affect epigenetic mechanisms, brain-derived neurotrophic factor levels and behavior of chronically stressed rats

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ABSTRACT

Epigenetic modulation of brain-derived neurotrophic factor (BDNF) provides one possible explanation for the dysfunctions induced by stress, such as psychiatric disorders and cognitive decline. Interestingly, social support can be protective against some of these effects, but the mechanisms of social buffering are poorly understood. Conversely, early isolation exacerbates the responses to stressors, although its effects in adulthood remain unclear. This study investigated the effects of social isolation and social buffering on hippocampal epigenetic mechanisms, BDNF levels and behavioral responses of chronically stressed young adult rats. Male Wistar rats (3 months) were assigned to accompanied (paired) or isolated housing. After one-month half of each group was submitted to a chronic unpredictable stress (CUS) protocol for 18 days. Among accompanied animals, only one was exposed to stress. Behavioral analysis encompassed the Open field, plus maze and inhibitory avoidance tasks. Hippocampal H3K9 and H4K12 acetylation, HDAC5 expression and BDNF levels were evaluated. Isolated housing increased HDAC5 expression, decreased H3K9 and H4K12 acetylation, reduced BDNF levels, and impaired long-term memory. Stress affected weight gain, induced anxiety-like behavior and decreased AcK9H3 levels. Interactions between housing conditions and social stress were seen only for HDAC5 expression, which showed a further increase in the isolated + CUS group but remained constant in accompanied animals. In conclusion, social isolation at adulthood induced epigenetic alterations and exacerbated the effects of chronic stress on HDAC5. Notwithstanding, social support counteracted the adverse effects of stress on HDAC5 expression.

1. Introduction

The reactions of the organism to stressful situations are usually analyzed based on the classic concept of the fight or flight response. In this concept, the brain perceives and determines what is threatening, and activates the sympathetic nervous system and the hypothalamic-pituitary-adrenocortical (HPA) axis, leading to the recruitment of different organs and systems for a concerted effort to combat or escape from threat. Although providing a good characterization of responses to stress, the fight or flight concept is incomplete from the standpoint of human beings [1]. A remarkable response of humans to stress is the tendency to affiliate, that is, to come together in groups to provide and receive joint protection in threatening times [2,3]. Social support seems to have a protecting effect against the negative outcomes of stress exposure [4–6]. Conversely, social isolation and feelings of loneliness are important stressors by itself, being associated with alterations of the

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neuroendocrine response to stress and predisposition to different mental health dysfunctions, such as anxiety, depression and cognitive decline [7,8].

The effects of social isolation have been investigated in many animal models. Studies with mice and rats have shown that social isolation is associated with important alterations in brain neurochemistry, structure and function, inducing behavioral changes manifested as depressive and anxiety-like symptoms [9–11]. It is also known that isolated animals usually show exacerbated behavioral and neuroendocrine responses to chronic stressors [12]. However, these studies were mostly conducted to investigate the effects of maternal separation or early isolation on behavioral and endocrine responses to stress at adulthood [13,14]. Thus, the effect of adult isolation on the behavioral and physiological responses to chronic stress remain unclear.

The social contact seeking that humans show after stress exposure can also be observed in other mammals with distinct levels of social bonding, including rats [15]. Additionally, social support, or group housing, can decrease plasma glucocorticoid levels and reduce the reactions to stress in different animal models [9,16–20]. Although the behavioral effects of social support are well documented, studies concerning the mechanisms implicated in social buffering of stress responses are mostly restricted to the role of the HPA axis, oxytocin and vasopressin [9]. However, in the last decade it became increasingly evident that epigenetic mechanisms provide one possible explanation for the lasting impact that a history of stress exposure can have on future stress reactivity and maladaptation [21]. Thus, it would be interesting to investigate if social buffering could also be acting through modulation of epigenetic mechanisms. If this is the case, it could be potentially effective to prevent exacerbated reactions or dysfunctional adaptations in response to stress.

One of the most susceptible brain regions to the effects of chronic stress is the hippocampus, a component of the limbic system that regulates motivation, emotion, and processing of declarative memories [22,23]. Chronic stress impairs neurogenesis, plasticity and neuronal survival in the hippocampus [24,25]. These changes have been associated with psychiatric and cognitive dysfunctions and, more recently, have been investigated from the epigenetic point of view [26].

Among the epigenetic mechanisms activated in the hippocampus by chronic stress is the modulation of histone acetylation [27]. Histone acetylation promotes gene transcription by reducing the interaction of histones with DNA (allowing the coupling of transcriptional machinery to DNA) or serving as a recognition site for gene transcription promoters [28]. Histone acetylation is modulated by the activity of acetyltransferases (HATs), responsible for the increase in acetylation, and deacetylases (HDACs), responsible for the decrease in acetylation [29].

Different stress protocols, including acute and chronic restraint, social defeat and chronic unpredictable stress (CUS), are able to simultaneously increase the activity of HDACs and decrease histone acetylation. In this context, the activity of HDAC5 and the acetylation of H3 (K9) and H4 (K12) has drawn attention because of their role in the regulation of brain-derived neurotrophic factor (BDNF) expression [27,30–35]. BDNF is an important modulator of neurotransmission [36–38], neuroplasticity [39,40] and neuronal survival [41]. Chronic stress was already shown to decrease BDNF levels in humans [42,43] and animals [44]. Moreover, lower levels of BDNF in serum were associated to neurodegenerative diseases [45,46] and psychiatric disorders [47–49]. Thus, there are suggestions that the maladaptive effects of chronic stress on mental health are, at least partially, associated to the epigenetic modulation of BDNF levels [12,50].

The current study was designed to explore the effects of social isolation and social buffering on epigenetic and behavioral responses to chronic stress. More specifically, we investigated hippocampal H3K9 and H4K12 acetylation, HDAC5 expression and BDNF levels, as well as behavioral responses, in young adult rats maintained in different housing conditions (isolation or accompanied housing) and exposed to chronic stress (CUS protocol). Our main hypotheses were that: (1) isolation and chronic stress would lead to negative outcomes on the investigated variables; (2) isolation would magnify the effects of chronic stress; (3) paired housing would be protective against epigenetic, BDNF and behavioral alterations induced by chronic stress.

2. Material and methods

2.1. Animals

Adult male Wistar rats (three-month-old, 465–573 g, n = 46) were obtained from the university breeding facility (Centro de Modelos Biológicos Experimentais/ Pontifícia Universidade Católica do Rio Grande do Sul, CeMBE/PUCRS). Animals were maintained in standard cages with sawdust bedding, room temperature of 21 ± 1 °C, a 12-h light/dark schedule and ad libitum access to standardized pellet food and water. The experiments were carried out in conformity with the Guide for the Care and Use of Laboratory Animals and performed according to the recommendations of the Brazilian Guidelines for the Care and Use of Animals in Research and Teaching (DBCA, published by CONCEA, MCTI). Experimental protocols were approved by the Ethics Committee for the Use of Animals of the Pontifical Catholic University (CEUA, registration No. 7142). All efforts were made to reduce sample size and minimize animal suffering.

2.2. Experimental design

All animals were weighted and randomly divided in two experimental groups: Accompanied (two animals/ home cage) and Isolated (one animal/home cage). After one month, half of the animals of each group were submitted to a 20 days' stress protocol (see below), whereas the other half remained in the standard housing conditions during this period. Thus, four experimental subgroups emerged: Accompanied (two animals/home cage); Accompanied + CUS (two animals/home cage and one of them daily submitted to the CUS protocol); Isolated (one animal/ home cage); Isolated + CUS (one animal/home cage daily submitted to the CUS protocol).

2.3. Chronic unpredictable stress (CUS) protocol

The CUS protocol, also known as chronic unpredictable mild stress protocol, is widely used [51]. Composed by diverse micro-stressors, presented in a random and unpredictable fashion, the CUS protocol was

Table 1
Schedule of stressors used during the chronic unpredictable stress (CUS) treatment.

Stress	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Water deprivation	10h- >	10h					
Wet bedding		13-17h					
Light 24h			8h- >	8h			
Imobilization				16-16:45h			
Food deprivation					11h- >	11h	
Strobe light						14-16h	
Cage tilt							7-11h

designed according to the literature to induce a mild to moderate intensity stress [52,53]. The stress protocol (Table 1) lasted 14 days, was interrupted during four days for behavioral tasks, and resumed for another 4 days. Two days after the end of the CUS protocol animals were weighted and euthanized by decapitation.

2.4. Behavioral tasks

The behavioral tasks were run on four consecutive days in the following sequence: Open field, plus maze, inhibitory avoidance training and testing sessions.

2.4.1. Open field

Open field testing was performed as previously described [54]. In short, animals were placed in a 40 × 45 × 50 cm high open field cage divided into 12 equal-sized sections under red lighting for 5 min. Between each session, feces and urine were removed from the apparatus. Animals were videotaped and locomotor and exploratory responses (latency to start locomotion, section crossings and rearings) were scored offline by blind experimenters with high inter-rater reliability (Pearson's $r > 0.9$).

2.4.2. Elevated plus maze

The elevated plus maze test is a standard method to assess the anxiety-like behaviors in rodents [55]. The apparatus consisted of two open and two closed arms with the same size (50 × 10 cm) elevated 50 cm above the floor. The closed arms were surrounded by 40 cm high walls. Animals were placed in the central square of the plus maze apparatus (10 × 10 cm), facing the open arm, and allowed to explore the maze during five minutes. Between animals, feces and urine were removed from the apparatus. All animals were videotaped and the number of entries and time spent in open versus closed arms were scored offline by blind experimenters with high inter-rater reliability (Pearson's $r > 0.9$).

2.4.3. Inhibitory avoidance

The inhibitory avoidance task was performed to evaluate long term aversive memory and followed the procedures previously described [56]. The apparatus was an acrylic box (50 × 25 × 25 cm) whose floor consisted of parallel-caliber stainless-steel bars (1 mm diameter) spaced 1 cm apart, and a platform that was 7 cm wide and 2.5 cm high. During the training session animals were placed on the platform and their latency to step down on the grid with all four paws was measured. Animals received a 0.4-mA, 3.0 s foot shock after stepping down on the grid and were immediately removed from the apparatus. The test session was carried out 24 h after training, no foot shock was given and the step-down latency (maximum of 180 s) was used as a measure of memory retention.

2.5. Biochemical analysis

Animals were euthanized by decapitation 48 h after the last session of the CUS protocol. Brains were immediately removed and the hippocampus rapidly dissected and snap-frozen in nitrogen. All samples were stored at −80 °C until further analysis, as explained bellow.

2.5.1. Analysis of histone acetylation by western blot

The dissected and nitrogen frozen hippocampi samples were homogenized, placed in EDTA-free (Sigma-Aldrich, St. Louis, MO, USA) solution 1x containing a protease inhibitor cocktail tablet, and stored at −80 °C for subsequent analysis. For histone extraction, PBS buffer (Phosphate-Buffered-Saline) containing 250 u L Triton and 10 mg NaN3 was added to the homogenate samples to a 50 mL final volume. After 10 min on ice, samples were centrifuged at 6500 *g* for 10 min at 4 °C. The supernatant was collected and acid extraction (0.2-N HCl) of histones was carried out overnight at 4 °C. Samples were centrifuged once again (6500 *g* for 10 min at 4 °C), supernatants saved, and the protein content was determined using the Coomassie Blue method, with bovine serum albumin as a standard [57]. Western blot analysis of acetylated H3K9 (AcK9H3) and H4K12 (AcK12H4) was done as follows. Twenty-five μg total protein was separated on a 10% SDS polyacrylamide gel and transferred electrophoretically to a nitrocellulose membrane. Membranes were blocked with 5% non-fat dry milk in TBS containing 0.05% Tween 20 and were incubated overnight with the following antibodies: anti-histone H3 (ab1791, Abcam) at 1:3000, anti-acetyl histone H3 (Lys-9, ab10812, Abcam) at 1:500, anti-histone H4 (ab10158, Abcam) at 1:200 and anti-acetyl histone H4 (Lys-12, K12, ab61238, Abcam) at 1:700. Goat anti-rabbit (ab97051, HRP) radish-conjugated secondary antibodies were used and detected using ECL Western Blotting Substrate Kit (Abcam, Cambridge, UK). Pre-stained molecular weight protein markers (SuperSignal Molecular Weight Protein Ladder, Thermo Scientific, Rockford, USA) were used to determine the detected bands molecular weight and confirm target specificity of antibodies. Analysis of band intensities were performed in a Carestream Gel Logic 2200 PRO Imaging System and the associated Image Analysis Software. Data for acetylated histones were corrected for the amount of total histone protein.

2.5.2. Analysis of HDAC5 gene expression by real-time PCR

Total cellular RNA of hippocampus was extracted with SV Total RNA Isolation System (Promega, Madison, WI, USA) according to the manufacturer's protocol. RNA was re-suspended in nuclease-free water and was quantitated by spectrophotometry. The total RNA was used for reverse transcription (RT) reactions. RT reactions were performed using Invitrogen Superscript IV One-Step RT-PCR System (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol, and this was followed by real-time PCR of the target gene. TaqMan probes and the One-Step RT-PCR System (Applied Biosystems, Foster City, CA) were used in our experiments. PCR reactions were performed using 20x Assays-On-Demand Gene Expression Assay Mix (containing unlabeled PCR primers and Taq-Man probe) and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. PCR conditions were 95 °C for 10 min, followed by 95 °C for 15 s and 60 °C for 1 min repeated for 40 cycles. Experiments were performed in duplicate for each data point. Beta-actin was evaluated as an internal RNA control. Quantitative values were obtained from the cycle number (CT value) at which the increment in fluorescent signal associated with an exponential growth of PCR products started to be detected. The amount of target gene mRNA expression was normalized to the endogenous level of Beta-actin. Analysis was performed by obtaining the relative threshold cycle (ΔCT), in relation to the CT of the control gene in order to measure the relative expression level ($2^{-\Delta\Delta CT}$) of the target gene [58]. Primer sequences for HDAC5 were: 5′ CAGCCAGAAGATGTACGCCA3′ (*forward*) and 5′GCTGTGATGGCTACG GAGTT3′ (*reverse*). For Beta-actin they were 5′ACCGAGCATGGCTACA GCGTCACC3′(*forward*), 5′GTGGCCATCTCTTGCTCGGAGTCT3′ (*reverse*).

2.5.3. Analysis of BDNF by ELISA

Hippocampus samples were homogenized by gently grinding in 0.1 M phosphate buffer solution with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). The homogenates were immediately centrifuged at 2000 *g* for 5 min and the supernatant was collected and frozen at −80 °C until further analysis. BDNF levels were evaluated with a commercial sandwich-ELISA kit (Milipore, USA) following the manufacturer's instructions. In short, samples were added in duplicate to the microtiter plates (96 well flat-bottom), incubated for 24 h at 4 °C and rinsed four times with wash buffer. After that, biotinylated mouse anti-human BNDF monoclonal antibody (diluted 1:1000 in sample diluent) was added to each well and incubated for 3 h at room temperature. Wells were once again washed and then incubated with streptavidin–horseradish peroxidase conjugate solution (diluted

1:1000) for 1 h at room temperature. After the addition of substrate and stop solution, the amount of BDNF was determined (absorbance set at 450 nm). The standard curve ranged from 15.63 to 500 pg/ml of BNDF and showed a direct relationship between optical density and BDNF concentration. Total protein was measured by Bradford's method [57] using bovine serum albumin as standard.

2.6. Statistical analysis

Parametric data are expressed as mean ± standard deviation and were analyzed with two-way ANOVAs, with housing conditions (accompanied or isolated) and stress (submitted or not submitted to the CUS protocol) as the between-group variables. The non-parametric data of the inhibitory avoidance test are expressed as median (interquartile ranges) and were analyzed with Kruskal-Wallis tests, followed by Wilcoxon (for dependent variables) and Mann-Whitney (for independent variables) tests whenever appropriate. $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Weight gain during the experimental period

The two-way ANOVA indicated a significant main effect of stress [$F(1,40) = 23.076$, $p < 0.001$] on weight gain. As can be seen in Fig. 1, animals submitted to the CUS protocol showed significantly less weight gain than animals that were not submitted to the stress protocol. However, there was no effect of housing condition or any interaction between housing condition and stress on weight gain (all $p > 0.05$).

3.2. Open field

The results obtained in the open field task can be seen in Table 2. The two-way ANOVAs identified neither significant effects of housing conditions and stress, nor significant interactions between housing conditions and stress, on latencies to start locomotion, crossings and rearings (all $p > 0.05$).

3.3. Elevated plus maze

The results of the Plus maze task can also be seen in Table 2. The two-way ANOVA indicated significant effects for stress only on the time spent in open [$F(1,39) = 6.436$, $p = 0.017$] and closed [$F(1,39) = 5.786$, $p = 0.023$] arms. Animals submitted to the CUS protocol spent significantly less time in the open arms, and consequently more time in the closed arms, than animals that were not submitted to the stress protocol. No significant effects of housing condition, or interactions between housing condition and stress, were seen on any of the Plus maze variables (all $p > 0.05$).

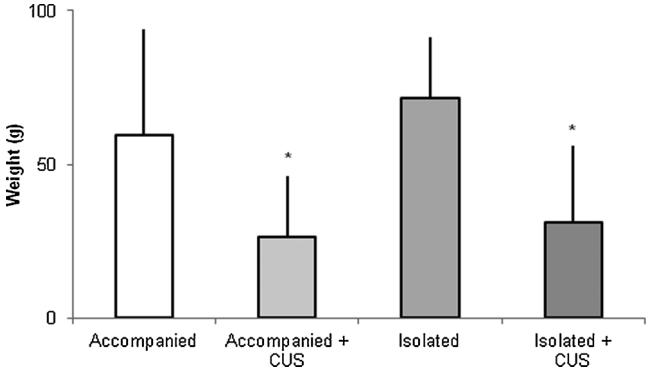

Fig. 1. Weight gain of rats during the experimental procedures, calculated as the difference between weight at the start (when animals were assigned to the different housing conditions) and at the end (immediately before animals were euthanized) of the experiment. Statistical analysis was performed using two-way analysis of variance with housing conditions (accompanied or isolated) and stress (submitted or not submitted to the CUS protocol) as fixed factors. Data are expressed as mean ± standard deviation. n = 10–12 *per* group. *$p < 0.001$ in comparison to the accompanied-only and isolated-only subgroups, indicating a significant effect of stress.

3.4. Inhibitory avoidance

As indicated by the Kruskal-Wallis test, latency to step down the platform in the training session was not significantly different ($p = 0.441$) between the accompanied [4.52 (3.41/5.99), accompanied + CUS [5.05 (4.36/6.295)], isolated[4.15 (3.49/5.48)] and isolated + CUS [3.84 (3.01/6.340] groups. Although the latency to step down increased significantly from the training to the test session in all experimental groups, as indicated by the Wilcoxon test (all $p < 0.05$), further analysis with the Mann-Whitney post hoc test indicated that the isolated and isolated + CUS group had a worse performance on the memory retention test than the accompanied and accompanied + CUS groups (all $p < 0.05$) (Fig. 2). On the other hand, no significant differences were identified between the accompanied and accompanied + CUS group ($p = 0.236$) and between the isolated and isolated + CUS groups ($p = 0.744$).

3.5. Histone acetylation

Significant main effects of housing condition were found for AcK9H3 [$F(1,14) = 26.473$, $p < 0.001$] and AcK12H4 [$F(1,18) = 11.733$, $p = 0.003$]. As can be seen in Fig. 3, isolated animals had lower levels of acetylated histones than accompanied animals. A main effect of stress was seen only on H3K9 acetylation [$F(1,14) = 6.752$, $p = 0.021$], which decreased in animals submitted to the CUS protocol. No significant interactions between housing conditions and stress were seen on H3K9 and H4K12 acetylation (all $p > 0.05$).

3.6. HDAC5 gene expression

The two-way ANOVA indicated significant effects of housing condition [$F(1,9) = 327.95$, $p < 0.001$] and stress [$F(1, 9) = 154,31$, $p < 0.001$] on the HDAC5 gene expression, as well as a significant interaction between housing condition and stress [$F(1,9) = 144.78$, $p < 0.001$]. As can be seen in Fig. 4, the expression of the HDAC5 gene was higher in isolated than in accompanied animals. Animals submitted to the CUS protocol also showed higher levels of HDAC5 expression than animals that were not submitted to this stress protocol. The interaction between housing condition and stress can also be seen in Fig. 4, which shows a greater effect of the CUS protocol on isolated animals in comparison to accompanied animals. This pattern of results suggests that accompaniment can mitigate the effects of CUS on HDAC5 expression.

3.7. BDNF levels

The results obtained for BDNF levels can be seen in Fig. 5. The two-way Anova indicated significant housing effects on hippocampal BDNF [$F(1,18) = 22.469$, $p < 0.001$], with higher levels of this neurotrophin in accompanied than in isolated animals. However, no significant effects of stress, or interactions between stress and housing conditions, were seen on the BDNF levels (all $p > 0.05$).

4. Discussion

One of the main hypotheses of this study was that social isolation and chronic unpredictable stress of young adult rats would lead to

Table 2
Effects of housing conditions and stress on Open Field and Plus maze parameters.

		Groups			
		Accompanied	Accompanied + CUS	Isolated	Isolated + CUS
Open Field	Latency(s)	3.5 ± 1.67	2.71 ± 1.05	3.20 ± 1.5	3.82 ± 2.03
	Crossing(n)	71.9 ± 34.39	90.61 ± 13.45	82.3 ± 18.12	91.54 ± 28.9
	Rearing(n)	29.9 ± 9.64	34.33 ± 7.4	29.25 ± 5.95	30.95 ± 7.05
Plus Maze	Open Arm Time (%)	14.23 ± 10.07	12.79 ± 6.27*	13.53 ± 3.84	4.56 ± 1.43*
	Closed Arm Time (%)	75.48 ± 13.96	76.46 ± 11.93**	78.33 ± 5.85	91.46 ± 6.69**
	Open Arm Entries (n)	1.50 ± 1.3	1.00 ± 1.41	2.00 ± 1.56	0.69 ± 0.85
	Closed Arm Entries (n)	4.12 ± 2.1	3.25 ± 2.52	3.20 ± 1.98	3.15 ± 2.3

Statistical analysis was performed using two-way analysis of variance, with housing conditions (accompanied or isolated) and stress (submitted or not submitted to the CUS protocol) as fixed factors. Data are expressed as mean ± standard deviation. n = 10–12 per group.

* $p < 0.05$ for the time spent in open arms in comparison to the accompanied-only and isolated-only subgroups, indicating significant effects of the CUS protocol.

** $p < 0.05$ for the time spent in closed arms in comparison to the accompanied-only and isolated-only subgroups, indicating significant effects of the CUS protocol.

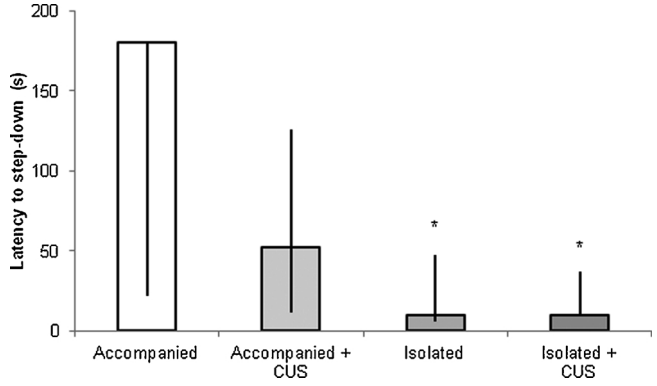

Fig. 2. Long-term retention of inhibitory avoidance memory in animals submitted to different housing and stress conditions. The retention test was run 24 h after the training session. Statistical analysis was performed using Kruskal-Wallis test and Mann-Whitney's post hoc test. Data are expressed as median and interquartile range. n = 10–12 *per* group. *$p < 0.05$ in comparison to the accompanied subgroups, indicating a significant housing effect.

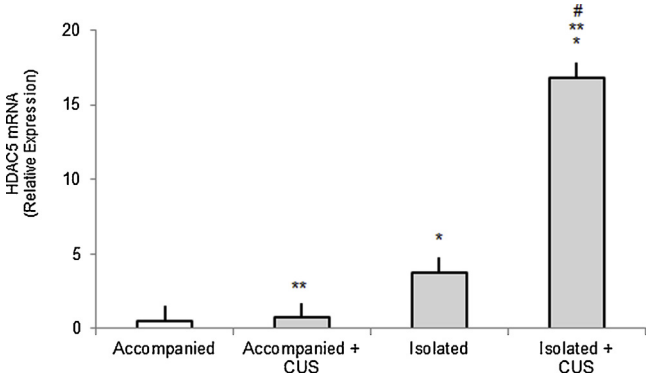

Fig. 4. Hippocampal alterations in the expression of the HDAC5 gene in response to different housing conditions and stress. Samples were normalized to Beta-actin expression and run in duplicate. Statistical analysis was performed using two-way analysis of variance with housing conditions (accompanied or isolated) and stress (submitted or not submitted to the CUS protocol) as fixed factors. Data are expressed as mean ± SD. *$p < 0.001$ indicating the housing effect; ** $p < 0.001$ indicating the stress effect; #$p < 0.001$ indicating the interaction between housing condition and stress.

negative outcomes on epigenetic mechanisms known to modulate hippocampal BDNF levels and affect behavior. Accordingly, our results indicated increased HDAC5 expression, decreased histone acetylation (AcK9H3 and AcK12H4), lower BDNF levels and impaired long-term memory in isolated animals. Moreover, the stress protocol used in this study was capable of affecting weight gain, inducing anxiety-like behavior and decreasing AcK9H3 levels and increasing HDAC5 expression. We also hypothesized that social isolation would magnify the effects of chronic stress on the investigated variables. However, further worsening of the effects of the CUS protocol by isolation were limited to the HDAC5 expression, as indicated by the significantly higher

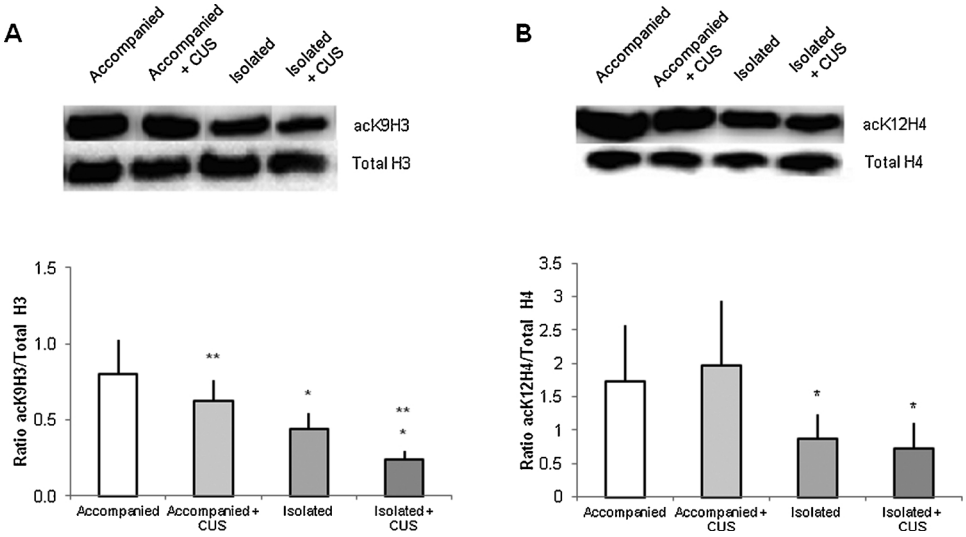

Fig. 3. Quantification and representative western blots of (A) acetylated histone 3 lysine 9 [AcK9H3] and (B) histone 4 lysine 12 [AcK12H4] in the hippocampus of rats exposed to different housing conditions and stress. Statistical analysis was performed using two-way analysis of variance with housing conditions (accompanied or isolated) and stress (submitted or not submitted to the CUS protocol) as fixed factors. n = 4–6 *per* group. Data are expressed as mean ± standard deviation. *$p < 0.01$ in comparison to the accompanied subgroups, indicating a significant housing effect; **$p < 0.05$ in comparison to the accompanied-only and isolated-only subgroups, indicating a significant effect of stress.

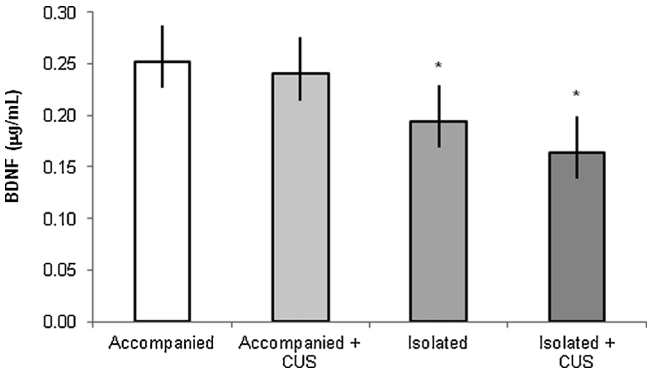

Fig. 5. Alterations of BNDF levels in the hippocampus of rats submitted to different housing conditions and stress. Statistical analysis was performed using two-way analysis of variance with housing conditions (accompanied or isolated) and stress (submitted or not submitted to the CUS protocol) as fixed factors. Data are expressed as mean ± standard deviation. n = 5–6 *per* group. *$p < 0.001$ in comparison to the accompanied subgroups, indicating significant housing effects.

expression in the isolated + CUS animals in comparison to the isolated-only animals. Our third assumption was that paired housing would be protective against the alterations induced by chronic stress. Support for this supposition was also limited to the HDAC5 expression, as suggested by the lack of significant effects of the CUS protocol on the HDAC5 expression of accompanied animals, in opposition to its effect on socially isolated animals.

Locomotor and exploratory activities in the open field were unchanged by chronic stress and housing conditions. Although different studies indicated decreased or increased open field activity after induction of social isolation [59–61] or CUS [51,62–65], our results are consistent with the findings of other research groups, which reported no alterations in locomotor and exploratory behavior of animals exposed to these treatments [66–69]. As pointed out by Hu and collaborators (2010), it is possible that these inconsistent findings among research groups are the result of confounding factors such as the modification of the stress protocol and stimuli intensity, behavioral measure methodology, the variety in animal species and the method of interpretation of results [66].

Analysis of weight gain and anxiety-like behaviors, evaluated with the plus maze task, also indicated no significant effects of isolation. In fact, this is not a surprising result. A recent review concluded that social isolation has only a small effect on rodent defense behavior [70], and the lack of significant effects on plus maze results is not uncommon [68,71,72]. However, animals submitted to CUS had a reduction in weight gain, indicating that this mild stress protocol had a negative impact on them. Moreover, animals exposed to the CUS protocol spent significantly less time in the open arms of the plus maze in comparison to animals that were not submitted to this stress protocol. Most studies that use CUS protocols report weight decreases [51,73–75] and increased anxiety-related defense behaviors [74,75].

Although locomotor and exploratory activities, weight gain and anxiety-like behaviors were not affected by social isolation, the negative effects of this experimental protocol became evident by the decreased hippocampal acetylation of H3K9 and H4K12, the increased HDAC5 expression and the decreased BDNF levels in comparison to accompanied animals. Interestingly, this pattern of results appears to be conserved from early development into adulthood. Maternal separation is associated with reduced levels of total, exon I and exon IV BDNF mRNA, lower BDNF protein levels, decreased acetylation of histone H3 and H4 at the BDNF promoter IV and increased HDAC5 mRNA [76,77]. Li and collaborators (2016) reported a decrease in histone acetylation and BDNF protein expression after social isolation in early adolescent animals and our results clearly suggest that the isolation of young adult rats has a negative impact on H3K9 and H4K12 acetylation, which is associated to an increase in HDAC5 expression and BDNF decrease [78]. In fact, decreases in hippocampal BDNF levels are a common finding in studies of social isolation and have been associated to impaired synaptic plasticity, neurogenesis and neuronal survival, besides behavioral dysfunctions such as depression, anxiety and memory impairments (for a review see reference [12]). In line with these evidences, our results indicated that animals of the isolated subgroups had impaired long-term memory in the inhibitory avoidance task when compared to the accompanied subgroups.

Consolidation of inhibitory avoidance memory is known to be dependent on the extracellular release of BDNF and its interaction with tropomyosin-related kinase B (TrkB) [79–81]. There are also evidences that histone acetylation (including H3K9 and H4K12) begins a gene expression program that leads to hippocampal memory consolidation [82]. Accordingly, factors that decrease histone acetylation (such as aging) are associated to impairment of aversive memories [83], whereas factors that are able to increase acetylation (such as physical exercise or HDAC inhibitors) are associated with inhibitory avoidance improvement [82,84,85]. Moreover, inhibition of HDACs facilitates long-term potentiation in the CA1 area of the dorsal hippocampus, a cellular plasticity mechanism involved in the establishment of inhibitory avoidance memory [86–88]. Thus, our results clearly show an association of social isolation with epigenetic mechanisms potentially involved in the decrease of BDNF levels and memory impairment. However, our study design does not allow the establishment of causal relationships between these variables. Thus, the clinical relevance of our findings should be further investigated in studies planned to evaluate the causal relations between epigenetic modifications, alterations in BDNF levels and behavioral outcomes. Therefore, it would be interesting to evaluate the effect of experimental procedures known to depress the expression of HDAC5 or increase the expression of BDNF, such as pharmacological interventions, viral-mediated BDNF overexpression or HDAC5 knockdown models [30,84,85,89–91], and verify if they are able to revert the effects of social isolation.

Histone modification of the BDNF gene in the hippocampus is likely to play a critical role in the response to stressful environments. Different stress protocols (including acute and chronic restrain, social defeat and CUS) are able to induce epigenetic effects through decreases in histone acetylation, increases in HDAC expression and/or reduction of BDNF expression [27,31,32,34]. In this study, stress effects decreased H3K9 acetylation and increased HDAC5 expression. However, the worsening of the epigenetic effects of the CUS protocol by social isolation were limited to the expression of HDAC5, which showed higher levels in the isolated + CUS subgroup in comparison to the isolated-only subgroup. Seo and collaborators (2016) combined maternal separation and chronic restrain stress and also observed that maternal separation exacerbated the effects of the stress protocol on HDAC5 expression. However, the authors also found a further reduction of histone H3 and H4 acetylation at BDNF promoter IV and a further decrease in BDNF mRNA (both total and at exon IV) in animals that were submitted to the restrain stress in addition to the maternal separation. [76]. The critical elements responsible for the extent of the effects of isolation on the responses of animals to other chronic stressors have not yet been identified, but it is likely that the developmental stage of the animals, the type and duration of the isolation and stress protocols play a significant role on the outcomes seen in different studies [51,92]. Moreover, the methods used to investigate the epigenetic (total histone acetylation vs chromatin immunoprecipitation assays directed to specific BDNF promoters) and BDNF alterations (protein levels vs mRNA expression, total hippocampus vs hippocampal subregions) could also contribute to some of the discrepancies seen between the studies [30–32]. Notwithstanding, this is the first study to explore the interactions of social isolation and chronic stress on adult animals. The results obtained for the HDAC expression in isolated + CUS animals warrant further investigations on the effects of isolation on epigenetic

mechanisms of chronically stressed animals. Thus, future studies should evaluate the effects of more intense stress protocols (the CUS protocol of this study can be classified as mild to moderate) and broaden the epigenetic variables to be investigated (e.g. evaluating histone methylation and demethylases).

Besides evaluating the effects of housing conditions and stress on animals, our study was also designed to evaluate the possible effects of social buffering on additional epigenetic and behavioral effects induced by the CUS protocol on isolated animals. However, the analysis of social buffering was limited by the fact that, out of all variables investigated (anxiety-like behaviors, memory, BDNF levels, epigenetic variables), only HDAC5 expression showed significant effects of isolation on the stressed animals. So, if social buffering effects were to occur, only HDAC5 expression would be capable to indicate them. Howsoever, no effects of the CUS protocol were seen on the HDAC5 expression of accompanied animals. This result suggests that social buffering could be potentially involved in the modulation of HDAC5 expression and warrants further investigation on this issue. It is important to note that the role of histone remodeling in the pathophysiology and treatment of psychiatric disorders has been underscored by studies showing that drugs, experimental (such as sodium butyrate) or therapeutic (such as antidepressant and anxiolytics), capable to inhibit HDAC5 effects can revert disturbances of the epigenetic control of BDNF levels [30,32,76,93]. Thus, our results suggest that social buffering could act on some of the mechanisms targeted by these pharmacological interventions, i.e. modulation of the effects of HDAC5.

In conclusion, the results of this study indicate that social isolation and mild CUS protocols are able to induce epigenetic alterations in the hippocampus of adult animals. However, social isolation effects were more extensive and the only ones that lead to decreased BDNF levels and memory impairment. They also worsened the effects of the CUS protocol on HDAC5 expression. Moreover, the lack of effects of the CUS protocol on HDAC5 expression suggest that social buffering can act through epigenetic mechanisms to counteract the harmful effects of stress. Thus, this study adds to the knowledge of the epigenetic effects of social isolation in adulthood, a developmental time window in which epigenetic mechanisms have been scarcely explored. Moreover, the possibility of social buffering effects on HDAC5 expression seen in this study warrant further investigations. There are surprisingly few studies on the mechanisms through which social support operates. Affiliative behavior, group cohesion and liking are natural responses seen in humans exposed or anticipating stressful events [1,15,94]. Moreover, the effects of social buffering in humans are far reaching, being able to aid in the health outcomes of diseases that affect different organs and systems and even increase longevity [9]. Thus, social support is a field that deserves much more attention than it has received until now.

Author contributions

E. Bromberg conceived, designed and supervised the experiments. J.V. Borges, B.S. de Freitas, V. Antoniazzi, C.S. dos Santos, K. Vedovelli, V.N. Pires and L. Paludo performed the experiments. E. Bromberg and J.V. Borges analyzed the data. M.N.M. de Lima contributed reagents and aided in manuscript writing. E. Bromberg and J.V. Borges wrote the manuscript. All authors provided final approval for the submission of the manuscript.

Competing financial interests

The authors declare no competing financial interests.

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References

[1] S.E. Taylor, Tend and befriend: biobehavioral bases of affiliation under stress, Curr. Dir. Psychol. Sci. 15 (6) (2006) 273–277 http://www.jstor.org/stable/20183134.
[2] R.F. Baumeister, M.R. Leary, The need to belong: desire for interpersonal attachments as a fundamental human motivation, Psychol. Bull. 117 (3) (1995) 497–529, https://doi.org/10.1037/0033-2909.117.3.497.
[3] S.E. Taylor, The Tending Instinct: How Nurturing Is Essential to Who We Are and How We Live, Holt, New York, 2002.
[4] M. Heinrichs, T. Baumgartner, C. Kirschbaum, U. Ehlert, Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress, Biol. Psychiatry 54 (2003) 1389–1398, https://doi.org/10.1016/S0006-3223(03)00465-7.
[5] A. Steptoe, A. Shankar, P. Demakakos, J. Wardle, Social isolation, loneliness, and all-cause mortality in older men and women, Proc. Natl. Acad. Sci. U. S. A. 110 (15) (2013) 5797–5801, https://doi.org/10.1073/pnas.1219686110.
[6] E.B. Thorsteinsson, J.E. James, M.E. Gregg, Effects of video-relayed social support on hemodynamic reactivity and salivary cortisol during laboratory-based behavioral challenge, Health Psychol. 17 (5) (1998) 436–444, https://doi.org/10.1037/0278-6133.17.5.436.
[7] J.T. Cacioppo, S. Cacioppo, J.P. Capitanio, S.W. Cole, The neuroendocrinology of social isolation, Annu. Rev. Psychol. 66 (2015) 733–767, https://doi.org/10.1146/annurev-psych-010814-015240.
[8] N. Leigh-Hunt, D. Bagguley, K. Bash, V. Turner, S. Turnbull, N. Valtorta, W. Caan, An overview of systematic reviews on the public health consequences of social isolation and loneliness, Public Health 152 (2017) 157–171, https://doi.org/10.1016/j.puhe.2017.07.035.
[9] A.K. Beery, D. Kaufer, Stress, social behavior, and resilience: insights from rodents, Neurobiol. Stress 1 (2015) 116–127, https://doi.org/10.1016/j.ynstr.2014.10.004.
[10] S.J. Lupien, B.S. McEwen, M.R. Gunnar, C. Heim, Effects of stress throughout the lifespan on the brain, behaviour and cognition, Nat. Rev. Neurosci. 10 (6) (2009) 434–445, https://doi.org/10.1038/nrn2639.
[11] T.W. Robbins, Neurobehavioural sequelae of social deprivation in rodents revisited: Modelling social adversity for developmental neuropsychiatric disorders, Psychopharmacology 30 (11) (2016) 1082–1089, https://doi.org/10.1177/0269881116664450.
[12] I. Zaletel, D. Filipović, N. Puškaš, Hippocampal BDNF in physiological conditions and social isolation, Rev. Neurosci. 28 (6) (2017) 675–692, https://doi.org/10.1515/revneuro-2016-0072.
[13] C.O. Ladd, K.V. Thrivikraman, R.L. Huot, P.M. Plotsky, Differential neuroendocrine responses to chronic variable stress in adult Long Evans rats exposed to handling-maternal separation as neonates, Psychoneuroendocrinology 30 (6) (2005) 520–533, https://doi.org/10.1016/j.psyneuen.2004.12.004.
[14] A.H. Veenema, S.O. Reber, S. Selch, F. Obermeier, I.D. Neumann, Early life stress enhances the vulnerability to chronic psychosocial stress and experimental colitis in adult mice, Endocrinology 149 (6) (2008) 2727–2736, https://doi.org/10.1210/en.2007-1469.
[15] B. Latané, J. Eckman, V. Joy, Shared stress and interpersonal attraction, J. Exp. Soc. Psychol. 1 (Suppl. 1) (1966) 80–94.
[16] C.A. Gonzalez, C.L. Coe, S. Levine, Cortisol responses under different housing conditions in female squirrel monkeys, Psychoneuroendocrinology 7 (2-3) (1982) 209–216.
[17] T.E. Smith, J.A. French, Social and reproductive conditions modulate urinary cortisol excretion in blacktufted-ear marmosets (Callithrixkuhli), Am. J. Primatol. 42 (4) (1997) 253–267, https://doi.org/10.1002/(SICI)1098-2345(1997)42:4<253::AID-AJP1>3.0.CO;2-W.
[18] N. Sachser, M. Durschlag, D. Hirzel, Social relationships and the management of stress, Psychoneuroendocrinology 23 (8) (1998) 891–904, https://doi.org/10.1016/S0306-4530(98)00059-6.
[19] T. Kikusui, J.T. Winslow, Y. Mori, Social buffering: relief from stress and anxiety, Philos Trans. R. Soc. London Biol. Sci. 361 (1476) (2006) 2215–2228, https://doi.org/10.1098/rstb.2006.1941.
[20] A.C. De-Vries, T.K. Craft, E.R. Glasper, G.N. Neigh, J.K. Alexander, Richter award winner: social influences on stress responses and health, Psychoneuroendocrinology 32 (6) (2007) 587–603, https://doi.org/10.1016/j.psyneuen.2007.04.007.
[21] J.D. Gray, J.F. Kogan, J. Marrocco, B.S. McEwen, Genomic and epigenomic mechanisms of glucocorticoids in the brain, Nat. Rev. Endocrinol. 13 (11) (2017) 661–673, https://doi.org/10.1038/nrendo.2017.97.
[22] J.G. Howland, Y.T. Wang, Synaptic plasticity in learning and memory: stress effects in the hippocampus, Prog. Brain Res. 169 (2008) 145–158, https://doi.org/10.1016/S0079-6123(07)00008-8.
[23] D. Sierra-Mercado, N. Padilla-Coreano, G.J. Quirk, Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression, Neuropsychopharmacology 36 (2) (2011) 529–538, https://doi.org/10.1038/npp.2010.184.
[24] J.D. Gray, T.A. Milner, B.S. McEwen, Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors, Neuroscience

239 (2013) 214–227, https://doi.org/10.1016/j.neuroscience.2012.08.034.
[25] J.B. Ortiz, C.D. Conrad, The impact from the aftermath of chronic stress on hippocampal structure and function: Is there a recovery? Front. Neuroendocrinol. 49 (2018) 114–123, https://doi.org/10.1016/j.yfrne.2018.02.005.
[26] C. Nasca, D. Zelli, B. Bigio, S. Piccinin, S. Scaccianoce, R. Nisticò, B.S. McEwen, Stress dynamically regulates behavior and glutamatergic gene expression in hippocampus by opening a window of epigenetic plasticity, Proc. Natl. Acad. Sci. U. S. A. 112 (48) (2015) 14960–14965, https://doi.org/10.1073/pnas.1516016112.
[27] R.G. Hunter, K.J. McCarthy, T.A. Milne, D.W. Pfaff, B.S. McEwen, Regulation of hippocampal H3 histone methylation by acute and chronic stress, Proc. Natl. Acad. Sci. U. S. A. 106 (49) (2009) 20912–20917, https://doi.org/10.1073/pnas.0911143106.
[28] J.E. Herrera, R.L. Schiltz, M. Bustin, The accessibility of histone H3 tails in chromatin modulates their acetylation by P300/CBP-associated factor, J. Biol. Chem. 275 (17) (2000) 12994–12999, https://doi.org/10.1074/jbc.275.17.12994.
[29] R.B. Selvi, T.K. Kundu, Reversible acetylation of chromatin: implication in regulation of gene expression, disease and therapeutics, Biotechnol. J. 4 (3) (2009) 375–390, https://doi.org/10.1002/biot.200900032.
[30] N.M. Tsankova, O. Berton, W. Renthal, A. Kumar, R.L. Neve, E.J. Nestler, Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action, Nat. Neurosci. 9 (2006) 519–525, https://doi.org/10.1038/nn1659.
[31] M. Fuchikami, S. Morinobu, A. Kurata, S. Yamamoto, S. Yamawaki, Single immobilization stress differentially alters the expression profile of transcripts of the brain-derived neurotrophic factor (BDNF) gene and histone acetylation at its promoters in the rat hippocampus, Int. J. Neuropsychophamarcol 12 (2009) 73–82, https://doi.org/10.1017/S1461145708008997.
[32] C.L. Ferland, L.A. Schrader, Regulation of histone acetylation in the hippocampus of chronically stressed rats: a potential role of sirtuins, Neuroscience 174 (2011) 104–114, https://doi.org/10.1016/j.neuroscience.2010.10.077.
[33] XXX.
[34] H.E. Covington, V.F. Vialou, Q.C. Laplant, Y.N. Ohnishi, E.J. Nestler, Hippocampal-Dependent Antidepressant-Like Activity of Histone Deacetylase Inhibition, Neurosci. Lett. 493 (3) (2012) 122–126, https://doi.org/10.1016/j.neulet.2011.02.022.
[35] H. Li, Q. Jiang, X. Fu, X. Jiang, Q.X. Zhou, H.M. Qiu, Abnormal modification of histone acetylation involved in depression-like behaviors of rats induced by chronically unpredicted stress, Neuroreport 28 (16) (2017) 1054–1060, https://doi.org/10.1097/WNR.0000000000000879.
[36] R. Mossner, S. Daniel, D. Albert, A. Heils, O. Okladnova, A. Schmitt, et al., Serotonin transporter function is modulated by brain-derived neurotrophic factor (BDNF) but not nerve growth factor (NGF), Neurochem. Int. 36 (3) (2000) 197–202, https://doi.org/10.1016/S0197-0186(99)00122-9.
[37] O. Guillin, J. Diaz, P. Carroll, N. Griffon, J.C. Schwartz, P. Sokoloff, BDNF controls dopamine D3 receptor expression and triggers behavioural sensitization, Nature 411 (6833) (2001) 86–89, https://doi.org/10.1038/35075076.
[38] A.L. Carvalho, M.V. Caldeira, S.D. Santos, C.B. Duarte, Role of the brain-derived neurotrophic factor at glutamatergic synapses, Br. J. Pharmacol. 153 (Suppl1) (2008) S310–24, https://doi.org/10.1038/sj.bjp.0707509.
[39] B. Lu, G. Nagappan, Y. Lu, BDNF and synaptic plasticity, cognitive function, and dysfunction, Handb. Exp. Pharmacol. 220 (2014) 223–250, https://doi.org/10.1007/978-3-642-45106-5_9.
[40] G. Leal, C.R. Bramham, C.B. Duarte, BDNF and hippocampal synaptic plasticity, Vitam. Horm. 104 (2017) 153–195, https://doi.org/10.1016/bs.vh.2016.10.004.
[41] S.J. Allen, J.J. Watson, D.K. Shoemark, N.U. Barua, N.K. Patel, GDNF, NGF and BDNF as therapeutic options for neurodegeneration, Pharmacol. Ther. 138 (2) (2013) 155–175, https://doi.org/10.1016/j.pharmthera.2013.01.004.
[42] F. Jeanneteau, V. ChaoM, Are BDNF and glucocorticoid activities calibrated? Neuroscience 239 (2013) 173–195, https://doi.org/10.1016/j.neuroscience.2012.09.017.
[43] M.S. Corrêa, B.L. Giacobbo, K. Vedovelli, D.B. Lima, P. Ferrari, I.I. Argimon, J.C. Walz, E. Bromberg, Age effects on cognitive and physiological parameters in familial caregivers of alzheimer's disease patients, PLoS One 11 (10) (2016) e0162619, , https://doi.org/10.1371/journal.pone.0162619.
[44] J.D. Gray, T.A. Milner, B.S. McEwen, Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors, Neuroscience 239 (2013) 214–227, https://doi.org/10.1016/j.neuroscience.2012.08.034.
[45] C. Zuccato, E. Cattaneo, Brain-derived neurotrophic factor in neurodegenerative diseases, Nat. Rev. Neurol. 5 (6) (2009) 311–322, https://doi.org/10.1038/nrneurol.2009.
[46] K. Sopova, K. Gatsiou, K. Stellos, C. Laske, Dysregulation of neurotrophic and haematopoietic growth factors in Alzheimer's disease: from pathophysiology to novel treatment strategies, Curr. Alzheimer Res. 11 (1) (2014) 27–39, https://doi.org/10.2174/1567205010666131120100743.
[47] A.O. Ahmed, A.M. Mantini, D.J. Fridberg, P.F. Buckley, Brain-derived neurotrophic factor (BDNF) and neurocognitive deficits in people with schizophrenia: a meta-analysis, Psychiatry Res. 226 (1) (2015) 1–13, https://doi.org/10.1016/j.psychres.2014.12.069.
[48] T.L. Huang, C.C. Lin, Advances in biomarkers of major depressive disorder, Adv. Clin. Chem. 68 (2015) 177–204, https://doi.org/10.1016/bs.acc.2014.11.003.
[49] B. Lima Giacobbo, J. Doorduin, H.C. Klein, R.A.J.O. Dierckx, E. Bromberg, E.F.J. de Vries, Brain-derived neurotrophic factor in brain disorders: focus on neuroinflammation, Mol. Neurobiol. (2018), https://doi.org/10.1007/s12035-018-1283-6 [Epub ahead of print].
[50] T.L. Roth, F.D. Lubin, A.J. Funk, J.D. Sweatt, Lasting epigenetic influence of early-life adversity on the BDNF gene, Biol. Psychiatry 65 (2009) 760–769, https://doi.org/10.1016/j.biopsych.2008.11.028.
[51] P. Willner, The chronic mild stress (CMS) model of depression: history, evaluation and usage, Neurobiol. Stress 6 (2016) 78–93, https://doi.org/10.1016/j.ynstr.2016.08.002.
[52] G.D. Gamaro, L.P. Manoli, I.L. Toerres, R. Silveira, C. Dalmaz, Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures, Neurochem. Int. 42 (2) (2003) 107–114, https://doi.org/10.1016/S0197-0186(02)00080-3.
[53] Q. Chen, L. Ren, S. Min, X. Hao, H. Chen, J. Deng, Changes in synaptic plasticity are associated with electroconvulsive shock-induced learning and memory impairment in rats with depression-like behavior, Neuropsychiatr. Dis. Treat. 14 (2018) 1737–1746, https://doi.org/10.2147/NDT.S163756.
[54] K. Vedovelli, E. Silveira, E. Velho, L. Stertz, F. Kapczinski, N. Schröder, et al., Effects of increased opportunity for physical exercise and learning experiences on recognition memory and brain-derived neurotrophic factor levels in brain and serum of rats, Neuroscience 199 (2011) 284–291, https://doi.org/10.1016/j.neuroscience.2011.08.012.
[55] J. Xu, L. Chen, J. Su, Z. Liu, J. Chen, Q. Lin, et al., The anxiolytic-like effects of ginsenoside Rg3 on chronic unpredictable stress in rats, Sci. Rep. 8 (1) (2018) 7741, https://doi.org/10.1038/s41598-018-26146-5.
[56] R. Roesler, N. Schröder, M.R. Vianna, J. Quevedo, E. Bromberg, F. Kapczinski, et al., Differential involvement of hippocampal and amygdalar NMDA receptors in contextual and aversive aspects of inhibitory avoidance memory in rats, Brain Res. 975 (1-2) (2003) 207–213, https://doi.org/10.1016/S0006-8993(03)02656-8.
[57] M.M. Bradford, A rapid and sensitive method for the quantitation microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 254 (1976) 248–254.
[58] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT–PCR, Nucleic Acids Res. 29 (9) (2001) 2002–2007 1.
[59] G.B. Varty, M.P. Paulus, D.L. Braff, M.A. Geyer, Environmental enrichment and isolation rearing in the rat: effects on locomotor behavior and startle response plasticity, Biol. Psychiatry 47 (10) (2000) 864–873, https://doi.org/10.1016/S0006-3223(99)00269-3.
[60] H. Arakawa, The effects of isolation rearing on open-field behavior in male rats depends on developmental stages, Dev. Psychobiol. 43 (2003) 11–19, https://doi.org/10.1002/dev.10120.
[61] L. Sun, L. Min, H. Zhou, M. Li, F. Shao, W. Wang, Adolescent social isolation affects schizophrenia-like behavior and astrocyte biomarkers in the PFC of adult rats, Behav. Brain Res. 333 (2017) 258–266, https://doi.org/10.1016/j.bbr.2017.07.011.
[62] R. Harris, Failure to change exploration or saccharin preference in rats exposed to chronic mild stress, Physiol. Behav. 63 (1) (1997) 91–100, https://doi.org/10.1016/S0031-9384(97)00425-3.
[63] J. Grønli, R. Murison, E. Fiske, B. Bjorvatn, E. Sørensen, C.M. Portas, et al., Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions, Physiol. Behav. 84 (4) (2005) 571–577, https://doi.org/10.1016/j.physbeh.2005.02.007.
[64] H. Dang, Y. Chen, X. Liu, Q. Wang, L. Wang, W. Jia, et al., Progress in neuropsychopharmacology and biological psychiatry antidepressant effects of Ginseng total saponins in the forced swimming test and chronic mild stress models of depression, Prog. Neuropsychopharmacol. Biol. Psychiatry 33 (8) (2009) 1417–1424, https://doi.org/10.1016/j.pnpbp.2009.07.020.
[65] Y.H. Jung, S.I. Hong, S.X. Ma, J.Y. Hwang, J.S. Kim, J.H. Lee, et al., Strain differences in the chronic mild stress animal model of depression and anxiety in mice, Biomol. Ther. 22 (5) (2004) 453–459, https://doi.org/10.4062/biomolther.2014.058.
[66] H. Hu, L. Su, Q. Xu, H. Zhang, L.W. Wang, Behavioral and [F-18] fluorodeoxyglucose micro positron emission tomography imaging study in a rat chronic mild stress model of depression, Neuroscience 169 (2010) 171–181, https://doi.org/10.1016/j.neuroscience.2010.04.057.
[67] J. Djordjevic, A. Djordjevic, M. Adzic, M.B. Radojcic, Effects of chronic social isolation on Wistar rat behavior and brain plasticity markers, Neuropsychobiology 66 (2012) 112–119, https://doi.org/10.1159/000338605.
[68] T.R. Butler, O.J. Ariwodola, J.L. Weiner, The impact of social isolation on HPA axis function, anxiety-like behaviors, and ethanol drinking, Front. Integr. Neurosci. 7 (2014) 102, https://doi.org/10.3389/fnint.2013.00102.
[69] O.S. Cevik, L. Sahin, L. Tamer, Long term treadmill exercise performed to chronic social isolated rats regulate anxiety behavior without improving learning, Life Sci. 200 (2018) 126–133, https://doi.org/10.1016/j.lfs.2018.03.029.
[70] F. Mohammad, J. Ho, J.H. Woo, C.L. Lim, D.J.J. Poon, B. Lamba, et al., Concordance and incongruence in preclinical anxiety models: systematic review and meta-analyses, Neurosci. Biobehav. Rev. 68 (2016) 504–529, https://doi.org/10.1016/j.neubiorev.2016.04.011.
[71] X. Zhang, B. Wang, J. Jin, S. An, Q. Zeng, Y. Duan, et al., Early deprivation reduced anxiety and enhanced memory in adult male rats, Brain Res. Bull. 108 (2014) 44–50, https://doi.org/10.1016/j.brainresbull.2014.08.005.
[72] N. Joshi, R.A. Leslie, T.S. Perrot, Analyzing the experiences of adolescent control rats: Effects of the absence of physical or social stimulation on anxiety-like behaviour are dependent on the test, Physiol. Behav. 179 (2017) 30–41, https://doi.org/10.1016/j.physbeh.2017.05.019.
[73] K. Matthews, N. Forbes, I.C. Reid, Sucrose consumption as an hedonic measure following chronic unpredictable mild stress, Physiol. Behav. 57 (2) (1995) 241–248, https://doi.org/10.1016/0031-9384(94)00286-E.
[74] J.J. Herrera-Pérez, V. Benítez-Coronel, G. Jiménez-Rubio, O.T. Hernández-Hernández, L. Martínez-Mota, Young-adult male rats' vulnerability to chronic mild stress is reflected by anxious-like instead of depressive-like behaviors, Neurosci. J.

(2017) 8952079, , https://doi.org/10.1155/2016/5317242.
[75] C. Hu, Y. Luo, H. Wang, S. Kuang, G. Liang, Y. Yang, et al., Re-evaluation of the interrelationships among the behavioral tests in rats exposed to chronic unpredictable mild stress, PLoS One 12 (9) (2017) e0185129, , https://doi.org/10.1371/journal.pone.0185129.
[76] M.K. Seo, N.N. Ly, C.H. Lee, H.Y. Cho, C.M. Choi, L.H. Nhu, et al., Early life stress increases stress vulnerability through BDNF gene epigenetic changes in the rat hippocampus, Neuropharmacology 105 (2016) 388–397, https://doi.org/10.1016/j.neuropharm.2016.02.009.
[77] M.K. Seo, Y.H. Kim, R.S. McIntyre, R.B. Mansur, Y. Lee, N.E. Carmona, et al., Effects of antipsychotic drugs on the epigenetic modification of brain-derived neurotrophic factor gene expression in the hippocampi of chronic restraint stress rats, Neural Plast. 2018 (2018) 2682037, , https://doi.org/10.1155/2018/2682037.
[78] H. Li, Q. Jiang, X. Fu, X. Jiang, Abnormal modification of histone acetylation involved in depression-like behaviors of rats induced by chronically unpredicted stress, Mol. Biol. 28 (16) (2017) 1054–1060, https://doi.org/10.1097/WNR.0000000000000879.
[79] L. Minichiello, TrkB signalling pathways in LTP and learning, Nat. Rev. Neurosci. 10 (12) (2009) 850–860, https://doi.org/10.1038/nrn2738.
[80] D. Panja, C.R. Bramham, BDNF mechanisms in late LTP formation:synthesis and breakdown, Neuropharmacology 76 (2014) 664–676, https://doi.org/10.1016/j.neuropharm.2013.06.024.
[81] Y. Zhang, P. Smolen, C.M. Alberini, D.A. Baxter, J.H. Byrne, Computational model of a positive BDNF feedback loop in hippocampal neurons following inhibitory avoidance training, Learn. Mem. 23 (12) (2016) 714–722, https://doi.org/10.1101/lm.042044.116.
[82] C.L. Meireles, K. Bertoldi, L.R. Cechinel, B.L. Schallenberger, V.K. da Silva, N. Schröder, et al., Treadmill exercise induces selective changes in hippocampal histone acetylation during the aging process in rats, Neurosci. Lett. 634 (2016) 19–24, https://doi.org/10.1016/j.neulet.2016.10.008.
[83] S. Peleg, F. Sananbenesi, A. Zovoilis, S. Burkhardt, S. Bahari-javan, R.C. Agis-Balboa, et al., Altered histone acetylation is associated with age-dependent memory impairment in mice, Science 328 (5979) (2010) 753–756, https://doi.org/10.1126/science.1186088.
[84] T. Barichello, J.S. Generoso, L.R. Simões, C.J. Faller, R.A. Ceretta, F. Petronilho, et al., Sodium butyrate prevents memory impairment by Re-establishing BDNF and GDNF expression in experimental pneumococcal meningitis, Mol. Neurobiol. 52 (1) (2015) 734–740, https://doi.org/10.1007/s12035-014-8914-3.
[85] F.S. Petry, A.S. Dornelles, M. Lichtenfels, F.E. Valiati, C. Brunetto, D. Farias, et al., Histone deacetylase inhibition prevents the impairing effects of hippocampal gastrin-releasing peptide receptor antagonism on memory consolidation and extinction, Behav. Brain Res. 307 (2016) 46–53, https://doi.org/10.1016/j.bbr.2016.03.041.
[86] J.M. Levenson, K.J. O'Riordan, K.D. Brown, M.A. Trinh, D.L. Molfese, J.D. Sweatt, Regulation of histone acetylation during memory formation in the hippocampus, J. Biol. Chem. 279 (39) (2004) 40545–40559, https://doi.org/10.1074/jbc.M402229200.
[87] C.G. Vecsey, J.D. Hawk, K.M. Lattal, J.M. Stein, S.A. Fabian, M.A. Attner, et al., Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation, J. Neurosci. 27 (2007) 6128–6140, https://doi.org/10.1523/JNEUROSCI.0296-07.2007.
[88] D.P. Stefanko, R.M. Barrett, A.R. Ly, G.K. Reolon, M.A. Wood, Modulation of long-term memory for object recognition via HDAC inhibition, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 9447–9452, https://doi.org/10.1073/pnas.0903964106.
[89] D. Liu, H. Qiu, H. Fei, X. Hu, H. Xia, L. Wang, et al., Histone acetylation and expression of mono-aminergic transmitters synthetases involved in CUS-induced depressive rats, Exp. Biol. Med. 39 (3) (2014) 330–336, https://doi.org/10.1177/1535370213513987.
[90] E. Tronci, F. Napolitano, A. Muñoz, C. Fidalgo, F. Rossi, A. Björklund, et al., BDNF over-expression induces striatal serotonin fiber sprouting and increases the susceptibility to l -DOPA-induced dyskinesia in 6-OHDA-lesioned rats, Exp. Neurol. 297 (2017) 73–81, https://doi.org/10.1016/j.expneurol.2017.07.017.
[91] D.G. Kabra, K. Pfuhlmann, C. García-Cáceres, S.C. Schriever, V. Casquero García, A.F. Kebede, et al., Hypothalamic leptin action is mediated by histone deacetylase 5, Nat. Commun. 7 (2016) 10782, https://doi.org/10.1038/ncomms1078.
[92] A.M. Stankiewicz, A.H. Swiergiel, P. Lisowskic, Epigenetics of stress adaptations in the brain, Brain Res. Bull. 98 (2013) 76–92, https://doi.org/10.1016/j.brainresbull.2013.07.003.
[93] F. Duclot, M. Kabbaj, Epigenetic mechanisms underlying the role of brain-derived neurotrophic factor in depression and response to antidepressants, J. Exp. Biol. 218 (2015) 21–31, https://doi.org/10.1242/jeb.10708.
[94] W.N. Morris, S. Worchel, J.L. Bois, J.A. Pearson, C. Alan, G.M. Samaha, et al., Collective coping with stress: group reactions to fear, anxiety, and ambiguity, J. Pers. Soc. Psychol. 33 (1976) 674–679.