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The role of sociability in social instability stress: Behavioral, neuroendocrine and monoaminergic effects

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ABSTRACT

Extensive literature has reported a link between social stress and mental health. In this complex relationship, individual strategies for coping with social stress are thought to have a possible modulating effect, with sociability being a key factor. Despite the higher incidence of affective disorders in females and sex-related neurochemical differences, female populations have been understudied. The aim of the present study was, therefore, to analyze the behavioral, neuroendocrine, and neurochemical effects of stress in female OF1 mice, paying special attention to social connectedness (female mice with high vs low sociability). To this end, subjects were exposed to the Chronic Social Instability Stress (CSIS) model for four weeks. Although female mice exposed to CSIS had increased arousal, there was no evidence of depressive-like behavior. Neither did exposure to CSIS affect corticosterone levels, although it did increase the MR/GR ratio by decreasing GR expression. Female mice exposed to CSIS had higher noradrenaline and dopamine levels in the hippocampus and striatum respectively, with a lower monoaminergic turnover, resulting in an increased arousal. CSIS increased serotonin levels in both the hippocampus and striatum. Similarly, CSIS was found to reduce kynurenic acid, 3-HK, and IDO and iNOS enzyme levels in the hippocampus. Interestingly, the observed decrease in IDO synthesis and the increased serotonin and dopamine levels in the striatum were only found in subjects with high sociability. These highly sociable female mice also had significantly lower levels of noradrenaline in the striatum after CSIS application. Overall, our model has produced neuroendocrine and neurochemical but not behavioral changes, so it has not allowed us to study sociability in depth. Therefore, a model that induces both molecular and behavioral phenotypes should be applied to determine the role of sociability.

1. Introduction

Epidemiological studies have shown that chronic stress is a risk factor for physical and mental health, both in early developmental stages and in adulthood [1]. The most common type of chronic stress in humans and other social animals is generated by socially demanding situations [2,3]. In addition to stress, several studies have also highlighted the influence of coping strategies and personality on physical and mental health [4].

Two main systems have been found to mediate most of the stress response mechanisms: the sympathetic-adrenomedullary (SAM) axis and the hypothalamic-pituitary-adrenal (HPA) axis. In a situation identified as dangerous, the sympathetic nervous system (SNS) releases noradrenaline (NA), which stimulates the adrenal glands to release adrenaline into the bloodstream. For its part, the activation of the HPA axis causes glucocorticoids (cortisol or corticosterone) to be secreted into the blood, an action that modifies the gene expression of virtually every cell and prepares the body for fight or flight. Following activation of the HPA axis, hypothalamic glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) are critical in the negative feedback

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process that inhibits further glucocorticoid release. Several studies in humans have demonstrated the association between chronic stress and high levels of NA and glucocorticoids [5,6]. These alterations in the activity of the SAM and HPA axes are associated with numerous deleterious psychological and physical health outcomes, including mood disorders [7,8].

Depression is a mood disorder characterized by an intrusive and persistent mood accompanied by low self-esteem and a loss of interest or pleasure (anhedonia). It is one of the most prevalent illnesses in the world, with a consistent gender pattern: women are more likely to suffer from depression, have more severe symptoms, and a poorer clinical outcome [9,10]. Of all stressors, chronic social stress is the one most strongly associated with depression [11–13]. In the same way, depression is associated with social risk factors, social impairment, and poor social functioning [14]. The clinical and etiological heterogeneity of major depressive disorder has made it difficult to elucidate its pathophysiology. Nevertheless, current neurobiological theories point out HPA axis and monoamine alterations, among others [15]. Increasing cortisol secretion and the activity of the sympathetic system induces changes in the immune response [16], which in turn stimulates the release of the enzyme indoleamine-2,3-dioxygenase (IDO) from macrophages. This enzyme degrades tryptophan, the amino acid precursor of serotonin (5-HT), and increases kynurenine levels and/or the kynurenine to tryptophan ratio, which could explain low 5-HT levels in the brain during depressive stages [17]. On the other hand, monoaminergic synthesis may also be compromised by the sequestration of its essential synthesis cofactor, tetrahydrobiopterin (BH4) by the inducible NOS (iNOS), which is also activated by proinflammatory cytokines. In this regard, chronic stress associated with depression is not only associated with altered 5HT neurotransmission [18], but with activation of the kynurenine [19] and/or BH4 pathways [20].

Although epidemiological studies have clearly shown that females are more vulnerable than males to stress-related psychopathologies, preclinical research is still mainly conducted with male animals. Consequently, the lack of preclinical research carried out with females may explain the poorer outcomes of treatments among subjects of this sex [21]. Given that the main source of the stress contributing to the development of mood disorders in humans is social in nature [8], animal models based on social stressors may be the most appropriate, as they represent situations that individuals may face in their daily lives. However, it is necessary to design and conduct experiments that take the ethology (natural behaviors) of the species into consideration, in order to obtain results that can eventually be successfully translated to the clinic [22]. Many experiments have, in the past, been based on the stress generated by agonistic interactions prompted by territorial aggression or dominance, or in other words, the social defeat model [23–25]. However, this model is not suitable for inducing chronic social stress in OF-1 females, as subjects from this population do not display territorial aggression [26]. Consequently, our knowledge of the specific mediators involved in the possible negative effects of social stress in females is very limited. In light of females' social nature, the chronic social instability stress (CSIS) model may be more appropriate and have greater ethological validity for this population. However, the results are not always consistent when applied to female mice [27]. Some authors [26,28,29] have reported an association between the CSIS model and depressive-like behavioral changes, although others have failed to find any such connection [30–32], a discrepancy that may indicate individual differences in intrinsic sociability. In light of this, the aim of the present study was to analyze the role of inherent sociability in behavioral and neurochemical responses to chronic social instability stress among female mice using our previous paradigm [31].

2. Material and methods

2.1. Subjects and husbandry

Eighty-one OF1 outbred female mice (Janvier Labs, France) were purchased at age 8 weeks. They were housed in groups of three in transparent plastic cages (24.5 × 24.5 × 15 cm), with black poplar/aspen shavings as litter bedding, two sheets of tissue as nesting material, and a sheet of cardboard as enrichment. Animals were provided with ad libitum access to water and food. The room was kept at a temperature of between 22°C and 24 °C, with a relative humidity level of 70% and a reversed 12-h light/dark cycle (white lights on from 20:00–08:00 h), including 20 min of progressively increasing light (dawn, 07:40–08:00 h) and 20 min of progressively decreasing light (dusk, 19:40–20:00 h). All procedures involving mice were performed in accordance with that established in the European Directive (2010/63/EU) and were approved by the Animal Welfare Ethics Committee of the University of the Basque Country (CEEA-UPV/EHU; M20/2018/090) and the Gipuzkoa Provincial Council (PRO-AE-SS-062).

2.2. Experimental procedure

After the 10-day acclimation period (day -1), animals were housed in groups of three (non-stressed animals, $n = 33$) or four (stressed animals, $n = 48$). Then, the Social Interaction Test (SIT) was performed to classify subjects into the high-sociability and low-sociability groups. Since the aim was to study the inherent social interaction of the animals, we analyzed this parameter according to the number of animals they would be living with during the experiment; non-stressed 3 and stressed 4. On day 0, the mice were divided into four experimental groups: Non-Stressed/Low-Sociability (NS/LS) ($n = 13$), Non-Stressed/High-Sociability (NS/HS) ($n = 20$), Stressed/Low-Sociability (S/LS) ($n = 20$), and Stressed/High-Sociability (S/HS) ($n = 28$). The S groups were subjected to the Chronic Social Instability Stress (CSIS) model for 28 days, whereas the NS mice remained in the same housing conditions as during the adaptation period (3 animals per cage). Behavioral assessment of all animals ($n = 81$) commenced once the CSIS period had ended, with the Sucrose Preference Test (SPT), the Open Field Test (OFT), and the Novel Object Recognition Test (NORT) being carried out between days 28 and 30. On day 31, blood samples of all animals were collected by a submandibular puncture to determine plasma corticosterone levels. All animals were then sacrificed by cervical dislocation. The brain was removed and the whole hypothalamus, hippocampi, and striata were dissected under sterile conditions and stored at −80 °C for biological determinations (Fig. 1).

2.3. Stress procedure

In order to increase unpredictability, this model was modified from Labaka et al. [26], applying seven variable periods of four days of isolation and overcrowding as previously described [31]. Thus, the S groups (both HS and LS] were exposed to the CSIS model over a 28-day period (Fig. 1). The mice were subjected to a highly unstable and unpredictable social situation, with alternating phases of isolation (1, 2, or 3 days) and crowding (4 per cage, during 1, 2, or 3 days). During each crowding phase, we ensured that four different mice that had no previous contact were placed together in a new clean cage. Meanwhile, control mice (NS) were housed in stable groups of three.

2.4. Behavioral assessment

Each animal's movements and behavior were recorded with an overhead video camera (GZ-MG773; JVC, Yokohama, Japan) for subsequent assessment using the ANY-maze© computerized version 4.96 video-tracking software program (Stoelting Europe, Dublin, Ireland). Behavioral assessment was performed during the dark phase (the

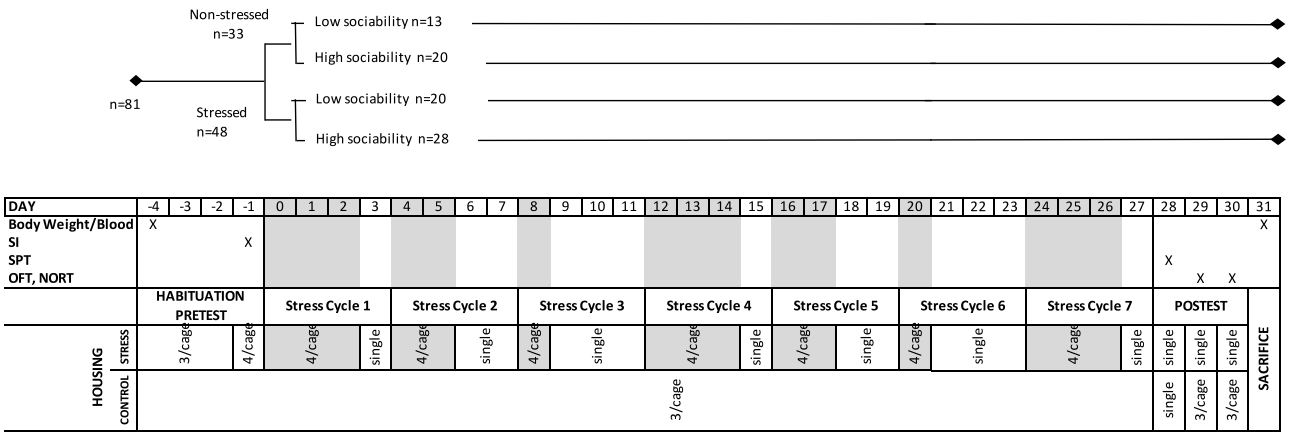

Fig. 1. Experimental procedure.

animals' active period) at 09:00 h and at 12:00 h (darkness under dim red lighting). All behavioral tests were performed in opaque plexiglass open-field box arenas (plexiglass boxes; 40 × 40 × 30 cm), except for the SPT and the SIT, which were conducted in the transparent plastic cages in which the mice were usually housed. Objects were cleaned between trials with a solution of 0.5% acetic acid.

2.4.1. Social interaction test (SIT)

On day -1, the mice were recorded in their home cages for 5 min. The behaviors assessed were: the time each subject spent attempting to maintain social contact with another mouse (interaction emitted), the time other subjects spent attempting to maintain social contact with the subject under study (interaction received), the time spent in non-social exploration and the time spent immobile [31]. Mice that emitted and received more social interaction and spent less time exploring non-social zones were categorized as High Sociability (HS), whereas those that emitted and received fewer social interaction behaviors and spent more time exploring their environment were assigned to the Low Sociability (LS) group. A multivariate discriminant analysis was performed (Wilk's Lambda method with step entry) to confirm the integrity of the groups derived from the cluster analysis and to determine which behavioral variables most efficiently discriminated between the clusters. In the NS group, the multivariate analysis carried out accounted for 100% of the cases obtained by the cluster solution, thereby confirming the statistical validity of the established groups. Social interaction emitted and received and non-social exploration were the variables that best discriminated between clusters ($p < 0.001$), followed by time spent immobile ($p = 0.024$). Regarding the S group, the discriminant model applied accounted for 89.6% of the cases obtained by the cluster solution, thereby confirming the statistical validity of these groups, as well as their behavioral description. Social interaction emitted and non-social exploration were the variables that best discriminated between clusters ($p < 0.001$), followed by social interaction received ($p = 0.002$). However, immobility made no significant contribution to the cluster differentiation ($p = 0.372$).

2.4.2. Sucrose preference test (SPT)

On day 28, all mice were individually housed and water bottles from the cages were removed. In their place, the mice were offered a free choice between two new bottles (both with a volume of 50 ml and identical in appearance to the mice's regular water bottles, to avoid the novelty factor) for a period of 24 h. One of the new bottles contained a 1% sucrose solution, and the other one contained water. The animals were not deprived of food or water before the test and the position of the bottles was counterbalanced to prevent the possible effect of a side preference. The consumption of the sucrose solution and water was measured by weighing the bottles at the beginning and at the end of the test. Each value was then divided by the mouse's body weight to calculate the relative intake. The sucrose preference discrimination index was calculated as follows: (sucrose consumption-water consumption)/total consumption * 100.

2.4.3. Open field test (OFT)

On day 29, the OFT was performed to assess behavioral responses, such as anxiety-like behaviors and mice locomotion. Animals were placed in the arena and were allowed to explore for 5 min [33]. Time spent in the center and time spent in the peripheral zone (area along the walls) of the arena were analyzed, along with locomotor activity (distance traveled).

2.4.4. Novel object recognition test (NORT)

On day 29, 1 hour after the OFT, NORT training was performed. The mice were placed in the open-field arena with 2 identical plastic caps, each measuring 4 cm in diameter. The caps served as familiar objects (F and F). The mice were allowed to explore the arena for 10 min, after which they were returned to their home cage. On day 30, the mice were returned to the same arena, but this time, one of the familiar objects (F) was replaced with a steel triangle that constituted the novel object (N). The total time spent exploring both objects was recorded over a 5 min period. Recognition memory was measured in terms of discrimination index: (time with N - time with F)/(time with N + time with F). Time spent in the F and N areas was also analyzed.

2.5. Physiological determinations

2.5.1. Sample collection

On day 31, blood samples were obtained by facial vein puncture with a lancet. Samples (15 μL) were collected using lithium heparin tubes (BD Microtainer®) between 09:00 h and 10:00 h. Samples were then centrifuged at 1800 g for 15 min at 4°C, and the resulting plasma was collected and stored in cryotubes (ClearLine®) (2 mL) at −80°C. Subsequently, the animals were sacrificed by cervical dislocation. The brain was removed and the hypothalamus, hippocampi, and striata were dissected under sterile conditions and stored at −80 °C.

2.5.2. Determination of plasma corticosterone concentrations

Plasma corticosterone levels were quantified using an enzyme immunoassay (Corticosterone Elisa Kit, ADI-900–097, Enzo Life Sciences), in accordance with the manufacturer's recommendations, and a Synergy HT microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). Data were analyzed by means of a 4-parameter logistic curve fit using MyAssays (Data Analysis Tools and Services for Bioassays; available at https://www.myassays.com/). The sensitivity of the assay was 27.0 pg/ml, and the intra and inter-assay variation coefficients were

between 7% and 8%.

2.5.3. Real-time RT−PCR measurements of mRNA expression

The total RNA of each structure was isolated using the NucleoSpin RNA Plus kit (Macherey Nagel, Germany). A spectrophotometric analysis was performed at 260 nm to determine RNA concentrations, while the 260:280 absorbance ratio was utilized to assess nucleic acid purity (Synergy HT, BioTek Instruments, Inc., Winooski, VT, USA). The total RNA was then reverse-transcribed using the PrimeScript RT reagent kit (Takara Bio Inc., Madrid, Spain). The resulting cDNA was quantified by SYBR Green-based (SYBR®Premix Ex TaqTM, Takara Bio Inc., Madrid, Spain) real-time PCR, and the formation of PCR products was monitored using the 7500 Real-Time PCR System (Applied Biosystems, Madrid, Spain). The cDNA sequences were obtained from GenBank at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). Both hypoxanthine phosphoribosyl transferase (HPRT) and glyceraldehyde-6-phosphate dehydrogenase (GAPDH) were used as reference genes. Primer sequences were designed using Primer Express Software v3.0 (Applied Biosystems, Madrid, Spain) and obtained from Applied Biosystems (Appendix Table A.1). The relative gene expression was determined using the $2^{-D\Delta t}$ method [34]).

2.5.4. Determination of monoamines and their metabolites by high-performance liquid chromatography (HPLC)

Brain structures were weighed and homogenized in a 60 μl solution (1% formic acid in acetonitrile). Zirconia Ceramic Balls (0.5 and 1.0 mm) were inserted into the sample tubes and then placed in the Bullet Blender for 3 min to bust the brain tissue. Immediately afterwards, the tubes were vortexed for 5 min (Vortex Genie-2; Scientific Industries, Bohemia, NY, USA) and, subsequently, the samples were centrifuged for 15 min at 15,000 × g and 4°C. The supernatants were dried for 30 min with compressed air to concentrate the samples and were then reconstituted with 30 μl of 0.05% trifluoroacetic acid. Next, the samples were again centrifuged for 20 min at 15,000 × g and 4°C and placed in the autosampler unit for analysis.

L-Phenylalanine (Phe), L-Tyrosine (Tyr), dopamine (DA), 3,4-Dihydroxyphenylacetic Acid (DOPAC), noradrenaline (NA), 3-methoxy-4-hydroxyphenylglycol (MHPG), Tryptophan (Tryp), kynurenine (Kyn), Kynurenic Acid (Kyna), 3-Hydroxykynurenine (3-HK), serotonin (5-HT), and 5-Hydroxyindoleacetic Acid (5-HIAA) were determined using 20 μL of each sample injected into the HPLC (Hewlett Packard 1100 System). The samples were separated on a Poroshell 120 EC—C18 column (100 × 4.6 mm, 2.7 μm), with an Analytical Guard Column (12.5 × 4.6 mm, 5 μm) being used for protection (Agilent Technologies). The mobile phase for this study comprised 0.05% trifluoroacetic acid (solvent A) and 99.9% acetonitrile (solvent B). The flow was maintained at a constant rate of 0.5 ml/min. The column was maintained at 25°C during the analysis, and the samples were maintained at 4°C in an autosampler unit. The samples were analyzed and monitored either by a fluorescence detector (FLD) at an emission wavelength of 320 nm or by a variable wavelength detector (VWD). The Phe (Excitation wavelength (Ex) 212 nm), NA, Tyr-and 5-HT (Excitation wavelength (Ex) 229 nm), and DA, MHPG and 5-HIAA (Excitation wavelength (Ex) 283 nm) effluents were monitored with the fluorescence detector at an Emission wavelength of 320 nm. The 3-HK, Kyn, DOPAC, Kyna, and Tryp effluent was monitored with a variable wavelength detector set at 230 nm. The total sample analysis time was 27 min. The final data were expressed as ng/ml.

2.6. Statistical analysis

A cluster analysis (Low or High Sociability) was performed using the SPSS 28.0 for Windows software package (SPSS Inc., Chicago, IL, USA). Statistical analyses of the behavioral and physiological variables and graphic visualization and design were performed using GraphPad Prism software (9.0, GraphPad Software, Inc). Study variables were first transformed into Z-scores and outlier values were adjusted to the median in accordance with the boxplot outlier labeling rule. Variables were analyzed using 2-way ANOVAs, the factors were stress and sociability. Specific comparisons between the NS/LS and S/LS groups, and between the NS/HS and S/HS groups were carried out using a post hoc Tukey test. Cohen's d test for effect size was performed to estimate the strength of the effects between two groups ("d" values > 0.8 are considered indicative of large effects, values of between 0.5 and 0.8 are considered indicative of moderate effects, and values < 0.5 are considered to indicate small effects). Values of $p < 0.05$ were considered statistically significant (95% confidence). Data are expressed as mean ± standard error (SEM). Only significant differences between groups are presented in the results section and small effect sizes are not considered.

3. Results

3.1. Behavioral assessment

No differences were found in either the SPT or the NORT (Fig. 2a, b). In contrast, in the OFT, stressed mice were observed to travel a greater distance ($F_{(3,77)} = 8.741$; $p = 0.004$; $\eta2 = 0.102$) than their non-stressed counterparts (Fig. 2c). No differences were observed in the time spent in periphery or in the center (Fig. 2d).

3.2. Biological assessment

3.2.1. Neuroendocrine effects

Overall, HS mice had higher corticosterone plasma levels than their LS counterparts ($F_{(3,76)} = 6424$; $p = 0.013$; $\eta2 = 0.078$) (Fig. 3a). Hypothalamic GR mRNA relative gene expression was lower in stressed than in non-stressed animals ($F_{(3,75)} = 23.49$; $p < 0.001$; $\eta2 = 0.24$) and the post hoc analysis revealed differences between the NS/HS and the S/HS groups ($p < 0.001$; $d = 1.20$; Fig. 3b). Similarly, MR mRNA

Table A1
PCR Primer specification.

Gene	Function	Primer sequences (5′−3′)	Analyzed structure	Gen Bank accession No.
GR	Glucocorticoid receptor	F: CCCATGGAGGTAGCGATTGT R: TGTAAAGGCTGCCCAATGTGT	Hypothalamus Hippocampus	DQ504162.1
MR	Mineralocorticoid receptor	F: ACCTGCAGAGAGGACCAATGA R: GGAGTAATTCGTGTTTTTCTTTGCT	Hypothalamus Hippocampus	AJ311855.1
IDO	Rate-limiting enzyme of tryptophan catabolism	F: AAAGCAATCCCCACTGTATCCA R: TGCCTTTTCCAATGCTTTCAG	Hippocampus Striatum	BC049931.1
iNOS	Inducible nitric oxide synthase	F: GGATCTTCCCAGGCAACCA R: CAATCCACAACTCGCTCCAA	Hippocampus Striatum	NM_010927.4
GAPDH	Catalyzing enzyme of glycolysis	F: CGGCCGCATCTTCTTGTG R: GTGACCAGGCGCCCAATAC	Hypothalamus Hippocampus Striatum	NM_001289726.1
HPRT	Catalyzing transferase of hypoxanthine	F: TGGGAGGCCATCACATTCT R: TCCAGCAGGTCAGCAAAAGAAC	Hypothalamus Hippocampus Striatum	NM _013556.2

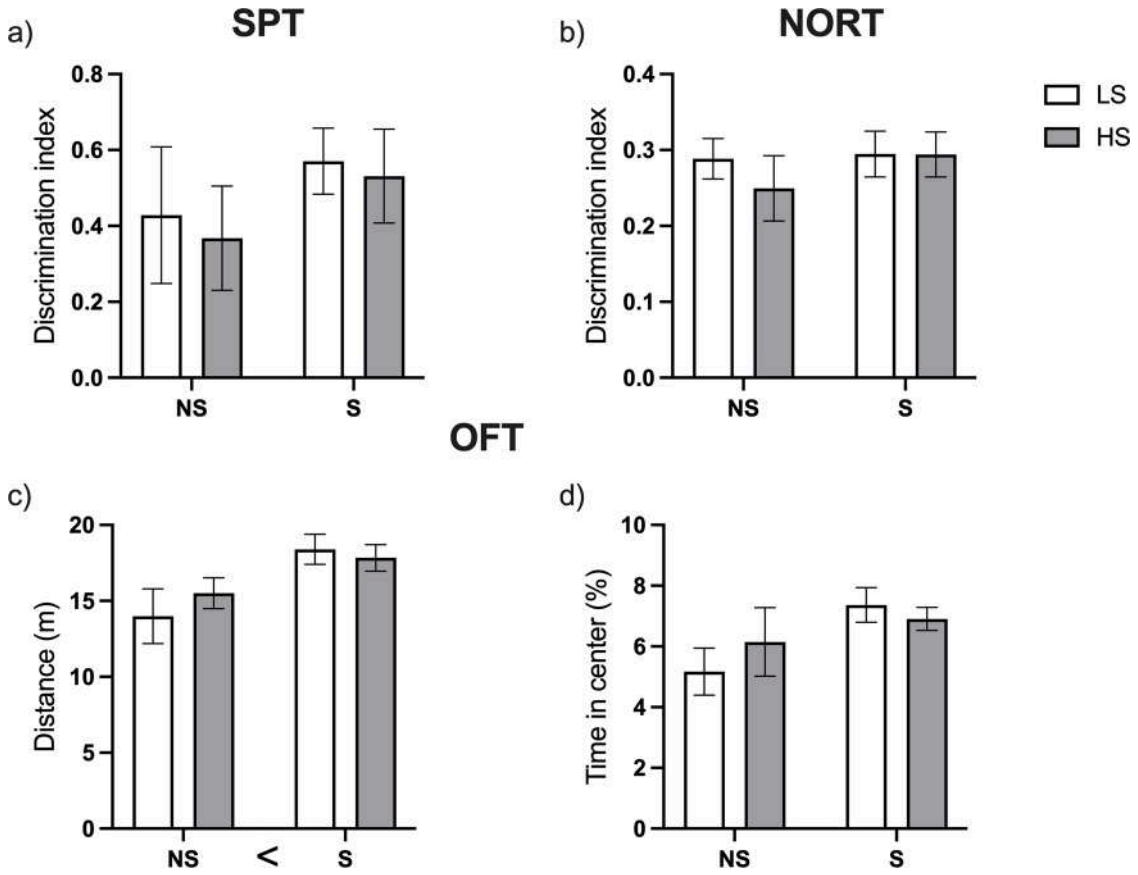

Fig. 2. a) SPT and b) NORT discrimination indexes, c) distance traveled, and d) the percentage of time spent in the center during the OFT. Stress factor significance is expressed as < or > according to directionality. Data are expressed as mean ±*S*.E.M.

expression was lower in stressed than in non-stressed animals ($F_{(3,75)}$ = 5.119; p = 0.027; η2 = 0.064), although in this case, the post hoc analysis did not reveal any differences between groups (Fig. 3c). The MR/GR ratio was higher in stressed than in non-stressed animals ($F_{(3,75)}$ = 7.917; p = 0.006; η2 =0.095), and the post hoc analysis revealed differences between the NS/HS and the S/HS groups ($p < 0.01$; $d = 1.04$; Fig. 3d). As in the hypothalamus, hippocampal GR mRNA relative gene expression was lower in stressed than in non-stressed animals ($F_{(3,75)}$ = 27.85; $p < 0.001$; η2 = 0.27) and the post hoc analysis revealed differences between the NS/LS and S/LS groups ($p < 0.01$; $d = 1.19$) and NS/HS and the S/HS groups ($p < 0.001$; d = 1.18; Fig. 3e). MR mRNA expression was higher in HS animals compared to LS ($F_{(3,75)} = 4.359$; p = 0.04; η2 = 0.06), although, the post hoc analysis did not reveal any differences between groups (Fig. 3f). Finally, hippocampal MR/GR ratio was higher in stressed than in non-stressed animals ($F_{(3,75)} = 14.10$; $p <$ 0.001; η2 = 0.16), and the post hoc analysis revealed differences between the NS/LS and the S/LS groups ($p < 0.05$; $d = 1.07$; Fig. 3g).

3.2.2. *Hippocampal and striatal monoamines and their metabolite levels*

3.2.2.1. Catecholamine levels in the hippocampus. Regarding catecholamines, the levels of both precursors, Phe ($F_{(3,72)} = 12.03$; $p < 0.001$;; η2 = 0.143; Fig. 4a) and Tyr ($F_{(3,73)}$ = 5.972; p = 0.017; η2 = 0.076; Fig. 4b), were significantly lower in stressed subjects than in controls. The post hoc analysis revealed that S/LS subjects had lower Phe-levels than their counterparts in the NS/LS group (p = 0.05; d = 1.00). No differences were observed in DA (Fig. 4c) or DOPAC levels (Fig. 4d), although the DOPAC/DA ratio was lower in the stressed group (4667.752 ± 742.232) than in the non-stressed one (16,388.747 ± 4320.515) ($F_{(3,69)} = 9.315$ $p = 0.006$; η2 = 0.119). Furthermore, stressed animals were found to have higher NA levels ($F_{(3,72)} = 8.165$; $p = 0.006$; η2 = 0.102; Fig. 4e) and lower MHPG levels ($F_{(3,72)} = 9.086$; $p = 0.004$; η2 = 0.112; Fig. 4f) than the non-stressed ones. Stressed mice (14.519 ± 1.938) also presented lower MHPG/NA ratio ($F_{(3,72)} = 6.784$; $p = 0.011$; η2 = 0.086) compared with non-stressed subjects (144.562 ± 71.441).

3.2.2.2. Catecholamine levels in the striatum. The statistical analyses revealed no differences in the catecholamine precursors (Fig. 5a,b), although the stressed group (1.072 ± 0.045) had a higher Tyr/Phe-ratio than the non-stressed mice (0.907 ± 0.0546) ($F_{(3,74)} = 4.805$; $p = 0.032$; η2 = 0.061). Stressed mice had higher DA levels than their non-stressed counterparts ($F_{(3,75)}$ = 11.35; p = 0.012; η2 = 0.131), and the S/HS group had significantly higher levels than NS/HS subjects ($p = 0.015$; d = 0.85; Fig. 5c). Moreover, although no differences were observed in DOPAC levels (Fig. 5d), stressed mice (7.104 ± 3.472) had a lower DOPAC/DA ratio than the non-stressed mice (283.491 ± 184.607) ($F_{(3,70)}$ = 4.248; p = 0.043; η2 = 0.057). Regarding NA, although no differences were observed in relation to the stress factor, they were observed in relation to the sociability factor ($F_{(3,74)} = 8.156$; $p = 0.006$; η2 = 0.099), with NS/LS and S/LS animals having higher NA levels than their NS/HS and S/HS counterparts ($t = 2.631$; $p = 0.013$ and $t = 2.040$; $p = 0.047$, respectively; Fig. 5e). No differences were observed in MHPG levels (Fig. 5f). Interestingly, stressed mice (32.295 ± 4.008) were observed to have a lower MHPG/NA ratio than the non-stressed mice (102.596 ± 16.882) ($F_{(3,73)} = 15.873$; $p < 0.001$; η2 = 0.179).

3.2.2.3. Indolamine levels in the hippocampus. Regarding indolamine levels, stressed animals had lower Tryp levels ($F_{(3,73)} = 13.91$; $p < 0.001$; η2 = 0.160), with differences being observed between the NS/LS and S/LS groups ($p = 0.03$; $d = 1.05$; Fig. 6a). No differences were observed in Kyn levels (Fig. 6b), although stressed animals had lower levels of Kyna ($F_{(3,71)} = 5.077$; $p = 0.027$; η2 = 0.67; Fig. 6c) and 3-HK ($F_{(3,73)} = 5.760$;

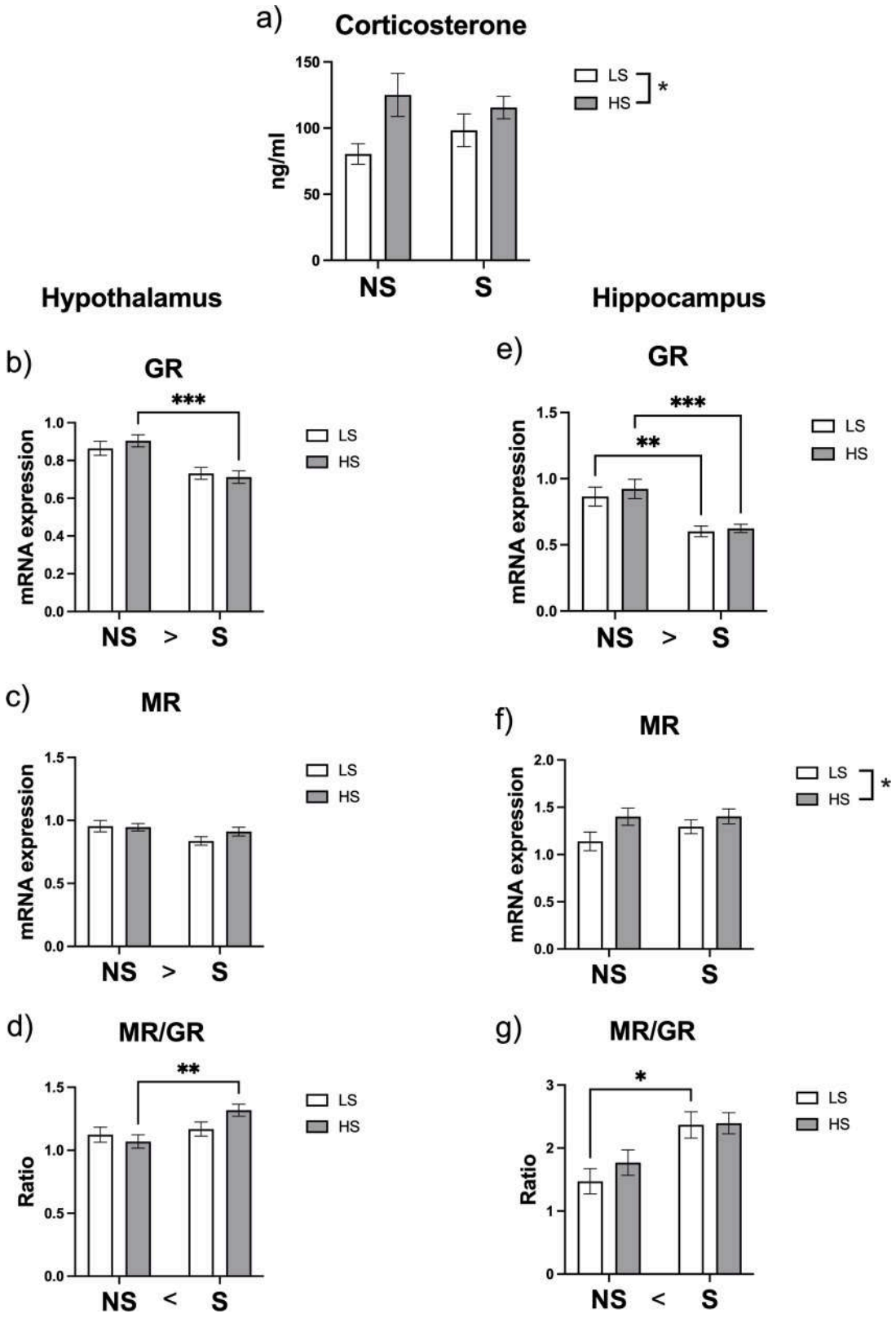

Fig. 3. a) Plasma corticosterone levels (ng/ml) at day 31. Hypothalamic b) GR and c) MR mRNA gene relative expression levels, and d) MR/ GR ratio. Hippocampal e) GR and f) MR mRNA gene relative expression levels, and g) MR/ GR ratio. Stress factor significance is expressed as < or > according to directionality. Data are expressed as mean ±*S*.E.M. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

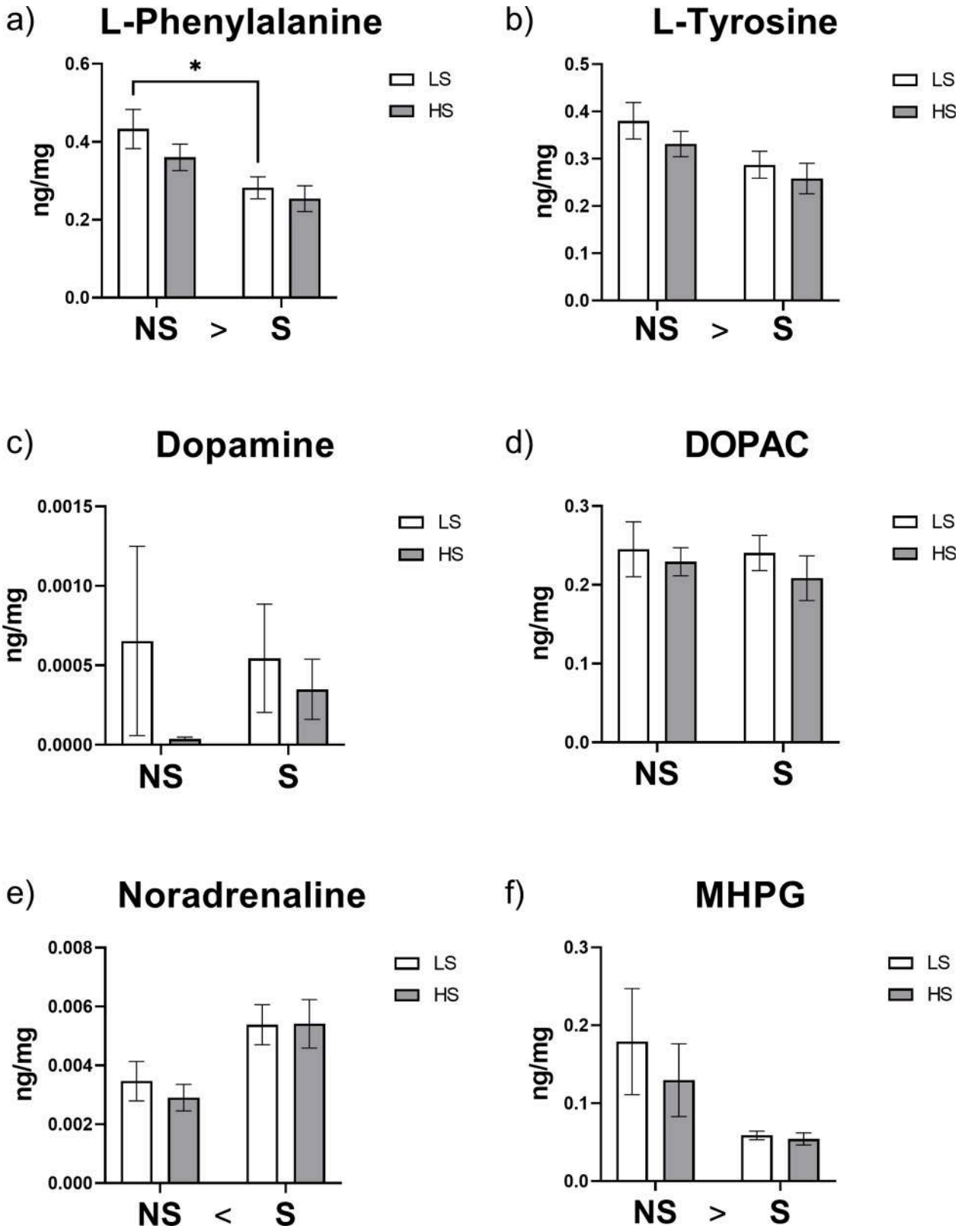

Fig. 4. Hippocampal a) L-Phenylalanine, b) L-Tyrosine, c) Dopamine, c) DOPAC, e) Noradrenaline, and f) MHPG levels expressed in ng/mg. Stress factor significance is expressed as < or > according to directionality. Data are expressed as mean ±*S*.E.M. *$p < 0.05$.

$p = 0.019$; $\eta 2 = 0.073$; Fig. 6d). They (0.010 ± 0.003) also showed a lower Kyna/3-HK ratio in comparison with non-stressed mice (0.017 ± 0.004) ($F_{(3,71)} = 3.998$; $p = 0.049$; $\eta 2 = 0.053$). Interestingly, the sociability factor was also significant for 3-HK ($F_{(3,73)} = 4.044$; $p = 0.048$; $\eta 2 = 0.052$), with LS animals having higher expression levels than their HS counterparts. Stressed mice had higher 5-HT levels ($F_{(3,72)} = 24.602$; $p < 0.001$; $\eta 2 = 0.255$), and differences were observed between the NS/LS and S/LS groups ($p = 0.001$; $d = 1.28$) and between the NS/HS and S/

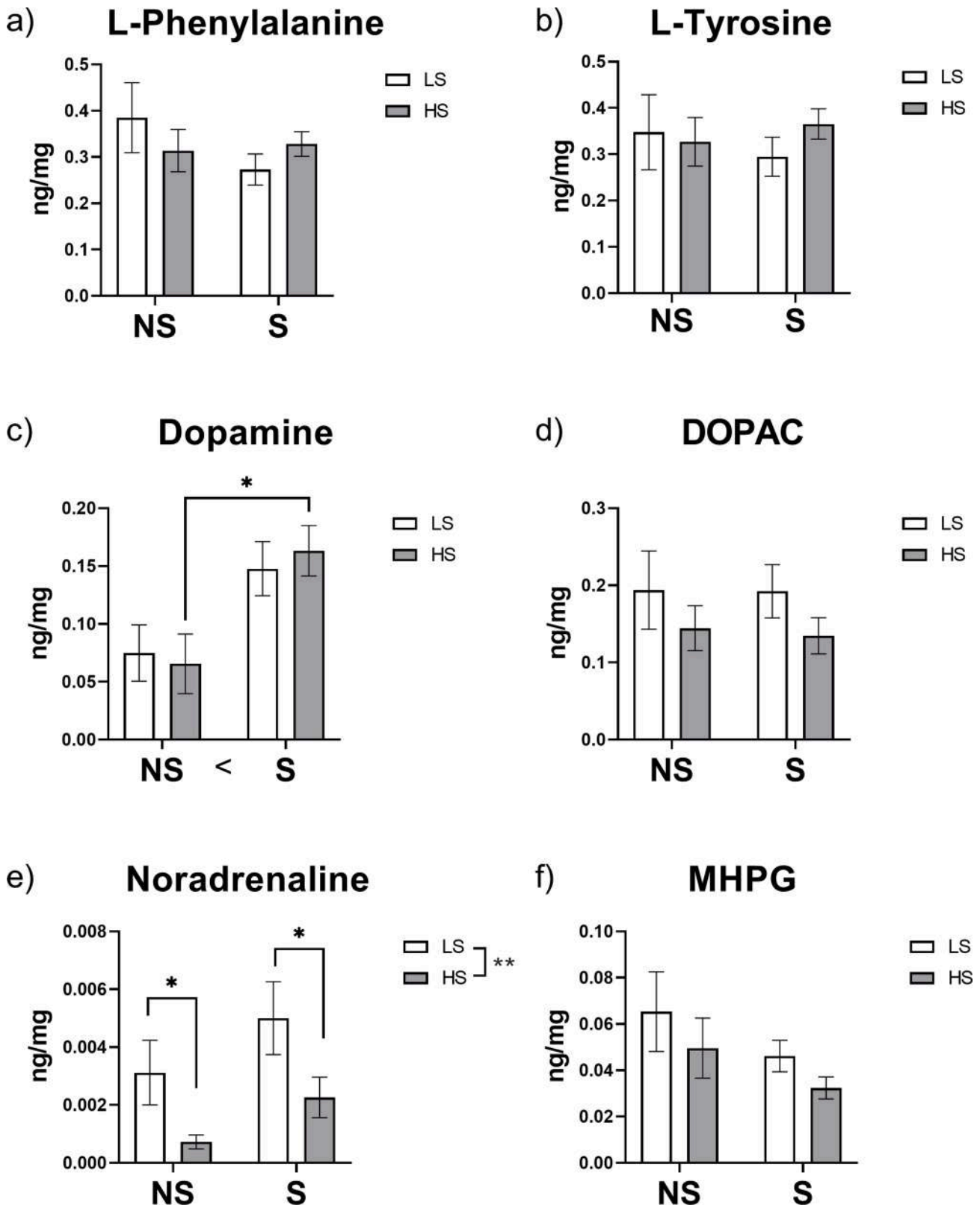

Fig. 5. Striatal a) L-Phenylalanine, b) L-Tyrosine, c) Dopamine, c) DOPAC, e) Noradrenaline, and f) MHPG levels expressed in ng/mg. Stress factor significance is expressed as < or > according to directionality. Data are expressed as mean ±*S*.E.M. *$p < 0.05$.

HS groups ($p = 0.04$; $d = 1.10$) (Fig. 6e). In contrast, stressed mice had lower 5-HIAA levels ($F_{(3,72)} = 5.692$; $p = 0.020$; $\eta 2 = 0.073$; Fig. 6f). Remarkably, stressed mice (0.232 ± 0.0417) were also found to have a higher 5-HT/Tryp ratio than non-stressed mice (0.189 ± 0.083) ($F_{(3,72)} = 6.676$; $p = 0.012$; $\eta 2 = 0.85$). Stressed group (0.228 ± 0.073) also showed a lower 5-HIAA/5H-T ratio compared with non-stressed subjects (1.338 ± 0.445) ($F_{(3,72)} = 10.009$; $p = 0.002$; $\eta 2 = 0.122$).

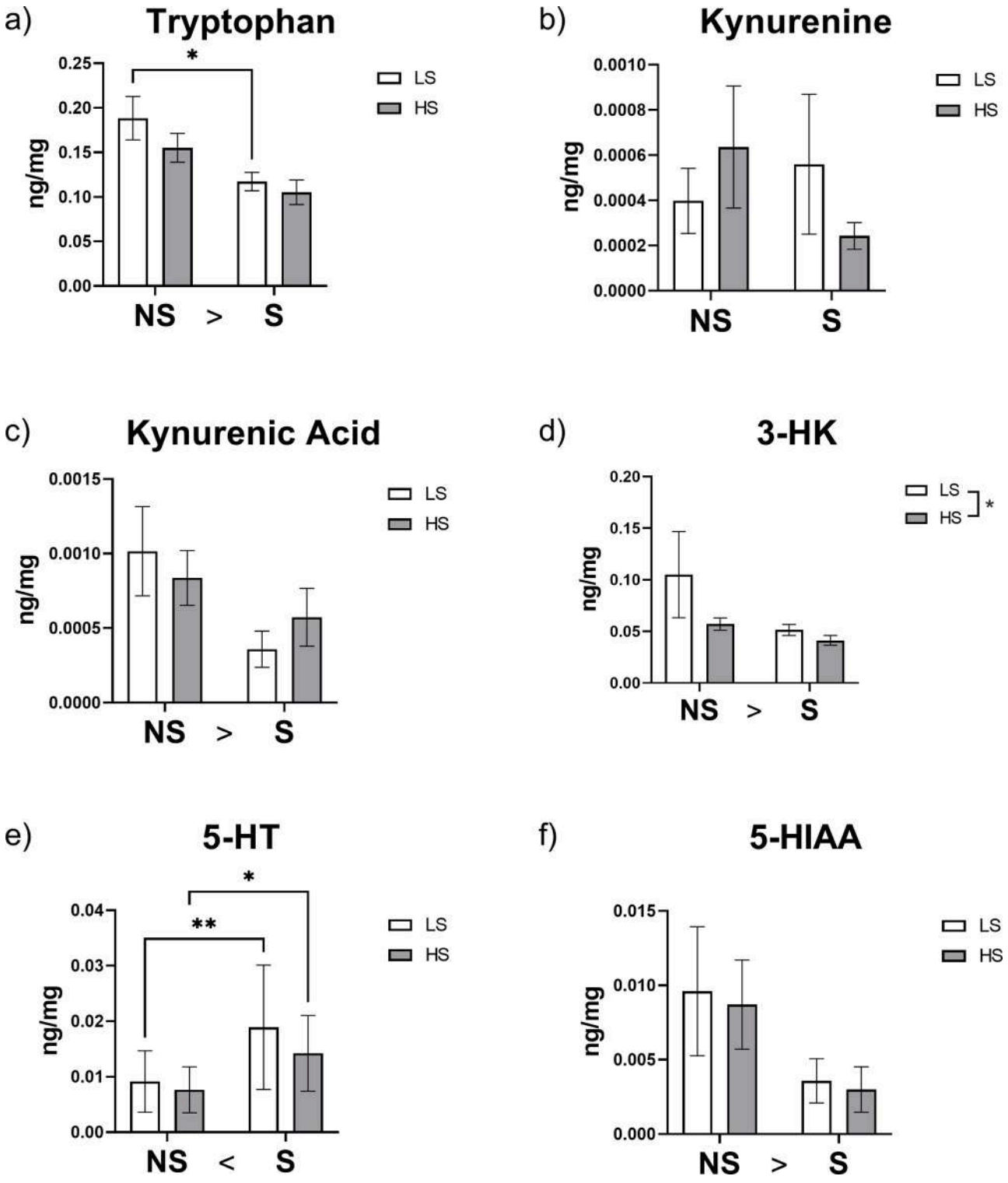

Fig. 6. Hippocampal a) Tryptophan, b), Kynurenine, c) Kynurenic Acid, d) 3-HK, e) 5-HT, and f) 5-HIAA levels expressed in ng/mg. Stress factor significance is expressed as < or > according to directionality. Data are expressed as mean ±*S*.E.M. *$p < 0.05$, and **$p < 0.01$.

3.2.2.4. Indolamine levels in the striatum. Regarding the indolamine pathway, no differences were observed in Tryp (Fig. 7a), Kyn (Fig. 7b) or Kyna levels (Fig. 7c), although stressed mice had higher 3-HK levels ($F_{(3,75)} = 8.483$; $p = 0.005$; $\eta2 = 0.105$) than the non-stressed ones. Moreover, stressed group ($0.003 \pm < 0.001$) showed a lower Kyna/3-HK ratio compared to non-stressed ones (0.007 ± 0.002) ($F_{(3,75)} = 4.139$; $p = 0.049$; $\eta2 = 0.103$; Fig. 7d). Similarly, stressed subjects had higher 5-HT levels than non-stressed mice ($F_{(3,74)} = 13.561$; $p < 0.001$; $\eta2 = 0.155$), and the post hoc analysis revealed differences between the NS/HS and the S/HS groups ($p = 0.01$; $d = 1.07$; Fig. 7e). Stressed mice (0.172 ± 0.013) also had a higher 5-HT/Tryp ratio than the non-stressed subjects (0.092 ± 0.017) ($F_{(3,72)} = 6.676$; $p = 0.012$; $\eta2 = 0.85$). In contrast, stressed mice had lower 5-HIAA levels ($F_{(3,73)} = 8.791$; $p = 0.004$; $\eta2 = 0.107$) and, interestingly, differences were also found for the stress x sociability interaction ($F_{(3,73)} = 4.839$; $p = 0.031$; $\eta2 = 0.062$). Post hoc analyses revealed significant differences between the NS/LS

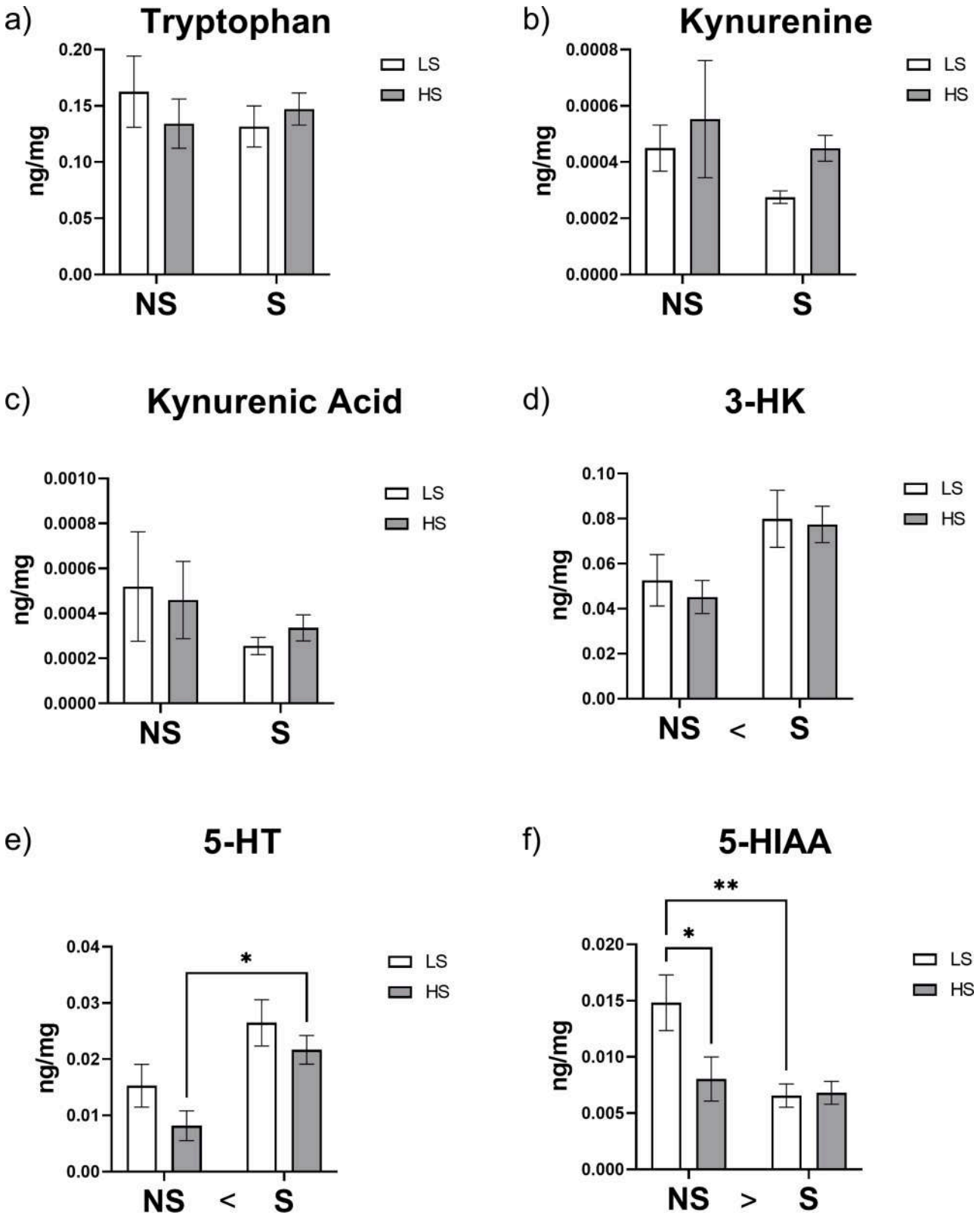

Fig. 7. Striatal a) Tryptophan, b), Kynurenine, c) Kynurenic Acid, d) 3-HK, e) 5-HT, and f) 5-HIAA levels expressed in ng/mg. Stress factor significance is expressed as < or > according to directionality. Data are expressed as mean ±*S*.E.M. *$p < 0.05$, and **$p < 0.01$.

and NS/HS groups ($p = 0.036$, $d = 0.784$), as well as between the NS/LS and S/LS groups ($p = 0.008$; $d = 1.217$; Fig. 7f). In terms of the ratio, stressed mice (0.553 ± 0.141) had lower 5-HIAA/5-HT levels than their non-stressed counterparts (2.895 ± 0.855) ($F_{(3,73)} = 6.638$; $p = 0.012$; $\eta 2 = 0.83$).

3.2.3. Hippocampal and striatal IDO and iNOS mRNA relative gene expression

Regarding the hippocampus, stressed mice had lower IDO levels than non-stressed mice ($F_{(3,76)} = 10.133$; $p = 0.002$; $\eta 2 = 0.118$), and significant differences were observed between the NS/HS and S/HS groups ($p < 0.05$; $d = 1.02$; Fig. 8a). iNOS mRNA expression was also lower in stressed mice ($F_{(3,76)} = 6653$; $p = 0.012$; $\eta 2 = 0.80$; Fig. 8b). In the striatum, no differences were observed between the NS and S groups in terms of either IDO or iNOS mRNA expression, although the sociability factor was significant for IDO mRNA expression levels, with HS mice having higher IDO levels than their LS counterparts ($F_{(3,76)} = 4.109$; $p = 0.046$; $\eta 2 = 0.051$; Fig. 8c, d).

4. Discussion

4.1. CSIS in female mice with high or low sociability: effects on behavior

The social instability stress model applied during 4 weeks did not produce the expected behavioral alterations in female mice, and no behavioral effects were observed as a function of sociability. We observed that females seem to be able to cope without inducing a depressive phenotype. These results underline the need, already pointed out by other authors [27,35], to establish a standardized CSIS protocol in order to achieve a more predictive model.

Individual differences are a fundamental aspect to consider when studying the negative effects of social stress. Although there is evidence to suggest that higher sociability levels may have a protective effect, particularly under conditions of social stress [36,37], we found no effect of either high or low sociability on any of the behavioral tests performed after the application of a chronic social instability stress model in female mice. The absence of statistically significant results may be due to the fact that none of these post-stress behavioral tests assess sociability specifically; also (and perhaps more likely), it may be that the CSIS model applied was not sufficient to elicit an allostatic load reflecting changes in behavior [31]. Although substantial evidence suggests that stressful life events predispose individuals to depression and anxiety-like behaviors [38,39], our CSIS model did not reveal anhedonia, a key index of depression-like behaviors [40]. The application of this stress paradigm has revealed positive [41] and negative [42,43] anhedonic effects, probably due to methodological (stress or anhedonia protocol, light or

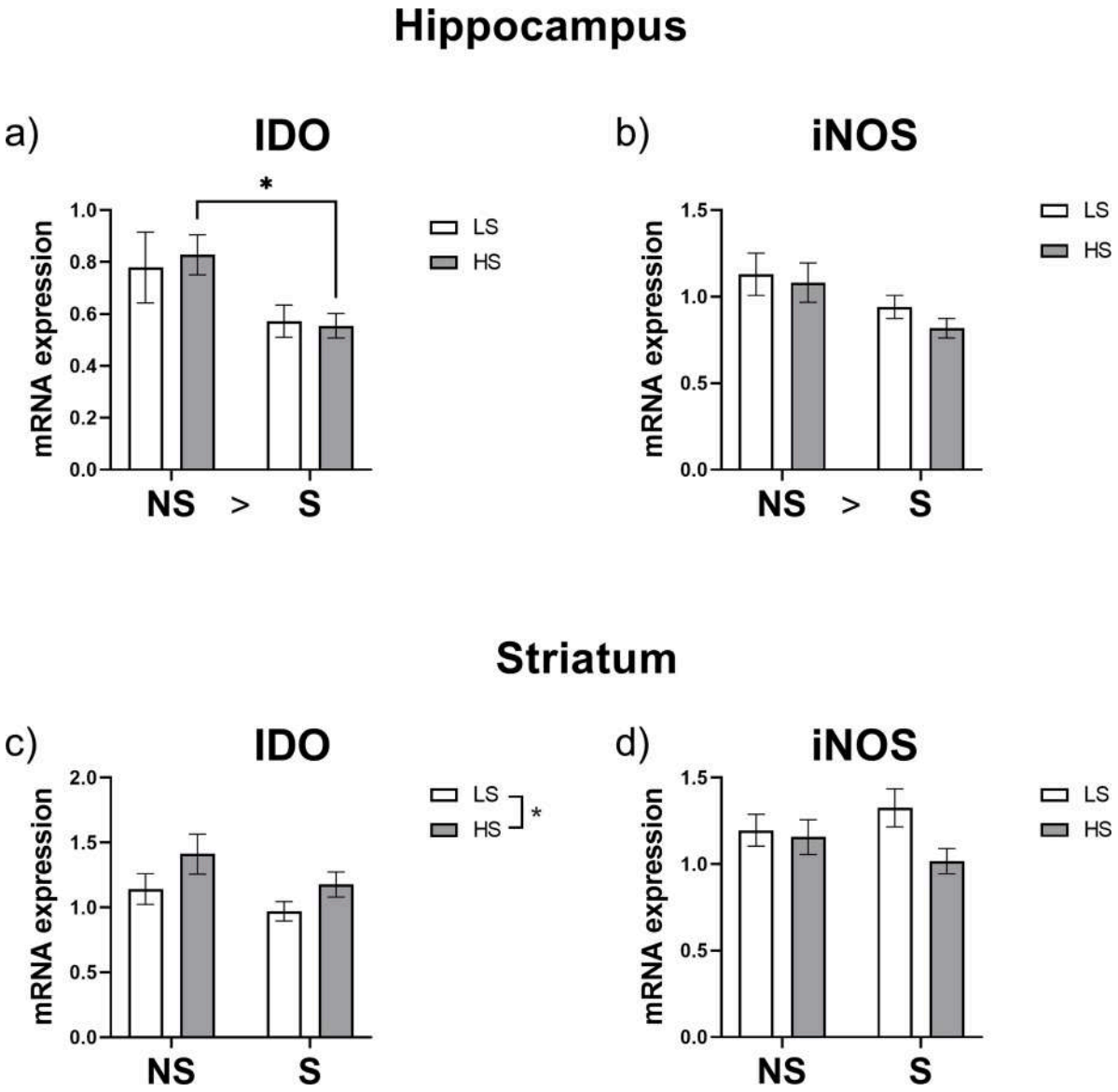

Fig. 8. a) IDO and b) iNOS mRNA expression levels in the hippocampus, and c) IDO and d) iNOS mRNA expression levels in the striatum. Stress factor significance is expressed as < or > according to directionality. Data are expressed as mean ±*S*.E.M. *$p < 0.05$.

dark phase behavioral testing) and individual (species, sex, stress coping strategies, phase of the females' estrous cycle) differences. In this regard, our lack of results in the sucrose preference test should also be interpreted with caution, since i) passive sucrose consumption tests without operant protocols may not be a good indicator of depressive-like behavior in laboratory mice [44], and ii) we did not use additional tests, such as forced swimming, because of their severity and because it is not clear whether immobility reflects a depressive phenotype or is a learned adaptive behavior [45].

Although stressed female mice displayed increased locomotor activity in the OFT, their lower thigmotaxis does not allow us to establish this observation as a symptom of anxiety-like behavior. Therefore, a more thorough assessment would have been necessary to rule out anxious behavior, using different test as elevated plus maze, zero maze or light and dark box [46]. Nevertheless, the active behavioral profile of female mice exposed to CSIS observed previously in our laboratory [26, 31], and by other authors [28], has been interpreted as indicative of higher arousal and precludes us from ruling out the possibility of our findings being indicative of anxiety-like behaviors. In this regard, the specific hyper-activation observed in females following emotional stress has been interpreted as a transitional phase towards a pathological stress response [47], or alternatively, as an adaptive coping strategy designed to manage and regulate pressures, demands, and emotions in response to stress [48]. Furthermore, although previous work in our laboratory found no effect of the estrous cycle on behavior following social stress due to instability [26], this variable was not considered in this study, and we cannot, therefore, rule out the possibility of the estrous cycle and estrogen levels having some effect on the active behavioral profile observed in female mice exposed to CSIS [49,50].

4.2. CSIS in female mice with high or low sociability: neuroendocrine effects

Consistently to that reported by other authors, stress did not alter corticosterone levels during exposure to CSIS [30,41,51]. As in previous studies conducted in our laboratory [31], among female mice, exposure to CSIS increased the MR/GR ratio, decreasing GR levels. Although stress has been commonly associated with a decrease in MR receptors relative to GR and a reduction in MR functionality, numerous studies have also observed sex differences in the physiological response to stress and its regulation [52]. Sex differences in the GR function also appear to make females more susceptible to dysregulation after a stressful event [53]. Following HPA axis activation, GRs are critical to the negative feedback process that inhibits additional glucocorticoid release. Thus, the significant reduction in GR expression previously observed in our laboratory in female mice [26,31] may attenuate the negative feedback process in response to a situation of chronic stress. Considering corticosterone plasma levels at the end of the CSIS model, our paradigm may have caused habituation of the HPA axis response that is often observed upon repeated exposure to the same stressor. In this sense, we cannot rule out a down-regulation of GR receptors expression in stressed subjects caused by glucocorticoids. The differential expression of receptors observed between stressed and non-stressed mice could indicate regulation of the HPA axis, with different consequences, since it has been associated with both, deleterious [54] and protective effects [55] in stressful situations. Furthermore, it is important to note that in the only work in which the effects of GR receptor deletion were studied in both sexes, they observed only deleterious effects in males, suggesting alternative mechanisms of GR regulation by females [56].

We did observe an effect of social connectedness, with high-sociability subjects having higher corticosterone levels and MR expression in the hippocampus. This receptor at the hippocampal CA2 region has been associated with social behavior [57]. However, as we have measured the expression at the whole hippocampus, we cannot determine if there are different expression patterns in the different regions of the hippocampus between groups. These results support the hypothesis that sociability may play a modulatory role in HPA activity, especially in situations of social stress [58–60]. If this were the case, the results obtained here would indicate that this change in female mice (allostatic load) depends on sociability. Considering that lower MR receptors expression has been commonly associated with mood disorders [61], and taking into account that we did observe a lower expression of MR receptors in the hypothalamus, the observed increased expression of these receptors in the hippocampus only in subjects with high sociability does not allow us to rule out a protective effect of high sociability in female mice submitted to CSIS model.

4.3. CSIS in female mice with high or low sociability: neurochemical effects

Although the release of catecholamines is a key initial event in response to stressors, it is now clear that different types of stressors and coping strategies elicit specific responses [62]. In our case, social instability resulted in an increase in NA in the hippocampus, as well as in a decrease in the levels of its metabolite MHPG and its precursors Phe-and Tyr, which rules out an over-activation of this pathway induced by social instability. In the striatum, we observed increased dopamine levels in subjects exposed to social instability, which may explain the increased locomotor activity observed in the OFT. However, as in the hippocampus, the reduced DA turnover observed in stressed subjects rules out the social instability-induced hyper-activation of the dopaminergic pathway. Interestingly, this increase in dopamine levels was only observed in subjects with high sociability. In this sense, sociability has been described as a behavioral characteristic that reflects a tendency to affiliate and is associated with positive affect, as well as with differences in the sensitivity of brain DA systems. Furthermore, in the striatum, and independently of stress, NA levels were significantly reduced in those subjects with high sociability, supporting the idea of the protective effect of this factor.

It is also well known that stressful situations can impact the Tryp-Kyn pathway, through glucocorticoids and cytokine-induced activation [63]. The stress model applied in the present study did not produce the expected changes in relation to the Tryp metabolic pathways. Social instability increased 5-HT levels in both the hippocampus and striatum, while reducing the levels of its metabolite 5-HIAA in both structures and its precursor Tryp in the hippocampus. Whereas in the hippocampus this effect was observed in subjects with high and low sociability, in the striatum it was only observed in subjects with high sociability, again highlighting the importance of this factor in the study of the negative effects of social stress. Although social stress has been commonly associated with an imbalance of the Tryp metabolic pathway, favoring Kyn synthesis over 5-HT, and therefore with an increase in its metabolites [64], our results reveal the opposite effect after the application of the social instability stress model. Interestingly, while no effect was observed in the Kyn/Tryp ratio, an activation biomarker of the Kyn pathway [65], increased activation of the 5-HT pathway (5-HT/Tryp ratio) was found in female mice subjected to CSIS. However, this increased serotoninergic production with respect to Kyn did not result in higher serotoninergic activity, since, similarly to that observed in the noradrenergic and dopaminergic pathways, a lower 5-HT turnover (5-HT/5-HIAA ratio) was observed in stressed subjects. Overall, these results do not allow us to rule out the possibility of a lower level of monoaminergic transmission in female mice subjected to social instability.

Interestingly, although social instability was not observed to affect Kyn levels in any of the structures, a reduction in both Kyna and 3-HK levels was found in the hippocampus. Although an increase in 3-HK in the striatum was observed after the application of the social instability model, the opposite effect observed in the hippocampus, together with the behavioral, neuroendocrine, and neurochemical results outlined above, allows us to rule out the possibly that the social instability model applied to female mice for 4 weeks does not have a deleterious effect at

any of the levels analyzed.

Stress-induced inflammatory activation is one of the main hypotheses in the study of the negative effects of social stress on health. Many studies have focused on the impact of pro-inflammatory cytokines on monoaminergic function, specifically through the activation of IDO. Firstly, this enzyme catalyzes the initial and rate-determining step of the Tryp metabolism via the Kyn pathway. And secondly, the nitric oxide (NO) produced by iNOS inhibits IDO activity by directly interacting with it and promoting its degradation. Contrary to the expected results, social instability stress decreased the synthesis of the IDO and iNOS enzymes when applied to female mice for 4 weeks. Consistently with the behavioral and neuroendocrine findings, these results support the idea that the social instability model applied did not elicit allostatic load. Although we did not observe any change in IDO or iNOS expression in the striatum, the lower expression of these enzymes in the hippocampus may reflect a resilient response to a mild stressor, regulating the IDO-mediated Tryp-degrading pathway. If this were indeed the case, it would indicate that the application of our psychosocial stress model did not promote the conversion of Tryp to Kyn and Kyna or 3-HK. The administration of IDO and iNOS inhibitors has been shown to alleviate the neurochemical and behavioral effects associated with chronic stress [66–68], and it has been suggested that social support may influence the expression of IDO [69]. Similarly, our results indicate that this reduction in IDO expression was significant only in those subjects with high sociability.

According to the behavioral and neuroendocrine results presented above, the neurochemical profile observed after the application of the CSIS model is not in line with the expected results and does not rule out that the level of stress was insufficient. Although there is extensive evidence, that stress and corticosterone are able to change the biosynthesis, release, and reuptake of monoamines [70–72], the absence of behavioral changes and effects on corticosterone levels following the application of the social instability stress model makes it difficult to establish a relationship between these variables (behavior, neuroendocrine and neurochemical).

Finally, we would like to point out the need to have predictive and translatable animal models of chronic social stress. To this end, we believe that it is essential to study both sexes but applying the same social stressor. In this sense, it would be interesting to explore the model of social chronic defeat in both males [73] and females [74]. Likewise, it is necessary to do a deeper phenotyping, since in neuroscience we tend to use relatively simple and quick tests [75], but we should focus more on the ethogram of the mice and expand the battery of tests for each behavioral domain to be studied.

In conclusion, although female mice exposed to CSIS showed increased arousal, there was no evidence of depressive-like behavior. Exposure to CSIS also did not affect corticosterone levels, although it increased the MR/GR ratio by decreasing GR expression. As a result of increased arousal, female mice exposed to CSIS had higher levels of NA and DA in the hippocampus and striatum respectively, although lower monoaminergic turnover was also observed. Contrary to the expected results, CSIS increased 5-HT levels in both the hippocampus and striatum, which may explain the lack of results in anhedonia. Similarly, and contrary to the expected results, CSIS was found to reduce kynurenic acid and 3-HK levels in the hippocampus, probably due to the observed decrease in the synthesis of the enzymes IDO and iNOS. Interestingly, the observed decrease in IDO synthesis and the increased 5-HT and DA levels in the striatum were only found in highly sociable subjects. Overall, our model has produced neuroendocrine and neurochemical but not behavioral changes, so it has not allowed us to study sociability in depth. Therefore, a model that induces both molecular and behavioral phenotypes should be applied to determine the role of sociability.

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Ethical approval

All procedures involving mice were performed in accordance with that established in the European Directive (2010/63/EU) and were approved by the Animal Welfare Ethics Committee of the University of the Basque Country (CEEA-UPV/EHU; M20/2018/090) and the Gipuzkoa Provincial Council (PRO-AE-SS-062).

Availability of data and materials

The study data will be made available upon reasonable request to the corresponding author.

CRediT authorship contribution statement

Alina Díez-Solinska: Investigation, Formal analysis, Data curation, Visualization, Writing – original draft. **Garikoitz Azkona:** Conceptualization, Writing – original draft, Visualization, Supervision. **Maider Muñoz-Culla:** Investigation, Writing – original draft. **Garikoitz Beitia-Oyarzabal:** Validation, Writing – original draft, Funding acquisition. **Olatz Goñi-Balentziaga:** Methodology, Supervision, Writing – review & editing. **Eneritz Gómez-Lazaro:** Supervision, Writing – review & editing, Funding acquisition. **Oscar Vegas:** Conceptualization, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A

Table A1

References

[1] L. Toussaint, G.S. Shields, G. Dorn, et al., Effects of lifetime stress exposure on mental and physical health in young adulthood: how stress degrades and forgiveness protects health, J. Health Psychol. 21 (2016) 1004–1014, https://doi.org/10.1177/1359105314544132, 20140819.

[2] L.C. Hawkley, S.W. Cole, J.P. Capitanio, et al., Effects of social isolation on glucocorticoid regulation in social mammals, Horm. Behav. 62 (2012) 314–323, https://doi.org/10.1016/j.yhbeh.2012.05.011, 2012/06/01.

[3] K.L. Tamashiro, M.M. Nguyen, R.R. Sakai, Social stress: from rodents to primates, Front. Neuroendocrinol. 26 (2005) 27–40, https://doi.org/10.1016/j.yfrne.2005.03.001.

[4] C.S. Carver, J. Connor-Smith, Personality and coping, Annu. Rev. Psychol. 61 (2010) 679–704, https://doi.org/10.1146/annurev.psych.093008.100352.

[5] M.R. Irwin, Cole SW, Reciprocal regulation of the neural and innate immune systems, Nat. Rev. Immunol. 11 (2011) 625–632, https://doi.org/10.1038/nri3042, 2011/08/05.

[6] J.T. Cacioppo, S. Cacioppo, J.P. Capitanio, et al., The neuroendocrinology of social isolation, Annu. Rev. Psychol. 66 (2015) 733–767, https://doi.org/10.1146/annurev-psych-010814-015240, 2014/08/22.

[7] V. Krishnan, E.J. Nestler, The molecular neurobiology of depression, Nature 455 (2008) 894–902, https://doi.org/10.1038/nature07455.

[8] A.K. Bekhet, J.A. Zauszniewski, W.E. Nakhla, Loneliness: a concept analysis, Nurs. Forum 43 (2008) 207–213, https://doi.org/10.1111/j.1744-6198.2008.00114.x.

[9] G. Parker, K. Fletcher, A. Paterson, et al., Gender differences in depression severity and symptoms across depressive sub-types, J. Affect. Disord. 167 (2014) 351–357, https://doi.org/10.1016/j.jad.2014.06.018, 2014/06/19.

[10] S.B. Patten, J.L. Wang, J.V. Williams, et al., Descriptive epidemiology of major depression in Canada, Can. J. Psychiatry 51 (2006) 84–90, https://doi.org/10.1177/070674370605100204.

[11] K.S. Kendler, J.M. Hettema, F. Butera, et al., Life event dimensions of loss, humiliation, entrapment, and danger in the prediction of onsets of major depression and generalized anxiety, Arch. Gen. Psychiatry 60 (2003) 789–796, https://doi.org/10.1001/archpsyc.60.8.789.

[12] G.M. Slavich, S.M. Monroe, H. GI, Early parental loss and depression history: associations with recent life stress in major depressive disorder, J. Psychiatr. Res. 45 (2011) 1146–1152, https://doi.org/10.1016/j.jpsychires.2011.03.004.

[13] G.M. Slavich, A. O'Donovan, E.S. Epel, et al., Black sheep get the blues: a psychobiological model of social rejection and depression, Neurosci. Biobehav. Rev. 35 (2010) 39–45, https://doi.org/10.1016/j.neubiorev.2010.01.003.

[14] A. Kupferberg, L. Bicks, G. Hasler, Social functioning in major depressive disorder, Neurosci. Biobehav. Rev. 69 (2016) 313–332, https://doi.org/10.1016/j.neubiorev.2016.07.002, 20160706.

[15] G. Hasler, Pathophysiology of depression: do we have any solid evidence of interest to clinicians? World Psychiatry 9 (2010) 155–161, https://doi.org/10.1002/j.2051-5545.2010.tb00298.x.

[16] R. Tian, G. Hou, D. Li, et al., A possible change process of inflammatory cytokines in the prolonged chronic stress and its ultimate implications for health, ScientificWorldJournal 2014 (2014), 780616, https://doi.org/10.1155/2014/780616, 2014/06/03.

[17] E. Haroon, C.L. Raison, A.H. Miller, Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior, Neuropsychopharmacology 37 (2012) 137–162, https://doi.org/10.1038/npp.2011.205, 2011/09/14.

[18] I. Mahar, F.R. Bambico, N. Mechawar, et al., Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects, Neurosci. Biobehav. Rev. 38 (2014) 173–192, https://doi.org/10.1016/j.neubiorev.2013.11.009, 20131201.

[19] A. Labaka, E. Gómez-Lazaro, O. Goñi-Balentziaga, et al., Venlafaxine reduces the striatal il6/il10 ratio and increases hippocampal GR expression in female mice subjected to chronic social instability stress, Stress 24 (2021) 561–571, https://doi.org/10.1080/10253890.2021.1895111, 20210326.

[20] S. Bhatt, A.N. Nagappa, C.R. Patil, Role of oxidative stress in depression, Drug Discov. Today 25 (2020) 1270–1276, https://doi.org/10.1016/j.drudis.2020.05.001, 20200508.

[21] A.K. Beery, Inclusion of females does not increase variability in rodent research studies, Curr. Opin. Behav. Sci. 23 (2018) 143–149, https://doi.org/10.1016/j.cobeha.2018.06.016, 2018/08/02.

[22] S.M. Peters, H.H. Pothuizen, B.M. Spruijt, Ethological concepts enhance the translational value of animal models, Eur. J. Pharmacol. 759 (2015) 42–50, https://doi.org/10.1016/j.ejphar.2015.03.043, 2015/03/28.

[23] A. Keeney, D.S. Jessop, M.S. Harbuz, et al., Differential effects of acute and chronic social defeat stress on hypothalamic-pituitary-adrenal axis function and hippocampal serotonin release in mice, J. Neuroendocrinol. 18 (2006) 330–338, https://doi.org/10.1111/j.1365-2826.2006.01422.x.

[24] N.N. Kudryavtseva, I.V. Bakshtanovskaya, L.A. Koryakina, Social model of depression in mice of C57BL/6 J strain, Pharmacol. Biochem. Behav. 38 (1991) 315–320, https://doi.org/10.1016/0091-3057(91)90284-9.

[25] Planchez B., Surget A. and Belzung C. Animal models of major depression: drawbacks and challenges. 2019; 126: 1383–1408.

[26] A. Labaka, E. Gómez-Lázaro, O. Vegas, et al., Reduced hippocampal IL-10 expression, altered monoaminergic activity and anxiety and depressive-like behavior in female mice subjected to chronic social instability stress, Behav. Brain Res. 335 (2017) 8–18, https://doi.org/10.1016/j.bbr.2017.08.002, 2017/08/05.

[27] O. Goñi-Balentziaga, J. Perez-Tejada, A. Renteria-Dominguez, et al., Social instability in female rodents as a model of stress related disorders: a systematic review, Physiol. Behav. 196 (2018) 190–199, https://doi.org/10.1016/j.physbeh.2018.09.001, 2018/09/06.

[28] H. Dadomo, L. Gioiosa, J. Cigalotti, et al., What is stressful for females? Differential effects of unpredictable environmental or social stress in CD1 female mice, Horm. Behav. 98 (2018) 22–32, https://doi.org/10.1016/j.yhbeh.2017.11.013.

[29] L. Saavedra-Rodriguez, L.A. Feig, Chronic social instability induces anxiety and defective social interactions across generations, Biol. Psychiatry 73 (2013) 44–53, https://doi.org/10.1016/j.biopsych.2012.06.035.

[30] J. Baranyi, N. Bakos, J. Haller, Social instability in female rats: the relationship between stress-related and anxiety-like consequences, Physiol. Behav. 84 (2005) 511–518, https://doi.org/10.1016/j.physbeh.2005.01.005.

[31] A. Díez-Solinska, A. Lebeña, L. Garmendia, et al., Chronic social instability stress down-regulates IL-10 and up-regulates CX3CR1 in tumor-bearing and non-tumor-bearing female mice, Behav. Brain Res. 435 (2022), 114063, https://doi.org/10.1016/j.bbr.2022.114063, 20220818.

[32] M.V. Schmidt, S.H. Scharf, C. Liebl, et al., A novel chronic social stress paradigm in female mice, Horm. Behav. 57 (2010) 415–420, https://doi.org/10.1016/j.yhbeh.2010.01.010.

[33] T.D. Gould, D.T. Dao, C.E. Kovacsics, The Open Field test. *Mood and Anxiety Related Phenotypes in mice: Characterization using Behavioral Tests*, Humana Press/Springer Nature, Totowa, NJ, US, 2009, pp. 1–20.

[34] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.

[35] A. Koert, A. Ploeger, C.L.H. Bockting, et al., The social instability stress paradigm in rat and mouse: a systematic review of protocols, limitations, and recommendations, Neurobiol. Stress 15 (2021), 100410, https://doi.org/10.1016/j.ynstr.2021.100410, 20211016.

[36] J.P. Capitanio, Personality dimensions in adult male rhesus macaques: prediction of behaviors across time and situation, Am. J. Primatol. 47 (1999) 299–320, https://doi.org/10.1002/(SICI)1098-2345(1999)47:4<299::AID−AJP3>3.0.CO;2-P.

[37] J.P. Capitanio, K. Abel, S.P. Mendoza, et al., Personality and serotonin transporter genotype interact with social context to affect immunity and viral set-point in simian immunodeficiency virus disease, Brain Behav. Immun. 22 (2008) 676–689.

[38] A. Chocyk, I. Majcher-Maoelanka, D. Dudys, et al., Impact of early-life stress on the medial prefrontal cortex functions-a search for the pathomechanisms of anxiety and mood disorders, Pharmacol. Rep. 65 (2013) 1462–1470, https://doi.org/10.1016/S1734-1140(13)71506-8.

[39] C. Heim, E.B. Binder, Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics, Exp. Neurol. 233 (2012) 102–111, https://doi.org/10.1016/j.expneurol.2011.10.032.

[40] P. Willner, Chronic Mild Stress (CMS) Revisited: consistency and behavioural-neurobiological concordance in the effects of CMS, Neuropsychobiology 52 (2005) 90–110, https://doi.org/10.1159/000087097.

[41] C.J. Herzog, B. Czh, S. Corbach, et al., Chronic social instability stress in female rats: a potential animal model for female depression, Neuroscience 159 (2009) 982–992, https://doi.org/10.1016/j.neuroscience.2009.01.059.

[42] G.M. Leedy, L.F. Barrows, S. Clark, Effects of social housing on hippocampal dendrites and behavior in ovariectomized rats, Brain Res. Bull. 92 (2013) 69–75, https://doi.org/10.1016/j.brainresbull.2012.11.006, 20121126.

[43] M.M. Nowacka, M. Paul-Samojedny, A.M. Bielecka, et al., Chronic social instability stress enhances vulnerability of BDNF response to LPS in the limbic structures of female rats: a protective role of antidepressants, Neurosci. Res. 88 (2014) 74–83, https://doi.org/10.1016/j.neures.2014.08.008, 20140828.

[44] F. Chaouloff, Social stress models in depression research: what do they tell us? Cell Tissue Res. 354 (2013) 179–190, https://doi.org/10.1007/s00441-013-1606-x, 20130327.

[45] M.L. Molendijk, E.R. de Kloet, Immobility in the forced swim test is adaptive and does not reflect depression, Psychoneuroendocrinology 62 (2015) 389–391, https://doi.org/10.1016/j.psyneuen.2015.08.028, 2015/09/02.

[46] G. Azkona, A. Amador-Arjona, C. Obradors-Tarragó, et al., Characterization of a mouse model overexpressing beta-site APP-cleaving enzyme 2 reveals a new role for BACE2, Genes Brain Behav. 9 (2010) 160–172, https://doi.org/10.1111/j.1601-183X.2009.00538.x, 2009/09/22.

[47] D.A. Bangasser, K.R. Wiersielis, S. Khantsis, Sex differences in the locus coeruleus-norepinephrine system and its regulation by stress, Brain Res. 1641 (2016) 177–188, https://doi.org/10.1016/j.brainres.2015.11.021, 20151121.

[48] M.M. Kelly, A.R. Tyrka, G.M. Anderson, et al., Sex differences in emotional and physiological responses to the trier social stress test, J. Behav. Ther. Exp. Psychiatry 39 (2008) 87–98, https://doi.org/10.1016/j.jbtep.2007.02.003, 20070312.

[49] I. Jaric, D. Rocks, H. Cham, et al., Sex and estrous cycle effects on anxiety-and depression-related phenotypes in a two-hit developmental stress model, Front. Mol. Neurosci. 12 (2019) 1–15, https://doi.org/10.3389/fnmol.2019.00074.

[50] S. Mora, N. Dussaubat, G. Diaz-Veliz, Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats, Psychoneuroendocrinology 21 (1996) 609–620, https://doi.org/10.1016/S0306-4530(96)00015-7.

[51] S. Bhatnagar, C. Vining, V. Iyer, et al., Changes in hypothalamic-pituitary-adrenal function, body temperature, body weight and food intake with repeated social stress exposure in rats, J. Neuroendocrinol. 18 (2006) 13–24, https://doi.org/10.1111/j.1365-2826.2005.01375.x.

[52] A.L. Heck, R.J. Handa, Sex differences in the hypothalamic – pituitary – adrenal axis ' response to stress: an important role for gonadal hormones, Neuropsychopharmacol. Rev. 44 (2018) 45–58, https://doi.org/10.1038/s41386-018-0167-9.

[53] Q. Wang, M. Joels, D.F. Swaab, et al., Hippocampal GR expression is increased in elderly depressed females, Neuropharmacology 62 (2012) 527–533, https://doi.org/10.1016/j.neuropharm.2011.09.014.

[54] C.L. Raison, A.H. Miller, When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders, Stress 160 (2003) 1554–1565, https://doi.org/10.1176/appi.ajp.160.9.1554.

[55] K.V. Wagner, X.D. Wang, C. Liebl, et al., Pituitary glucocorticoid receptor deletion reduces vulnerability to chronic stress, Psychoneuroendocrinology 36 (2011) 579–587, https://doi.org/10.1016/j.psyneuen.2010.09.007.

[56] M.B. Solomon, A.R. Furay, K. Jones, et al., Deletion of forebrain glucocorticoid receptors impairs neuroendocrine stress responses and induces depression-like behavior in males but not females, Neuroscience 203 (2012) 135–143, https://doi.org/10.1016/j.neuroscience.2011.12.014, 20111224.

[57] K.E. McCann, D.J. Lustberg, E.K. Shaughnessy, et al., Novel role for mineralocorticoid receptors in control of a neuronal phenotype, Mol. Psychiatry 26 (2021) 350–364, https://doi.org/10.1038/s41380-019-0598-7, 20191119.

[58] J.P. Capitanio, S.P. Mendoza, N.W. Lerche, et al., Social stress results in altered glucocorticoid regulation and shorter survival in simian acquired immune deficiency syndrome, Proc. Natl Acad. Sci. U S A 95 (1998) 4714–4719, https://doi.org/10.1073/pnas.95.8.4714.

[59] J.P. Capitanio, Nonhuman Primate Personality and Immunity: Mechanisms of Health and Disease, Springer New York, New York, NY, 2011, pp. 233–255.

[60] D.L. Hill, N. Pillay, C. Schradin, Glucocorticoid levels predict subsequent social tactic in females of a facultatively social mammal, Funct. Ecol. 35 (2021) 650–662, https://doi.org/10.1110/1365-2435.13744.

[61] F. ter Heegde, R.H. De Rijk, C.H. Vinkers, The brain mineralocorticoid receptor and stress resilience, Psychoneuroendocrinology 52 (2015) 92–110, https://doi.org/10.1016/j.psyneuen.2014.10.022, 20141107.

[62] S. Cabib, S Puglisi-Allegra, The mesoaccumbens dopamine in coping with stress, Neurosci. Biobehav. Rev. 36 (2012) 79–89, https://doi.org/10.1016/j.neubiorev.2011.04.012, 20110504.
[63] M.I. Butler, C. Long-Smith, G.M. Moloney, et al., The immune-kynurenine pathway in social anxiety disorder, Brain Behav. Immun. 99 (2022) 317–326, https://doi.org/10.1016/j.bbi.2021.10.020, 20211107.
[64] G. Bergamini, J. Mechtersheimer, D. Azzinnari, et al., Chronic social stress induces peripheral and central immune activation, blunted mesolimbic dopamine function, and reduced reward-directed behaviour in mice, Neurobiol. Stress 8 (2018) 42–56, https://doi.org/10.1016/j.ynstr.2018.01.004, 20180202.
[65] L. Lionetto, M. Ulivieri, M. Capi, et al., Increased kynurenine-to-tryptophan ratio in the serum of patients infected with SARS-CoV2: an observational cohort study, Biochim. Biophys. Acta Mol. Basis Dis. 1867 (2021), 166042, https://doi.org/10.1016/j.bbadis.2020.166042, 20201216.
[66] A. Laugeray, J.M. Launay, J. Callebert, et al., Chronic treatment with the IDO1 inhibitor 1-methyl-D-tryptophan minimizes the behavioural and biochemical abnormalities induced by unpredictable chronic mild stress in mice - comparison with fluoxetine, PLoS ONE 11 (2016), e0164337, https://doi.org/10.1371/journal.pone.0164337, 20161109.
[67] M. Ohnishi, M. Akagi, M. Kotsuki, et al., Indoleamine 2, 3-dioxygenase is responsible for low stress tolerance after intracerebral hemorrhage, PLoS ONE 18 (2023), e0273037, https://doi.org/10.1371/journal.pone.0273037, 20230208.
[68] N. Gilhotra, H. Jain, D. Dhingra, Differential effects of nitric oxide synthase inhibitors on anxiety in unstressed and stressed mice, Indian J. Exp. Biol. 48 (2010) 365–372.
[69] K. Zhang, N. Lei, M. Li, et al., Cang-Ai volatile oil ameliorates depressive behavior induced by chronic stress through IDO-mediated tryptophan degradation pathway, Front. Psychiatry 12 (2021), 791991, https://doi.org/10.3389/fpsyt.2021.791991, 20211215.
[70] A. Czyrak, M. Maćkowiak, A. Chocyk, et al., Role of glucocorticoids in the regulation of dopaminergic neurotransmission, Pol. J. Pharmacol. 55 (2003) 667–674.
[71] R. Kvetnansky, E.L. Sabban, M. Palkovits, Catecholaminergic systems in stress: structural and molecular genetic approaches, Physiol. Rev. 89 (2009) 535–606, https://doi.org/10.1152/physrev.00042.2006.
[72] J.J. Bonfiglio, C. Inda, D. Refojo, et al., The corticotropin-releasing hormone network and the hypothalamic-pituitary-adrenal axis: molecular and cellular mechanisms involved, Neuroendocrinology 94 (2011) 12–20, https://doi.org/10.1159/000328226, 20110513.
[73] E. Gómez-Lázaro, A. Arregi, G. Beitia, et al., Individual differences in chronically defeated male mice: behavioral, endocrine, immune, and neurotrophic changes as markers of vulnerability to the effects of stress, Stress 14 (2011) 537–548, https://doi.org/10.3109/10253890.2011.562939, 20110327.
[74] A.Z. Harris, P. Atsak, Z.H. Bretton, et al., A novel method for chronic social defeat stress in female mice, Neuropsychopharmacology 43 (2018) 1276–1283, https://doi.org/10.1038/npp.2017.259, 20171101.
[75] G. Azkona, R. Sanchez-Pernaute, Mice in translational neuroscience: what R we doing? Prog. Neurobiol. 217 (2022), 102330 https://doi.org/10.1016/j.pneurobio.2022.102330, 20220721.