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Curcumin Modulates Oxidative Stress, Cortical Activity and Corticosterone in Mice Exposed to Chronic Social Stress in the Spontaneous Aggressiveness Model.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ABSTRACT

Stress constitutes a fundamental physiological mechanism essential for survival and environmental adaptation. Chronic social stress is a major risk factor for emotional and behavioral disturbances, including aggressiveness. Curcumin, a bioactive polyphenol derived from Curcuma longa, exhibits antioxidants, anti-inflammatory, and antidepressant properties, making it a promising neuroprotective agent. Previous studies using the Spontaneous Aggressiveness Model (MSA) demonstrated increased production of reactive oxygen species (ROS) and c-Fos expression in aggressive mice, suggesting a role for oxidative stress. This study investigated the effects of curcumin supplementation on neuroendocrine and oxidative parameters in male Swiss Webster mice subjected to chronic social stress in MSA. Animals (n = 60, 3 weeks old) were allocated to control and curcumin-treated groups. The treated group received a 1.5% curcumin-enriched diet for ten days before social regrouping in week 10. Behavioral categorization into non-regrouped (NR), aggressive (AgR), subordinate (Sub), and harmonious (Har) profiles

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were performed at week 16. Plasma corticosterone was quantified by ELISA, ROS production by DHE assay, and c-Fos expression by immunofluorescence. All procedures were approved by the Ethics Committee on Animal Use of the Oswaldo Cruz Institute (CEUA/IOC L-022/2025). Curcumin supplementation reduced attack intensity, prevented hypercortisolemia in NR mice, and decreased ROS levels in aggressive animals, supporting its antioxidant and neuroendocrine modulatory effects as a potential neuroprotective strategy under chronic social stress.

KEYWORDS: Stress, Curcumin, ROS, c-FOS, Corticosterone.

Significance Statement

Chronic social stress represents a major environmental factor contributing to the onset of psychiatric and inflammatory disorders through persistent disruption of neuroendocrine and immune homeostasis. Prolonged activation of the hypothalamic–pituitary–adrenal axis and sustained glucocorticoid release led to oxidative and neuroinflammatory damage, promoting behavioral dysfunctions such as aggression. The Model of Spontaneous Aggressiveness (MSA) provides a robust and reproducible framework for investigating the biological mechanisms underlying stress-induced aggression by distinguishing individual behavioral phenotypes in socially challenged mice. Within this context, curcumin, a polyphenolic compound derived from Curcuma longa, has emerged as a promising therapeutic agent due to its antioxidant, anti-inflammatory, and neuroprotective properties. By examining corticosterone levels, reactive oxygen species production, and c-Fos expression in the prefrontal cortex, this study identifies potential neurobiological pathways through which curcumin modulates the effects of chronic social stress. These findings highlight the translational relevance of nutritional interventions as complementary approaches for preventing stress-related neuropsychiatric dysfunction.

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1. INTRODUCTION

Stress is recognized as a natural physiological response of the organism to situations perceived as threatening or challenging, characterized by a state in which homeostasis is compromised. This response is essential for promoting efficient adaptation to the stressor, restoring physiological balance, and protecting the organism [1].

According to the intensity and duration of exposure, stress can be classified as acute or chronic. Acute stress is characterized by a short-term response to a highly intense stimulus. Chronic stress results from prolonged exposure to less intense stressors and is widely prevalent in densely populated urban areas, often linked to social stress as a determining factor [2–4].

Among the primary triggers of chronic social stress are interpersonal conflicts, hostile environments, occupational pressures, financial difficulties, and social isolation — the latter intensified during the COVID-19 pandemic [3]. When faced with stress, the central nervous system (CNS) activates specific circuits mediated by neurotransmitters and hormones, resulting in adaptive physiological and behavioral responses. These responses involve structures such as the amygdala (emotional regulation), the hippocampus (learning and memory), and the prefrontal cortex (decision-making) [5].

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The hypothalamic-pituitary-adrenal (HPA) axis is the primary neuroendocrine system responsible for adaptation to stress. Its activation leads to the release of cortisol into the systemic circulation. Although cortisol plays a crucial role in modulating physiological processes during stress, its sustained release can cause neuroendocrine and neurobiological alterations that are detrimental to health [6,7].

Chronic stress has been associated with a wide range of inflammatory diseases, such as rheumatoid arthritis, cardiovascular disease, multiple sclerosis, asthma, and psoriasis [2]. Furthermore, substantial evidence points to its contribution to the development of psychiatric disorders, such as depression, anxiety, and panic disorder, often accompanied by aggressive behavior [8,9]. Aggression, in turn, can cause physical or psychological harm to others and, when exacerbated, is considered pathological [10–12]. In contexts of chronic social stress, aggression can evolve into violence, representing a significant risk to the physical and mental integrity of individuals [9]. Understanding the mechanisms involved in the manifestation of aggression under chronic social stress is therefore essential. However, such mechanisms are complex and involve multiple physiological, biochemical, anatomical, and genetic factors [9,10]. Murine models, such as the one developed by our team, have been valuable for studying this behavior [11]. During the observation of Swiss Webster mice kept in a vivarium, a pattern of spontaneous aggressive behavior was identified among animals exposed to the same environmental conditions. This motivated the development, in 2012, of the Model of Spontaneous Aggressiveness (MSA), a reproducible and innovative method for investigating aggressive behavior in response to chronic social stress [12–17]. MSA consists of grouping mice while they are still juvenile, followed by regrouping in adulthood, which generates a scenario of social stress. Individual responses to regrouping enable the categorization of animals into distinct behavioral profiles (aggressive, subordinate, harmonious, and non-regrouped), allowing for an in-depth investigation of the neurobiological mechanisms involved in stress-induced aggression [13–17].

In parallel with the advancement of behavioral models, interest has increased in non-pharmacological therapeutic strategies, such as nutritional interventions, that can promote resilience and mitigate the deleterious effects of chronic stress [18,19]. A frequently used example of a behavioral model is the social defeat model, in which mice are classified as resilient or susceptible to stress. However, methodological variations in this model can compromise the reliability of the results [20,21].

Within nutritional interventions, compounds with anti-inflammatory and antioxidant properties have been gaining prominence, considering the strong correlation between central and peripheral inflammation and stress-related psychiatric disorders [22,23]. The association of these compounds with well-established experimental models may contribute to the development of more effective and safe therapeutic approaches.

In addition to nutritional strategies, conventional treatment of impulsive aggression involves psychotropic drugs such as anxiolytics, antidepressants, mood stabilizers, and antipsychotics, which modulate central neurotransmitter systems [24–26]. Treatment options vary from addressing the underlying disorder (such as bipolar disorder) to directly managing aggression as an independent condition, considering common dysfunctions like serotonergic hypoactivity [24,25]. Despite the availability of various drugs (lithium, valproate, clozapine, beta-blockers, omega-3), clinical evidence shows limited success in controlling impulsive aggression [26]. Among the most promising natural compounds, curcumin—the main curcuminoid found in the rhizomes of Curcuma longa—stands out. This plant, native to South Asia, has been used for centuries in traditional medicine and contains, in addition to curcumin (CUR), the curcuminoids

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desmethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), which provide antioxidant, anti-inflammatory, and neuroprotective properties [27,28].

Curcumin constitutes approximately 2% of the dry weight of the rhizomes and can be obtained commercially as a standardized mixture: CUR (~77%), DMC (~17%), and BDMC (~3%) [27,28]. Preclinical studies suggest that this compound can modulate the neuroendocrine response to stress, reduce oxidative stress, and regulate the expression of proteins associated with neuronal activation, including c-Fos.

We hypothesize that a curcumin-enriched diet during infancy and adolescence influences reactive oxygen species (ROS) production, regulates corticosterone, and c-Fos protein expression in aggressive animals under stress during MSA regrouping, favoring an adaptive stress response. Therefore, we investigated whether pretreatment with curcumin, through a diet enriched with this compound, in Swiss Webster mice in the MSA after regrouping would regulate ROS production, c-Fos protein expression in the prefrontal cortex, and plasma corticosterone levels in these animals. During behavioral data analysis, we observed that the extent of lesions in the attacked animals in the control group was approximately three times greater than that in animals that received curcumin-supplemented food, despite the latter performing more attacks. Curcumin may not reduce the onset of aggressive behavior. Still, it does somehow influence the level of aggression, as these attacks cannot produce more severe injuries, as observed in control animals.

In this context, the present study aimed to investigate the effects of curcumin supplementation in Swiss Webster mice subjected to chronic social stress in the MSA. Plasma corticosterone levels, ROS production, and c-Fos protein expression in the prefrontal cortex were analyzed to assess the neuroendocrine and oxidative mechanisms underlying the modulation of aggressive behavior. The findings may contribute to the understanding of the therapeutic potential of curcumin in preventing damage associated with chronic social stress.

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2. MATERIAL AND METHODS

2.1 Animals

Male Swiss Webster mice (n= 60/assay) were obtained by the Multidisciplinary Center for Biological Research in the Area of Laboratory Animal Science (CEMIB/UNICAMP). They were housed at the Animal Experimentation Center of the Cell Biology Laboratory and the Laboratory of Innovations in Therapies, Teaching, and Bioproducts (LBC/LITEB – IOC). All animals were acclimated for seven days before the start of the experiments, under stable temperature (20–22 °C) and humidity (40–60%) conditions, with a 12-hour light/dark cycle regulated by ventilated racks and ad libitum access to food. The animals were individually identified (C1 to C5) and randomly assigned into 12 groups, with six belonging to the curcumin group (CURA1 to CURA6) (n= 30/assay) and 6 to the control group (A1 to A6) (n= 30/assay). Independent cohorts of animals were used for corticosterone, ROS, and c-Fos analyses. All experiments with animals were reviewed and approved by the Animal Use Ethics Committee of the Oswaldo Cruz Institute (CEUA/IOC) under license number L-022/2025. All methods were performed in accordance with the relevant guidelines and regulations. All experimental procedures adhere to the ARRIVE guidelines and were performed following the recommendations of the National Council for the Control of Animal Experimentation.

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2.2 Model of Spontaneous Aggression (MSA)

The MSA was performed according to the method described by Mélo et al. (2024) [29]. MSA consists of grouping young mice, followed by posterior regrouping of the same animals into new social groups in adulthood. At three weeks old, mice were divided into six groups (A1 to A6), with five animals per cage (C1-C5). At the fourth, sixth, and eighth weeks of age, we performed the Tail Suspension Test (TST), the ethogram, and the Pattern of Aggressive Behavior (PAB) tests. At the age of ten weeks, the animals were regrouped according to the TST, followed by regrouping these animals into 12 boxes, six from the curcumin group (CURR1 to CURR6) and six from the control group (R1 to R6), each containing four animals, with one hyperactive individual (Hyper), one hypoactive individual (Low), and two individuals with median activity (Median) in each cage. In this model, the stress-inducing factor is the regrouping procedure performed during the tenth week of life, disrupting previously established social hierarchies and promoting the emergence of aggressive interactions. Two cages with five animals were not regrouped (NR), which became our supplemented negative control (CUR NR) and non-supplemented negative control (NR).

At twelve, fourteen, and sixteen weeks of age, the ethogram and PAB were repeated. At sixteen weeks of age, the animals were categorized according to their behavior. Harmonious (Har) animals were those with reduced aggression and discrete or absent body lesions. Har animals were considered resilient individuals and served as our positive control. Defeated animals (AgD) were those showing moderate and intense body lesions caused by being subjected to aggression. Aggressive animals (AgR) were those exhibiting highly aggressive behavior towards other animals in the cage. NR and Har groups were used as negative and positive controls, respectively. Because this classification was based on spontaneous behavioral responses in the Model of Spontaneous Aggressiveness, the number of animals included in each behavioral category varied among groups and experimental phases. After categorization, all animals were euthanized, and brain tissues were collected (Fig. 1).

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2.3 Tail Suspension Test (TST)

The TST was implemented during the grouping phase and was used to characterize the animals' reactions. As described by Steru et al. (1985) [35], animals were suspended by the final third of the tail at the top of the apparatus. The test lasted five minutes, and the time in which the animal maintained a posture of mobility/immobility was quantified. Three categories were statistically defined depending on the animal’s physical reaction: (a) low activity (hypoactive), 104–150 s of immobility; (b) median activity (median), between 51 and 103 s of immobility; and (c) high activity (hyperactive), 0–50 s of immobility. After characterization of the activity animal profile, the results were used to standardize the regrouping MSA phase [12].

2.4 Ethogram and Pattern of Aggressive Behavior (PAB)

The ethogram and PAB were performed according to Felippe (2021) [14]; the ethogram was carried out during the grouping and regrouping phases [15]. In the ethogram, each cage is continuously filmed (top view) using a Canon camera PowerShot SX20 IS® (Lake Success, New York- USA) for 30 minutes, during which we can identify the aggressor animal, the defeated, and harmonious animals, quantify the number of attacks carried out

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by aggressive animals, and measuring of the lesion extension in cm^2 with a digital caliper (Digimess-China). The PAB was characterized for the individual physical animal evaluation, and we quantified the aggression according to predeterminate scores: (0) individual without lesions body; (1) individual with no-genital lesions in the tail or back; (2) individuals with moderate lesions caused by aggressive events in the tail and scrotum; (3) severe lesions caused by aggressive events in the back, tail, and scrotum.

2.5 Supplementation protocol

The therapeutic protocol was based on a previously published study [36]. Curcumin was incorporated into the chow and provided to group-housed animals throughout the experimental period, which depends on continuous social interaction and stable group housing conditions. Ten days before regrouping, the standard laboratory chow was replaced by a diet with 18% protein (adapted AIN-93M, Rhoster Indústria e Pesquisa) or a diet with 18% protein enriched with 1.5% curcumin (adapted AIN-93M + 1.5% curcumin, Rhoster Indústria e Pesquisa), both on pallets. Based on the average daily food intake of approximately 8 g of chow per mouse, each animal consumed an estimated total of 3528 g of chow over the 63-day experimental period. Considering the 1.5% curcumin enrichment, the estimated cumulative curcumin intake per animal was approximately 53 g during the treatment period. No absorption-enhancing adjuvants. The mice remained on their respective diets throughout the experiment (Fig. 1).

2.6 Collection and processing of brain tissue for fluorescence immunohistochemistry

For c-Fos protein analysis, animals were anesthetized 1 h after completion of the behavioral tests (16 who). Anesthesia was induced using a ketamine (75 mg/kg) and xylazine (10 mg/kg) mixture, followed by transcardiac perfusion with 4% paraformaldehyde (PFA) [14]. The brains of the animals used for this specific experiment were collected. They underwent a series of fixations: by immersion in 4% PFA (pH 7.4) for 48 hours, followed by cryoprotection with gradients in sucrose solution (10 to 30%) in PBS and, subsequently, embedded in OCT (Optimal Cutting Temperature) (Tissue Tek, Japan) and immersed in liquid nitrogen until completely frozen.

2.6.1 Fluorescence immunohistochemistry

To standardize the c-Fos protein, brain sections previously fixed by immersion in 4% PFA (pH 7.4) and cryoprotected with 10-30% sucrose gradients in PBS were used. Slides containing 7 μm brain sections were subjected to antigen retrieval by immersion in a citrate buffer solution (pH 6.0) using immunohistochemistry-compatible staining jars. Then, these tanks with the slides immersed in citrate buffer were placed in a container with water at 60 °C for 30 minutes, which allowed for antigen retrieval. The tissue sections were subjected to three consecutive washes with PBS, each lasting 10 minutes. They were then permeabilized using 0.1% Triton X-100 (Sigma-Aldrich, Brazil), a biological detergent, and marked with a hydrophobic pen (Sigma-Aldrich, Brazil) in distilled water. For blocking, a solution composed of 2% skim milk, 2.5% BSA, and 8% fetal bovine serum in PBS was used. After this step, the sections were incubated overnight at 4°C with the primary polyclonal anti-cFos antibody (Thermo Fisher Scientific, USA)

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at a dilution of 1:200 in PBS containing 0.1% Triton. Subsequently, the slides were washed in PBS and incubated for 1 hour at 37°C with the secondary anti-rabbit IgG antibody conjugated to the fluorophore Alexa Fluor 488 (Thermo Fisher, USA), at a dilution of 1:500, prepared in PBS with 0.1% Triton solution. To minimize autofluorescence, the sections were stained with Evans Blue at a ratio of 1:1,000 (in PBS) for 10 seconds and washed once. Immediately afterwards, the DAPI probe was applied at a dilution of 1:20,000 for 5 minutes at room temperature to highlight the cell nuclei, with subsequent three washes with PBS for 5 minutes each. The slides were then mounted with Faramount aqueous mounting medium (Dako/Agilent Technologies – USA) and analyzed under a laser confocal microscope, model LSM 710 (Zeiss – Germany), using a 40X objective. The analysis included the determination of the proportion of positive and negative nuclei for c-Fos protein, as well as the quantification of the density of c-Fos-positive cells per area, using the ImageJ software (version 2.9.0).

2.7 Analysis of ROS production by DHE assay

For the analysis of reactive oxygen species (ROS) production, animals were euthanized with isoflurane 5% overdose chamber at 16 weeks of age, and brain collection and dissection were quickly performed. Brain tissues were embedded in OCT and then immersed in liquid nitrogen until they were completely frozen. Then, 5-µm-thick sections of the brain tissue were made in the coronal plane using the cryostat for the detection of ROS. The DHE (Dihydroethidium) assay was employed, which is based on the chemical reaction between DHE and superoxide present in the tissue. DHE is a non-fluorescent chemical compound that, when interacting with a superoxide molecule, converts into a radical (DHE radical). This radical, when reacting again with another superoxide molecule, gives rise to a highly fluorescent compound called 2-hydroxy ethidium (2-OH-E^+). The quantification of superoxide production in the samples was performed using the mean fluorescence intensity (MIF) of the compound 2-hydroxy ethidium (2-OH-E^+). The tissue sections were marked with a hydrophobic pen (Sigma-Aldrich, Brazil) and then treated with the DHE solution at a concentration of 5 µM, diluted in Dulbecco's Modified Eagle Medium (DMEM). The samples were incubated at 37°C for 30 minutes, shaking at 50 rpm. Followed by three washes with 1X PBS, 5 minutes each, to complete the reaction at room temperature. Then, the slides were fixed with 4% paraformaldehyde (PFA) for 10 minutes, washed, and mounted in Prolong Gold with DAPI (4',6'-diamino-2-phenyl-indole). The slides were photographed using a laser confocal microscope, model LSM 710 (Zeiss - Germany). ROS production in the frontal cortex region was analyzed by the mean fluorescence intensity (MIF) of the fluorescent compound 2-hydroxy ethidium. This measurement was performed using ImageJ software (version 2.9.0).

2.8 Collection and processing of biological material for ELISA

Plasma corticosterone levels were determined using an ELISA kit according to the manufacturer’s instructions and results are expressed as ng/mL. The plasma corticosterone concentration was assessed at two time points, considering the rodents' circadian cycle: 12 hours of light (EXP), when there is no variation in corticosterone release, and 12 hours of darkness (PEAK), when there is an increase in corticosterone. Therefore, we collected the exponential increase (EXP) of plasma corticosterone from 12:00 AM onwards. And for the peak of plasma corticosterone from 7:00 PM onwards. At 16 weeks of age, the animals were anesthetized with a solution of xylazine (10 mg/kg)

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and ketamine (75 mg/kg) intraperitoneally. After anesthesia, cardiac puncture was performed to collect blood in heparin-coated tubes. After blood collection, the tubes were centrifuged for 10 minutes at 1000 x g and 4°C, and the supernatant (plasma) was collected and stored at -80°C until ELISA was performed. Circulating plasma corticosterone was quantified using an ELISA kit (EIACORT, ThermoFisher) according to the manufacturer's protocol. The plate was read in a spectrophotometer at 450 nm.

2.9 Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test, and is presented as mean ± standard deviation (SD). As the data did not consistently meet the assumptions of normality, comparisons among behavioral categories within each treatment group were performed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test. Comparisons between control and curcumin-treated groups were performed using two-way ANOVA with mixed-effects analysis. Statistical analyses were performed using SPSS software (version 16.0; IBM, CA, USA) and GraphPad Prism (version 8.0.1; GraphPad Software, Inc.). Statistical significance was considered at $p < 0.05$.

3. RESULTS

3.1 Categorization of Aggressive Animals

The highly aggressive animal selection was obtained through MSA application, as described in Fig. 1. During the grouping phase, behavioral tests were performed at the 4th, 6th, and 8th stages, including TST, ethogram, and PAB. The animal mobility evaluation was based on TST results. In the group of animals without curcumin supplementation, we observed that 42.42% of animals were hyperactive, 45.45% of animals had median mobility, and 12.12% were hypoactive (Fig. 2a). Neither aggressive events nor lesions were observed in any of the groups. After regrouping, the ethogram and PAB were performed again, and the animals were selected based on their aggressiveness profile: harmonious (Har), defeated (AgD), and aggressive. The ethogram shows that AgR animals increased the number of attacks to 5.0 ± 4.3 attacks/30min ($p<0.0001$) compared to other categories (NR: 0.0 ± 0,0; Har: 0.0 ± 0.0; AgD: 0.0 ± 0.0 attacks/30min) (Fig. 2b). In addition, the AgD animals significantly increased the extension lesion to 132.7 ± 128.84 cm² ($p <0.0001$) compared to other categories (NR: 0.0± 0.0; Har: 0.0 ± 0.0; AgR: 0.0 ±0.0 cm) (Fig. 2c). PAB, after regrouping, showed a significantly increased score to 2.90 ± 0.31 compared to other categories NR (0.0 ± 0.0), Har (0.0 ± 0.0) ($p<0.0001$) e AgR (0.0 ± 0.0) ($p<0.001$) (Fig. 2d).

In the group of animals supplemented with curcumin, 32.25% of the animals exhibited a hyperactive profile, 58.06% showed medium mobility, and 9.67% were hypoactive (Fig. 3a). We did not observe any aggressive events. Consequently, no lesions were identified in the mice during the grouping phase. After regrouping, the PCA and Ethogram behavioral tests were repeated. The PBA showed that the defeated animals (AgD) presented a significantly higher score of 2.83 ± 0.38, in relation to the NR (0.0 ± 0.0), Har (0.0 ± 0.0) and AgR (0.0 ± 0.0) animals ($p < 0.0001$) (Fig. 3b). Regarding the ethogram results of the curcumin group, the AgD animals presented a significantly greater lesion extension of 44.22 ± 19.89 cm², $p <0.0001$, in relation to the animals of the NR (0.0 ± 0.0), Har (0.0 ± 0.0) categories and, with $p <0.001$, regarding AgR (0.0 ± 0.0 cm²) (Fig. 3c). Consequently, the AgR animals presented a significant increase in the number

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of attacks of 8.4 ± 3.7/30min in relation to the other categories (NR: 0.0 ± 0.0; Har: 0.0 ± 0.0; AgD: 0.0 ± 0.0 attacks/30min) ($p < 0.0001$) (Fig. 3d).

After analyzing the behavioral data obtained from the ethogram of the supplemented and non-supplemented groups, we observed a significant difference in the extent of lesions on the attacked animals between the groups. From this, we analyzed these values and found that in the attacked animals of the control group, the lesions were significantly more extensive (33.17±66.33 cm²) than in the attacked animals of the curcumin-supplemented group (11.06±22.11 cm²) ($p<0.01$) (Fig. 4a). We also analyzed the number of attacks carried out by the attacking animals of both groups, and, interestingly, the supplemented group showed a significant increase of 2.1±4.2 compared to the AgR of the control group (1.2±2.5) ($p<0.5$) (Fig. 4b).

3.2 Plasma Corticosterone Levels

The regulation of cortisol (corticosterone in animals) is essential for a positive adaptive response to stress. A biologically effective clock controls production and release, dependent on the stimulation of light reaching receptors in the retina [37]. Studies are investigating the regulatory potential of curcumin in this process, preventing excessive levels of this hormone from influencing the neurophysiology of the adaptive response to stress and resulting in organismal damage [22,23].

Therefore, we decided to analyze plasma corticosterone levels in curcumin-supplemented and control animals, as the levels of this hormone in these animals occur in the opposite direction to those in humans. In rodents, basal and exponential levels are measured during the light period (morning/afternoon), while elevated and peak levels are measured at night. From the collections and analyses, we observed that, in the group of animals control (without curcumin), there was no significant difference in the EXP period, as well as in the PEAK period (Fig. 5).

In the CUR group, we observed a significant increase in corticosterone in the exponential period in non-regrouped animals (1.19 ± 0.27 µg/dL) compared to Har and AgD (0.50 ± 0.19 µg/dL and 0.29 ± 0.15 µg/dL, respectively) (Fig. 6a), however, no significant difference was observed in the peak period (Fig. 6b). When comparing the plasma concentration of corticosterone between the two periods (EXP and PEAK), the harmonious and defeated animals showed a significant increase in the time of most excellent corticosterone release (HAR: 1.04 ± 0.34 µg/dL) ($p<0.01$) (AgD: 0.86 ± 0.27 µg/dL) ($p<0.05$), compared to the time of exponential release, of their respective categories: HAR: 0.50 ± 0.19 µg/dL ($p<0.01$) and AgD: 0.29 ± 0.15 µg/dL ($p<0.05$) (Fig. 6c).

We then decided to compare the plasma corticosterone concentration between the control and curcumin groups at the exponential and peak increase times. We observed that in the exponential period, there was a significant increase in the plasma corticosterone levels of the supplemented NR animals compared to the NR control and the same was observed between the AgD control and AgD supplemented groups (NR CUR: 1.19 ± 0.27 µg/dL; NR CON: 0.68 ± 0.16 µg/dL; AgD CON: 0.83 ± 0.52 µg/dL; AgD CUR: 0.29 ± 0.15 µg/dL) (Fig. 7a). However, in the peak period, no statistical difference in plasma corticosterone levels was observed between the categories (Fig. 7b).

3.3 Analysis of ROS Production by DHE Assay

As previously described, we observed that social stress resulting from regrouping can lead to reduced activity of cytochrome c oxidase IV (COX IV), an enzyme involved

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in the mitochondrial electron transport system, particularly in mice with heightened aggression [13]. Recognizing that mitochondrial dysfunction leads to an increased production of reactive oxygen species (ROS), a recent study conducted by our team revealed an increase in reactive oxygen species in the frontal cortex of highly aggressive MEA animals, indicating possible oxidative stress [13]. Therefore, we propose investigating whether curcumin, with its well-described antioxidant potential in literature [27,38], can attenuate the oxidative stress previously observed in the prefrontal cortex of MEA animals. We analyzed the presence of superoxide, a type of ROS, in the frontal cortex of MEA mice from the control and curcumin groups using the DHE assay. Our results demonstrated that curcumin reduced ROS expression in AgR-supplemented individuals compared to AgR-supplemented individuals in the control group (Fig. 8e).

In a quantitative analysis, our individual results from the control group and the group supplemented with curcumin did not show a significant difference in mean fluorescence (Fig. 9a- b, respectively). However, there was a substantial reduction in the mean fluorescence intensity (MIF) of the aggressive animals in the supplemented group compared to the same category, in the control group, complementing what was seen qualitatively in the previous figure (AGR CUR: 47.23 ± 24.45; AGR CON: 95.61 ± 8.51) (Fig. 9c).

3.4 Fluorescence Immunohistochemistry

For c-Fos analysis, we focused on the prefrontal cortex because it plays a central role in top–down regulation of stress responses, impulse control, and inhibition of pathological aggression, exerting inhibitory control over limbic and hypothalamic circuits [30, 31, 32]. Chronic stress preferentially disrupts prefrontal cortex function, leading to impaired cognitive control and increased impulsivity [30, 32]. Thus, prefrontal cortex c-Fos expression provides a mechanistically relevant readout of stress adaptation [33, 34]. We followed the protocol described by Felippe et al. (2021) and we were able to observe nuclear and perinuclear c-Fos staining in cells of the frontal cortex, unlike the negative control, thereby achieving the standardization necessary for the present study [14]. Figure 10 shows representative images of c-Fos protein expression in the mouse frontal cortex, obtained by immunofluorescence. Cell nuclei were stained with DAPI (blue), while c-Fos expression was visualized in green. The images show a comparison of two experimental groups: the control group (CON) and the curcumin-supplemented group (CUR), distributed according to the different behavioral conditions—NR, Har, AgD, and AgR—induced by MEA. We can observe a greater c-Fos marking in the aggressive animals in the group supplemented with curcumin, primarily in relation to the same category in the control group (Fig. 10e). We analyzed the percentage data of c-Fos-positive cells as well as their distribution across the brain tissue examined in the different experimental subgroups, comparing animals from the control group (CON) with those supplemented with curcumin (CUR), under the following conditions: Non-Regrouped (NR), harmonious (Har), defeated (AgD), and aggressors (AgR) (Fig. 11). In this analysis, we can observe that there was no statistical difference in the distribution of cells stained for c-Fos across the tissue between the categories within their CON and CUR groups (Fig. 11 a, b). However, when comparing categories and experimental groups, the aggressor animals supplemented with curcumin had higher c-Fos expression when compared to the control aggressors (AgR CUR: 581.81 ± 24.5 mm^2; AgR CON: 247.1 ± 15.86 mm^2) (Fig. 11c). This profile was also seen in relation to the percentage of positive cells in general, where this difference was seen within the supplemented group between AgR and AgD

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(AgR: 77.29 ± 8.728 %; AgD: 56.52 ± 11.67 %) (Fig. 11e), as well as between AgR, CUR and AgR CON (AgR CUR: 77.29 ± 8.728 %; AgR CON: 46.05 ± 3.508 %) (Fig. 11f).

4. DISCUSSION

Social stress is defined as stress resulting from interactions with conspecifics and environmental challenges [1,2,4]. When exposure to such stressors is perceived as inescapable, it can become chronic. Chronic social stress constitutes a significant risk factor for the development of neuropsychiatric disorders, which often manifest with aggressive behavior as a prominent symptom [39,40,41]. Aggression is a multidimensional behavior comprising both adaptive and maladaptive components, with attack frequency and attack intensity reflecting partially distinct neurobiological processes [44,45].

In this study, we demonstrate that curcumin supplementation modulates the neurobiological consequences of chronic social stress without preventing aggressive behavior in the MSA. Animals receiving a curcumin-supplemented diet showed reduced lesion severity despite a higher number of attacks, indicating that attack frequency was not directly related to aggression intensity. Although curcumin did not reduce attack frequency, it markedly attenuated injury severity, suggesting a dissociation between the initiation and intensity of aggression. Reduced injury severity may reflect modulation of impulsivity and executive control, consistent with a shift toward a more regulated form of aggressive behavior mediated by prefrontal circuits involved in aggression control and emotion regulation. Such forms of aggression are considered adaptive in social hierarchies, whereas excessive injury-producing aggression is typically associated with stress-related neurobiological dysregulation [24, 31, 46, 47, 48, 49, 51].

The dietary concentration of curcumin adopted in the present study (1.5%) is in line with doses routinely used in murine models assessing behavioral, inflammatory, and oxidative parameters, and has been consistently reported as both biologically active and well tolerated in rodents [36,42]. Although the extrapolation of dietary concentrations from mice to humans is inherently limited by interspecies differences in metabolism, absorption, and bioavailability, clinical evidence indicates that orally administered curcumin exhibits a favorable safety profile in humans. Phase I and dose-escalation clinical trials have demonstrated that curcumin can be safely administered over a broad dosage range, from approximately 0,5 g to 12 g per day [43]. Collectively, these findings support the translational relevance and safety of the curcumin dose employed in the present experimental model.

Curcumin supplementation increased c-Fos expression in the prefrontal cortex (PFC) of aggressive animals, indicating enhanced neuronal engagement within circuits that are critically involved in cognitive control, emotion regulation, and inhibition of impulsive aggression [50, 51, 52]. Given the established role of the PFC in top-down regulation of stress and aggressive behavior, this increased neuronal activity may contribute to the protective effects observed in curcumin-treated animals, including reduced oxidative stress, attenuation of injury severity, and modulation of plasma corticosterone levels following regrouping. Convergent evidence indicates that effective recruitment of prefrontal networks supports adaptive stress responses and limits maladaptive behavioral and physiological outcomes associated with chronic or social stress exposure [32,53]. In this context, c-Fos induction in the PFC is widely recognized as a functional marker of adaptive neuronal activation in response to environmental challenges rather than pathological hyperactivation [54]. Together, these findings support

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the interpretation that curcumin promotes a neuroprotective and stress-adaptive response by facilitating prefrontal control over aggression- and stress-related neural circuits [55].

In previous studies, we have shown increased dopamine levels in the frontal cortex and reduced aggressive behavior after the use of first-line antipsychotics (haloperidol—a dopamine D2 receptor antagonist) and second-generation antipsychotics (risperidone—a dopamine D2 receptor antagonist and serotonin 5-HT1A and 5-HT2A receptor antagonists) in aggressive animals [12,56]. Knowing that dopamine release in the ventromedial cortex prevents excessive activation of the hypothalamic-pituitary-adrenal (HPA) axis during stress [57], we suggest that in our model (MEA), increased dopamine in the cerebral cortex of aggressive animals may prevent an increase in corticosterone levels in AgR mice, unlike what was observed in other categories (AgD and Har) of animals under social stress due to regrouping [17].

Dopamine released in the cortex can bind to dopaminergic receptors in the dorsal hypothalamic region, decreasing the production and release of corticotropin-releasing factor (CRF) [58]. CRF is involved in the activation of the HPA axis, which is the primary adaptive response to stress observed in animals and humans, culminating in the release of corticosterone in animals and cortisol in humans [57]. The fact that curcumin can promote resilience to chronic stress by reducing HPA axis activation, minimizing oxidative damage, and modulating neuroinflammatory pathways [36, 58, 59, 60] led us to investigate corticosterone levels in MSA animals, whether supplemented with or without curcumin.

Corticosterone regulation differed according to behavioral phenotype and sampling time, indicating that curcumin modulates hypothalamic–pituitary–adrenal (HPA) axis activity in a stress- and aggression-dependent manner. In non-supplemented animals, corticosterone levels did not differ significantly among behavioral categories at either the exponential (EXP) or peak (PEAK) phases, suggesting limited endocrine flexibility in response to regrouping. In contrast, curcumin supplementation revealed a differentiated and dynamic pattern of corticosterone regulation. During the EXP phase, curcumin increased corticosterone levels in non-regrouped (NR) animals, while reducing EXP corticosterone levels in defeated animals (AgD), indicating bidirectional modulation depending on stress exposure and behavioral vulnerability.

In non-stressed NR animals, the curcumin-induced increase in corticosterone occurred without exceeding peak concentrations, suggesting a controlled activation of glucocorticoid signaling rather than maladaptive hypercortisolemia. Such moderate elevations are known to exert permissive and preparative effects, facilitating physiological readiness and promoting adaptive stress responses, in contrast to the deleterious consequences of prolonged corticosterone elevation associated with chronic stress exposure [61,62]. Accordingly, curcumin appears to prevent the onset of dysregulated glucocorticoid responses in non-stressed animals by maintaining corticosterone within an adaptive range.

Conversely, in animals exposed to chronic social stress, particularly AgD animals, curcumin reduced corticosterone levels during the EXP phase, suggesting an attenuation of stress-induced HPA axis overactivation. This reduction may limit the detrimental effects of excessive glucocorticoid signaling, including oxidative stress and tissue damage, thereby contributing to improved stress coping. Notably, during the PEAK phase, curcumin supplementation increased corticosterone levels in harmonious and injured animals compared to their respective controls, indicating preservation of an appropriate peak endocrine response. This temporal pattern suggests that curcumin does not blunt HPA axis reactivity but rather refines its dynamics—attenuating excessive early

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activation while maintaining the capacity for a physiologically relevant peak response, a hallmark of adaptive HPA axis regulation [34].

In contrast, aggressive animals (AgR) exhibited no significant changes in corticosterone levels at either time point, regardless of curcumin supplementation. This finding is consistent with previous work from our group showing that highly aggressive animals fail to mount a typical corticosterone response following stress exposure [56]. Together, these observations support the notion that pathological aggression is associated with HPA axis dysregulation and reduced endocrine flexibility, a condition that curcumin is unable to reverse once established.

In summary, curcumin supplementation did not alter the development of aggressive or resilient behavioral phenotypes in the MSA but significantly reduced injury severity in subordinate animals exposed to chronic social stress. This protective effect was associated with a phenotype-dependent modulation of the HPA axis, characterized by attenuation of excessive corticosterone responses in defeated animals and prevention of hypercortisolemia in non-stressed individuals, without restoring corticosterone responsiveness in aggressive phenotypes. At the central level, curcumin increased c-Fos expression in the prefrontal cortex and reduced oxidative stress, supporting an adaptive neurobiological response linked to improved stress coping and impulse regulation.

Together, these findings support our central hypothesis that curcumin supplementation modulates the neuroendocrine and neurobiological consequences of chronic social stress. Although curcumin did not alter the development of aggressive or resilient behavioral phenotypes in the MSA, it reduced the physiological burden of stress through coordinated peripheral and central mechanisms, including phenotype-dependent regulation of the HPA axis, attenuation of oxidative stress, and increased c-Fos expression in the prefrontal cortex. These effects indicate that curcumin promotes adaptive stress coping and neurobiological resilience even in the absence of overt behavioral changes.

This study has some limitations that should be considered when interpreting the present findings. Curcumin was administered by incorporation into the chow of group-housed animals, which precluded direct measurement of individual food intake and, consequently, of the exact dose consumed by each animal. In addition, curcumin and its metabolites were not quantified in plasma or brain tissue; therefore, central nervous system exposure could not be confirmed experimentally and was inferred indirectly from the modulation of oxidative stress and c-Fos expression observed in the prefrontal cortex. Despite these limitations, the consistency of the endocrine, oxidative, and neuronal activation findings supports the conclusion that curcumin modulates neurobiological responses associated with chronic social stress.

5. Conclusion

The results of the ROS analysis in this study confirmed the antioxidant effect of curcumin on brain oxidative stress induced by regrouping stress in highly aggressive animals. Furthermore, in these individuals, curcumin's ability to increase c-Fos expression in a manner dissociated from the elevation of reactive oxygen species indicates a profile of action focused on the functional and adaptive modulation of cortical activity, rather than simply a response to oxidative damage.

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Overall, the results presented here reinforce the idea that curcumin acts as a multifunctional compound, promoting neuroendocrine and neurochemical adaptations that favor emotional control and resilience in the face of chronic social stress. These findings significantly contribute to the understanding of curcumin's effects in contexts of behavioral vulnerability, opening promising prospects for its use as a therapeutic adjunct in neuropsychiatric disorders associated with stress and behavioral changes.

Author contributions

RB, RM and VF contributed to Methodology, Formal analysis, Investigation, Writing - Original Draft, Review & Editing, and Visualization. **RB, RM, LS, SH, GO and VF** contributed to the Investigation. **TA-J** contributed to Writing - Review & Editing**. GO and VF** contributed to Methodology, Formal analysis, writing of the original draft, review and editing, Visualization, and Supervision. **VF and TA-J** contributed to the acquisition of Conceptualization, Resources, and Funding. **VF** contributed to Project administration. All authors read and approved the manuscript.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to the raw data containing detailed records of behavioral observations, videos, and laboratory notes that may include identifiable information about the experimenters, environmental conditions, and proprietary laboratory methodologies, protected by institutional confidentiality. However, the processed data and statistical analyses are described in the body of the work, ensuring transparency and reproducibility of the results within the current ethical and institutional limits. The raw data is available from the corresponding author on reasonable request.

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REFERENCES

[1] McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev. Jul;87(3):873-904, 2007. DOI: 10.1152/physrev.00041.2006.

[2] Antunes J. Stress and Disease: What Does Evidence Say? Psico saúde & doenças, 20(3), 590-603, 2019. DOI: 10.15309/19psd200304.

[3] Williams, W.A & Potenza, M.N. Neurobiology of Impulse Control Disorders. Rev. Bras. Psiquiatr. São Paulo, v. 30, supl. 1, p. S24-S30, May 2008.

[4] Souza, G. F. d. A., Praciano, G. d. A. F., Revorêdo, L. M. Y., Ramos, I. T. d. F., Neto, O. d. C. F., Rego, J. S. d. O et al. Factors Associated with Stress, Anxiety, and Depression

ARTICLE IN PRESS

in Patients with Chronic Diseases during the Social Distancing Period. Research, Society and Development, 2021; 10(14), e339101422211. https://doi.org/10.33448/rsd-v10i14.22211.

[5] Joëls M & Baram, TZ. The Neurosymphony of Stress. Nat Rev Neurosci. 2009; 10 (6): 459-466. DOI: 10.1038 / nrn2632.

[6] Guest FL; Martins-de-Souza DRH; Bahn S; Guest PC. Effects of Stress on Hypothalamic-Pituitary-Adrenal Axis Function in Individuals with Schizophrenia. Rev Psiq Clín. 2013;40(1):20-7. DOI: 10.1590/S0101-60832012005000002.

[7] Patriquin MA & Mathew SJ. The Neurobiological Mechanisms of Generalized Anxiety Disorder and Chronic Stress. Chronic Stress (Thousand Oaks). 2017 Jan-Dec; 1:2470547017703993. DOI: 10.1177/2470547017703993.

[8] McCall JG. et al. CRH Engagement of the Locus Coeruleus Noradrenergic System Mediates Stress-Induced Anxiety. Neuron, v.87, p. 605-620, 2015. DOI: 10.1016/j.neuron.2015.07.002.

[9] World Health Organization (WHO). The World mental health report: transforming mental health for all. Acesso em 15 de fevereiro de 2025. Disponível em: ISBN 978-92-4-004933-8.

[10] World Health Organization (WHO). WHO – Mental Disorders. Acesso em 15 de fevereiro de 2025. Disponível em: https://www.who.int/news-room/fact-sheets/detail/mental-disorders.

[11] Blanchard RJ, Hori K, Blanchard DC. Social dominance and individual aggressiveness. Aggression Behavior. 1988; 14:195-203.

[12] Fragoso VM, Hoppe LY, de Araújo-Jorge TC, de Azevedo MJ, Campos JD, Cortez CM et al. Use of haloperidol and risperidone in highly aggressive Swiss Webster mice by applying the model of spontaneous aggression (MSA). Behavioral Brain Research. v.01, p. 110-118, 2016. DOI: 10.1016/j.bbr.2015.12.010.

[13] Mendonça APM.; Hoppe LY; Gaviraghi A; Araújo-Jorge TC; Oliveira GM.; Felippe RM, et al. (2019). Highly aggressive behavior induced by social stress is associated to reduced cytochrome c oxidase activity in mice brain cortex. Neurochemistry International, v. 26, n. 126, p. 210-217. DOI: 10.1016/j.neuint.2019.03.017.

[14] Felippe RM, Oliveira GM, Barbosa RS, Esteves BD, Gonzaga BMS, Horita SIM et al. Experimental Social Stress: Dopaminergic Receptors, Oxidative Stress, and c-Fos Protein Are Involved in Highly Aggressive Behavior. Fron in Cel Neur. 2021; 15: 1-14.

[15] Felippe RM, Barbosa RS, Esteves BD, Oliveira GM, Araújo-Jorge TC, Fragoso VMS. Analysis of the Mice Activity Profile in the Model of Spontaneous Aggression (MSA). American Journal of Biomedical Science & Research. 2022;15: 444-451.

[16] Batista WS, Silva LCCP, Demarque KC, Oliveira FS, Acquarone M, Bôas FV et al. Study of Aggressive Behavior in Laboratory Mice: Application of the Model

ARTICLE IN PRESS

Spontaneous Aggressiveness (MSA).**Revista da Sociedade Brasileira de Ciências em Animais de Laboratório. 2012; 1(4): 322-335.

[17] Oliveira FS, Silva AB, Costa LM, Pereira JT, Souza RD, Almeida VC. Characterization of dominant and subordinate social status and the structure of the social hierarchy in Swiss Webster mice. *Rev Soc Bras Cienc Anim Lab.* 2013; 2:136–46.

[18] Toyoda A. Nutritional interventions for promoting stress resilience: Recent progress using psychosocial stress models of rodents. Anim Sci J. 2020 Jan;91(1): e13478. Doi: 10.1111/asj.13478.

[19] Goto T. & Toyoda A. A mouse model of subchronic and mild social defeat stress for understanding stress-induced behavioral and physiological 6mune6nc. Journal of Visualized Experiments. 2025;105, e52973 10.3791/52973.

[20] Sial OK; Warren BL; Alcantara LF; Parise EM & Bolanos-Guzman CA. Vicarious social defeat stress: Bridging the gap between physical and emotional stress. Journal of Neuroscience Methods. 2016; 258, 94–103. 10.1016/j.jneumeth.2015.10.012.

[21] Harris AZ; Atsak P; Bretton ZH; Holt ES; Alam R; Morton MP et al. A novel method for chronic social defeat stress in female mice. Neuropsychopharmacology. 2018; 43, 1276–1283. 10.1038/npp.2017.259.

[22] Hodes GE; Pfau ML; Leboeuf M; Golden SA; Christoffel DJ; Bregman D et al. Individual 7mune7nces in the peripheral 7mune system promote resilience versus susceptibility to social stress. Proceedings of the National Academy Science of the United States of America. 2014;111, 16136–16141. 10.1073/pnas.1415191111.

[23] Zhang L; Virgous C & Si H . Synergistic anti-inflammatory effects and mechanisms of combined phytochemicals. Journal of Nutritional Biochemistry. 2019;69, 19–30. 10.1016/j.jnutbio.2019.03.009.

[24] Siever LJ. Neurobiology of Aggression and Violence. American Journal of Psychiatry. 2008 Apr;165(4):429–42.

[25] Ferris CF, Stolberg T, Kulkarni P, Murugavel M, Blanchard R, Blanchard DC, Febo M, Brevard M, Simon NG. Imaging the neural circuitry and chemical control of aggressive motivation. BMC Neurosci. 2008 Nov 13;9:111. doi: 10.1186/1471-2202-9-111. PMID: 19014547; PMCID: PMC2601047.

[26] Goedhard L, Stolker J, Nijman H, Egberts A, Heerdink E. Aggression of Psychiatric Patients Associated with the Use of As-needed Medication. Pharmacopsychiatry. 2007 Jan;40(1):25–9.

[27] Sueth-Santiago V, Mendes-Silva GP, Decoté-Ricardo D, Lima MEF de. CURCUMIN, THE GOLDEN POWDER FROM TURMERIC: INSIGHTS INTO CHEMICAL AND BIOLOGICAL ACTIVITIES. Química Nova. 2015;38(4).

[28] Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as “Curecumin”: From kitchen to clinic. Biochemical Pharmacology. 2008 Feb;75(4):787–809.

ARTICLE IN PRESS

[29] Mélo, R.M.F. et al. Influence of leptin and its receptors on individuals under chronic social stress behavior. Front. Endocrinol. 2024; 15:1281135. doi: 10.3389/fendo.2024.1281135.

[30] Arnsten AFT. Stress signalling pathways that impair prefrontal cortex structure and function. Nat Rev Neurosci. 2009;10:410–422. doi:10.1038/nrn2648.

[31] Davidson RJ, Putnam KM, Larson CL. Dysfunction in the neural circuitry of emotion regulation—a possible prelude to violence. Science. 2000;289(5479):591–594. doi:10.1126/science.289.5479.591.

[32] McEwen BS, Morrison JH. The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course. Neuron. 2013;79(1):16–29. doi:10.1016/j.neuron.2013.06.028.

[33] Quirk GJ, Beer JS. Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. Curr Opin Neurobiol. 2006;16(6):723–727. doi:10.1016/j.conb.2006.07.004.

[34] Herman JP, et al. Regulation of the hypothalamic–pituitary–adrenocortical stress response. Compr Physiol. 2016;6(2):603–621.

[35] Steru, L.R.; Chermat, B.T.; Simon, P. The tail suspension test: a new method for screening antidepressants in mice, Psychopharmacol. 1985; v. 85, n.3, p.367–370. https://doi.org/10.1007/BF00428203.

[36] Aubry, AV.; Khandaker, H.; Ravenelle, R.; Grunfeld, I.S.; Bonnefil, V.; Chan, K.L. & Burghardt, NS (2019). A diet enriched with curcumin promotes resilience to chronic social defeat stress. Neuropsicofarmacologia, 44, 733-742.10.1038/s41386-018-0295-2.

[37] Loures DL, et al. Mental stress and the cardiovascular system. Arq Bras Cardiol. 2002;78(5):525–530. doi:10.1590/S0066-782X2002000500012.

[38] Zhou H, Beevers CS, Huang S. The targets of curcumin. Curr Drug Targets. 2011;12(3):332-347. doi:10.2174/138945011794815356.

[39] Ryan, T.A. et al. A MAPK/c-Jun-mediated switch regulates the initial adaptive and cell death responses to mitochondrial damage in a neuronal cell model—The International Journal of Biochemistry & Cell Biology. 2018; v. 104, p. 73–86.

[40] Yang, C.R. et al. Enhanced Aggressive Behaviour in a Mouse Model of Depression. Neurotoxicity Research. 2015; v. 27, n. 2, p. 129-142. doi: 10.1007/s12640-014-9498-4.

[41] Cassiello-Robbins, C. et al. The effects of aggression on symptom severity and treatment response in a trial of cognitive behavioral therapy for panic disorder. Comprehensive Psychiatry. 2015; v. 60, p. 1-8. Doi: 10.1016/j.comppsych.2015.04.012.

[42] Ejaz A, Wu D, Kwan P, Meydani M. Curcumin inhibits adipogenesis in 3T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice. J Nutr. 2009;139(5):919–925.

ARTICLE IN PRESS

[43] Hewlings SJ, Kalman DS. Curcumin: A review of its effects on human health. Foods. 2017;6(10):92.

[44] Miczek KA, Fish EW, De Bold JF, De Almeida RMM. Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and GABA systems. Psychopharmacology (Berl). 2002;163(3–4):434–458.

[45] Nelson RJ, Trainor BC. Neural mechanisms of aggression. Nat Rev Neurosci. 2007;8(7):536–546.

[46] Miczek KA, et al. Neurobiology of escalated aggression and violence. J Neurosci. 2007;27(44):11803–11806.

[47] Blanchard RJ, Wall PM, Blanchard DC. Problems in the study of rodent aggression. Horm Behav. 2003;44(3):161–170.

[48] Haller J. The role of central and peripheral factors in aggression-related pathology. Curr Top Behav Neurosci. 2014;17:31–45.

[49] Haller J, Tóth M, Halász J, De Boer SF. Patterns of violent aggression-induced brain c-Fos expression in male mice selected for aggressiveness. Physiol Behav. 2006;88(1–2):173–182.

[50] Li X, Xiong L, Li Y. The role of the prefrontal cortex in modulating aggression in humans and rodents. Behav Brain Res. 2025;476:115285.

[51] Chen Z, et al. Brain network localization of structural and functional alterations underlying aggression and impulsivity in humans. Transl Psychiatry. 2025;15:136.

[52] Denson TF. Interventions for reducing interpersonal reactive aggression: a neurocognitive perspective. Clin Psychol Rev. 2025;50:101980.

[53] Lupien SJ, et al. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci. 2009;10(6):434–445.

[54] Kovács KJ. c-Fos as a transcription factor: a stressful (re)view from a functional map. Neurochem Int. 1998;33(4):287–297.

[55] Islam MR, et al. Neuroprotective potential of curcumin: molecular insights and therapeutic opportunities. J Biochem Mol Toxicol. 2025;39:e23456.

[56] Oliveira, F.S. et al. HPLC description of norepinephrine, dopamine, and serotonin concentrations in different brain regions of highly aggressive Swiss Webster mice. Revista- Sociedade Brasileira de Ciência em Animais de Laboratório.2014; v.2, n. 4, p. 322-335.

[57] Tielbeek, J.J. et al. The impact of chronic stress during adolescence on the development of aggressive behavior: A systematic review on the role of the dopaminergic system in rodents. Neuroscience and Biobehavioral Reviews, v.91, p. 187-197, 2018.

ARTICLE IN PRESS

[58] Asai A, Miyazawa T. Occurrence of orally administered curcuminoid as glucuronide and glucuronide/sulfate conjugates in rat plasma. Life Sciences [Internet]. 2000 Oct 27 [cited 2020 Dec 3];67(23):2785–93. Available from: https://pubmed.ncbi.nlm.nih.gov/11105995/.

[59] Lopresti AL. Curcumin for neuropsychiatric disorders: a review of in vitro, animal and human studies. J Psychopharmacol. 2017;31(3):287–302.

[60] Xu Y, et al. Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor expression in chronically stressed rats. Brain Res. 2018;1687:84–95.

[61] Goh C, Agius M. The stress-vulnerability model how does stress impact on mental illness at the level of the brain and what are the consequences? Psychiatr Danub. 2010 Jun;22(2):198-202. PMID: 20562747.

[62] Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev. 2000;21(1):55–89.

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Fig. 1 Experimental Design of the MSA. In each trial, male Swiss Webster mice were used from the juvenile stage (3rd postnatal week) to adulthood (16th postnatal week). Behavioral tests, including TST, ethogram analysis, and PAB, were conducted at the 4th, 6th, and 8th weeks of age (woa). Ten days before regrouping, the supplementation protocol was initiated: one group (n = 30) received a curcumin-supplemented diet (1.5% curcumin + 15% protein), while the other group (n = 30) received a control diet containing 15% protein. On the 10th postnatal week, animals were regrouped based on their TST results. Ethogram and PAB tests were repeated on the 12th, 14th, and 16th week. The curcumin supplementation protocol was maintained throughout the MSA. After behavioral categorization, all animals were euthanized, and blood and brain samples were collected for analysis of target proteins and hormones using ELISA, fluorescence microscopy, and DHE staining. Adapted from Mélo et al., 2024 [29].

Fig. 2 Behavioral assessment of control animals (CON) before and after the implementation of the MSA, without supplementation. (A) Percentage of mice according to mobility assessment during the regrouping phase: Hyperactive – medium green; Median, median mobility – light green; Hypoactive – dark green (n=66). (B) Evaluation of the Pattern of Aggressive Behavior (PAB) based on animal categorization after regrouping: NR (non-regrouped), Har (harmonious), AgD (defeated), and AgR (aggressor). Score 0: no biting or visible body injuries; Score 1: low frequency of aggressive events; Score 2: moderate aggression and moderate injuries on the tail, back, and scrotal sac; Score 3: intense aggression and severe injuries on the tail, back, and scrotal sac (n =36). (C) Lesion area (cm^2) across the different behavioral categories. (D) Number of attacks initiated by AgR animals toward their cage mates (n=34).

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Values are expressed as means from three independent experiments. *** Indicates statistical significance ($p < 0.001$) between AgD and AgR groups. **** Indicates statistical significance ($p < 0.0001$) between AgD and both NR and Har groups, as well as between AgR and all other categories, according to the Kruskal-Wallis test followed by Dunn's multiple comparisons post hoc test.

Fig. 3 Behavioral assessment of animals supplemented with curcumin (CUR) before and after the implementation of the MSA. (A) Percentage of mice based on mobility evaluation during the regrouping phase, according to categorization into NR (non-regrouped), Har (harmonious), AgD (defeated), and AgR (aggressor): Hyperactive – medium green; Median, average mobility – light green; Hypoactive – dark green (n=31). (B) Evaluation of the Pattern of Aggressive Behavior (PAB) based on the same categorization (NR, Har, AgD, and AgR) after regrouping. Score 0: no bites or visible injuries on the body; Score 1: low frequency of aggressive events; Score 2: moderate aggression and moderate injuries on the tail, back, and scrotal sac; Score 3: severe aggression and extensive injuries on the tail, back, and scrotal sac (n=39). (C) Lesion area (cm^2) across categories (n=34). (D) Number of attacks performed by AgR animals toward other individuals (n=34). Values are expressed based on results from three independent experiments. *** Indicates statistical significance ($p < 0.001$) between AgD and AgR groups. **** Indicates statistical significance ($p < 0.0001$) between AgD and both NR and Har groups, as well as between AgR and all other categories, according to the Kruskal–Walli's test followed by Dunn's multiple comparisons post hoc test.

Fig. 4 Comparative analysis of aggressive behavior through ethogram between the control (CON) and curcumin-supplemented (CUR) groups after the application of the MSA.
(A) Lesion area (cm^2) among the categories NR (non-regrouped), Har (harmonic), AgD (defeated), and AgR (aggressive), and between the experimental control and curcumin groups. (B) Number of attacks by AgR animals compared to other individuals, between the control and curcumin experimental groups.
Values represent results from three independent experiments.
*Indicates statistical significance ($p < 0.05$) between the AgR groups of the control and curcumin groups. **Indicates statistical significance ($p < 0.01$) between animals from the control and curcumin groups, by Two-way ANOVA with mixed-effects analysis.

Fig. 5 Evaluation of plasma corticosterone concentration (µg/dL) during light/dark periods (EXP/PEAK) in control animals (CON) - without curcumin supplementation. (A) Plasma corticosterone measured in CON animals during the light period (EXP): NR (non-regrouped) (light brown), Har (harmonious) (light pink), AgD (defeated) (light orange), and AgR (aggressive) (orange). (B) Plasma corticosterone measured in CON animals during the dark period (PEAK): NR (non-regrouped) (light brown), Har (harmonious) (light pink), AgD (defeated) (light orange), and AgR (aggressive) (orange). (C) Comparative analysis of plasma corticosterone concentration in the control group between EXP and PEAK periods. Values correspond to 2 independent experiments (n=42). Comparisons among behavioral categories were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons post hoc test, while comparisons between EXP and PEAK periods were analyzed using two-way ANOVA with mixed-effects analysis. Statistical significance was set at $p < 0.05$.

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Fig. 6 Evaluation of plasma corticosterone concentration (µg/dL) during light/dark periods (EXP/PEAK) in curcumin-supplemented (CUR) animals. (A) Plasma corticosterone measured in CUR animals during the light period (EXP): NR (non-regrouped) (light brown), Har (harmonious) (light pink), AgD (defeated) (light orange), and AgR (aggressive) (orange). (B) Plasma corticosterone measured in CUR animals during the dark period (PEAK): NR (non-regrouped) (light brown), Har (harmonious) (light pink), AgD (defeated) (light orange), and AgR (aggressive) (orange). (C) Comparative analysis of plasma corticosterone concentration in the group during EXP and PEAK periods. Values correspond to 2 independent experiments (n=59). Comparisons among behavioral categories were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons post hoc test, while comparisons between EXP and PEAK periods were analyzed using two-way ANOVA with mixed-effects analysis. $^{**}p < 0.01$ (AgD PEAK vs. AgD EXP); $^{*}p < 0.05$ for indicated comparisons.

Fig. 7 Comparative analysis of plasma corticosterone concentration (µg/dL) between CON and CUR groups during light/dark periods (EXP/PEAK). (A) Plasma corticosterone measured during the exponential period (EXP), and (B) plasma corticosterone measured during the peak period (PEAK) in control and curcumin-supplemented animals across the categories NR (non-regrouped), Har (harmonious), AgD (defeated), and AgR (aggressive): CON (control) (light orange striped bars) and CUR (curcumin) (dark orange dotted bars). Indicates statistical significance ($^{*}p < 0.05$) between NR CUR EXP and NR CON EXP, and also between AgD CON EXP and AgD CUR EXP. Comparisons between experimental groups (CON vs. CUR) were analyzed using two-way ANOVA with mixed-effects analysis.

Fig. 8 Superoxide analysis as mean fluorescence intensity in the prefrontal cortex of control (CON) and curcumin-supplemented (CUR) mice. Representative images showing superoxide production labeling (in red): (a) Negative Control (DAPI - blue) CON and CUR, respectively; (b) non-regrouped (NR) CON and CUR; (c) harmonious (Har) CON and CUR; (d) defeated (AgD) CON and CUR; and (e) aggressive (AgR) CON and CUR—scale bar: 20 µm.

Fig. 9 Quantification of superoxide production as mean fluorescence intensity in the prefrontal cortex of control (CON) and curcumin-supplemented (CUR) mice. Superoxide production was quantified by the DHE assay through mean fluorescence intensity (MFI) as follows: (A) Individual comparison of MFI for each category of control group mice: NR (non-regrouped) (black bar), Har (harmonious) (gray bar), AgD (defeated) (dark gray bar), and AgR (aggressive) (light gray bar); (B) Individual comparison of MFI for each category of curcumin group mice; and (C) Comparison of MFI between control (black bars) and curcumin (gray bars) group mice for each category. Values are expressed as mean ± standard deviation (SD) from two independent experiments (n=25). Comparisons among behavioral categories within the same experimental group were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons post hoc test, whereas comparisons between CON and CUR groups were analyzed using two-way ANOVA with mixed-effects analysis. $^{*}p < 0.05$ indicates statistical significance for the indicated comparisons.

Fig. 10 Expression of c-Fos-positive cells in the frontal cortex of mice. Representative images showing labeling of cells expressing c-Fos protein (green) in the frontal: (a) Negative Control (DAPI-blue); (b) non-regrouped (NR) CON and CUR, respectively; (c)

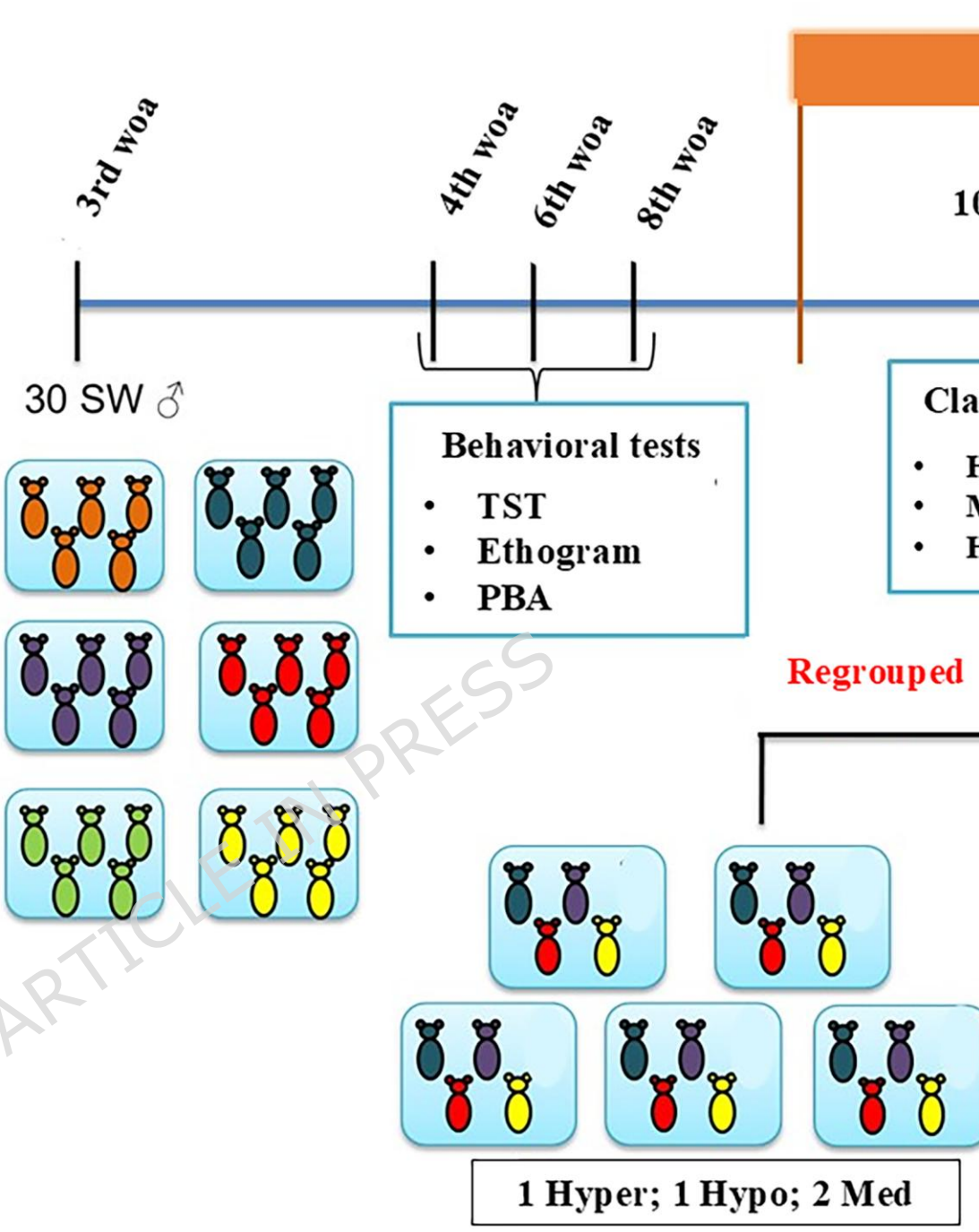
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harmonic (Har) CON and CUR; (d) defeated (AgD) CON and CUR; and (e) aggressive (AgR) CON and CUR. Scale bar = 50 μm.

Fig. 11 Quantification of c-Fos protein expression in the frontal cortex of mice as the number of positive cells per mm².
Quantification of c-Fos-positive cells in the frontal cortex, expressed as the area (mm²) occupied by immunolabeled cells, analyzed within the control (CON) group, within the curcumin-supplemented (CUR) group, and by comparison between CON and CUR groups across behavioral categories: non-regrouped (NR) (dark green bar), harmonious (Har) (green bar), defeated (AgD) (medium green bar), and aggressive (AgR) (light green bar). Values are expressed as mean ± standard deviation. Comparisons among behavioral categories (n= CON -16 ; CUR -15) within the same experimental group were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons post hoc test, whereas comparisons between CON and CUR groups within the same behavioral category were analyzed using two-way ANOVA with mixed-effects analysis. Statistical significance was set at $p < 0.05$.

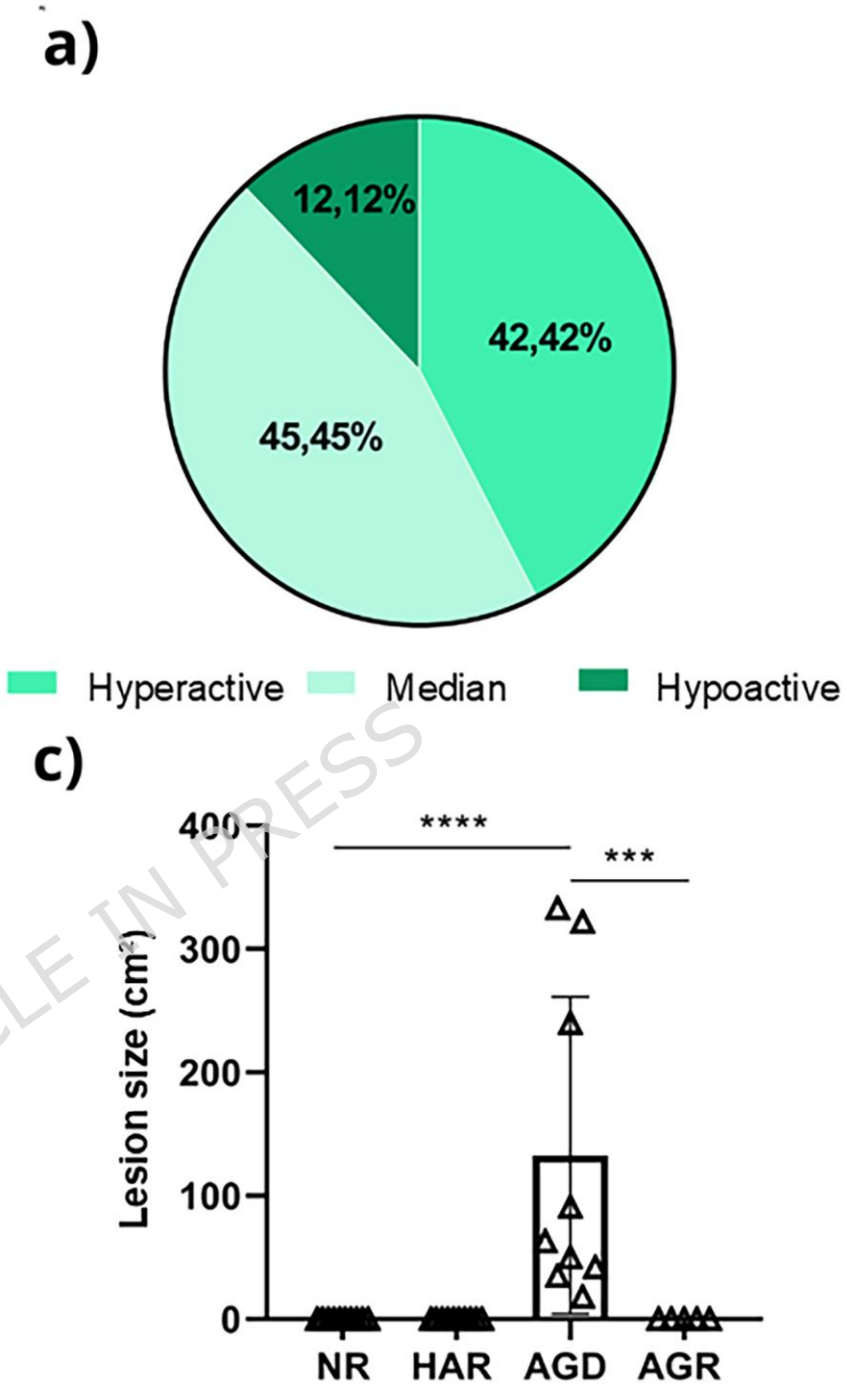
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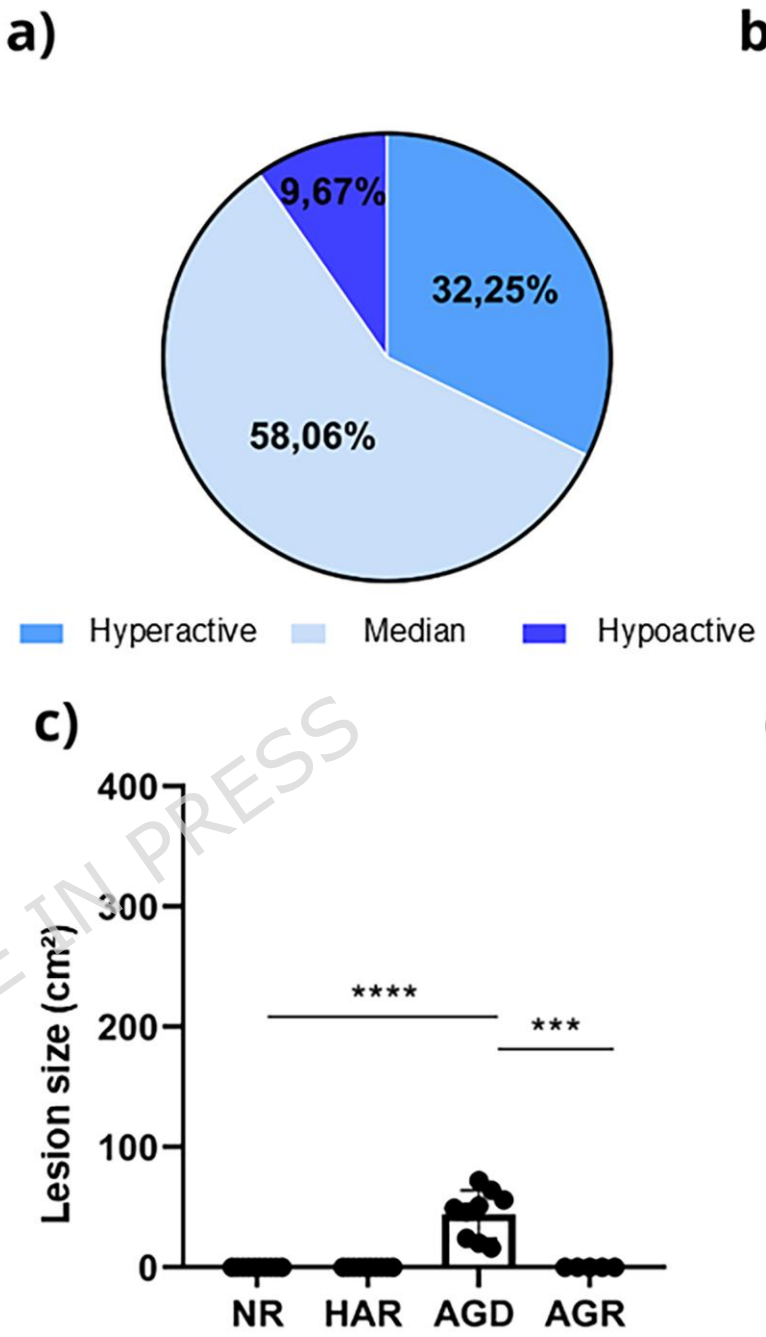
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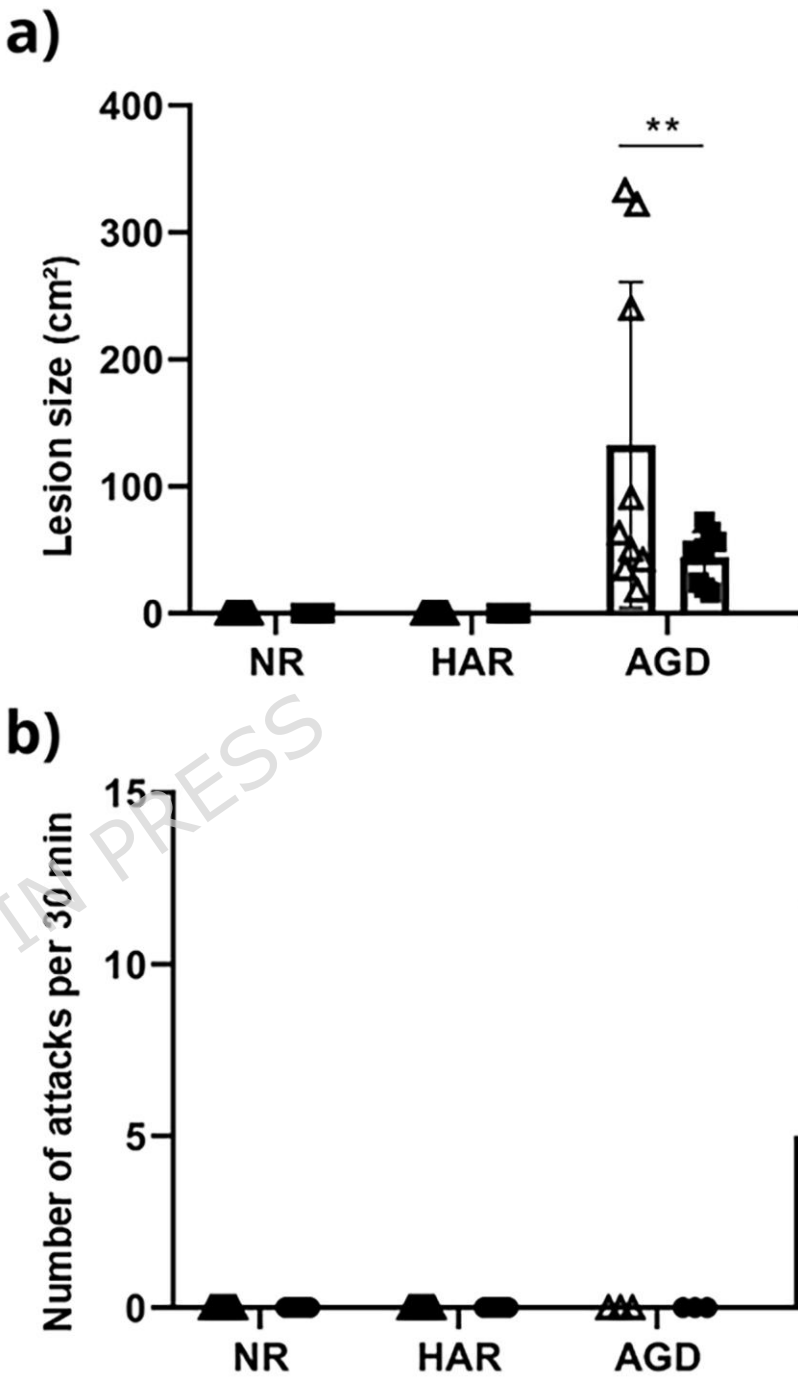
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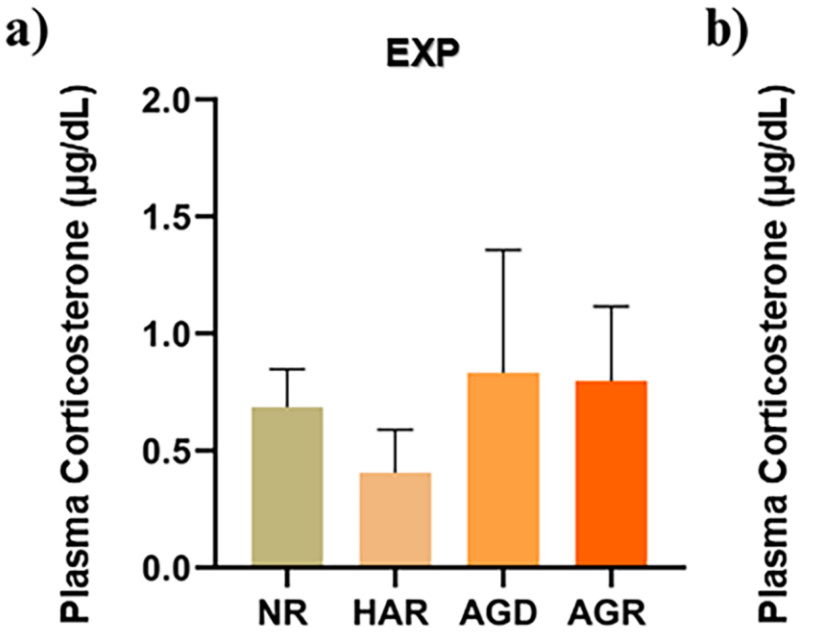
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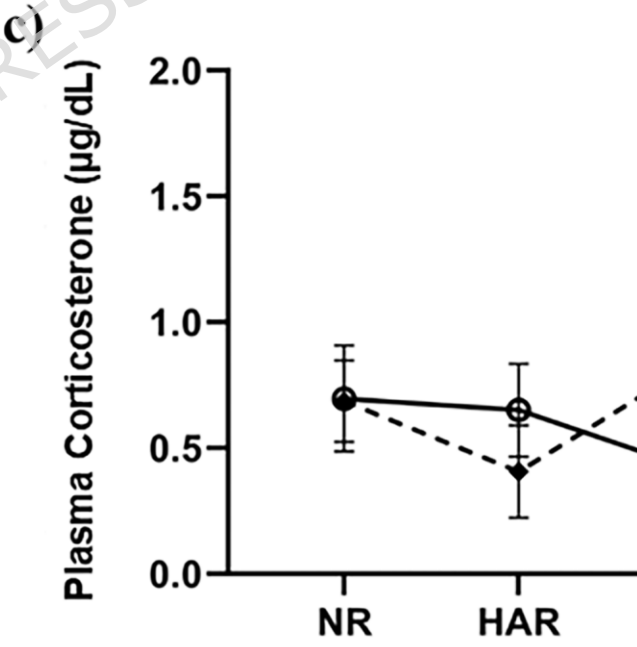
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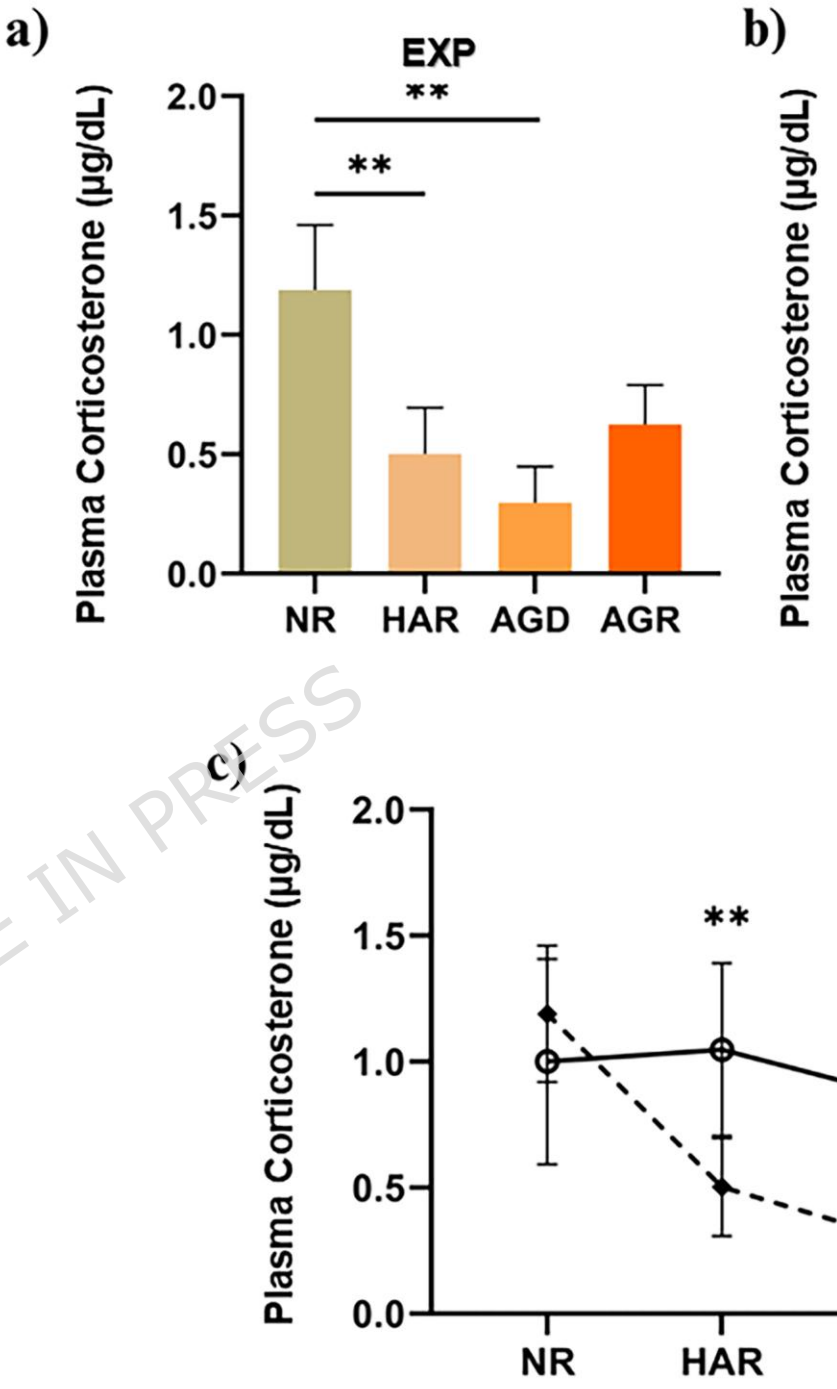
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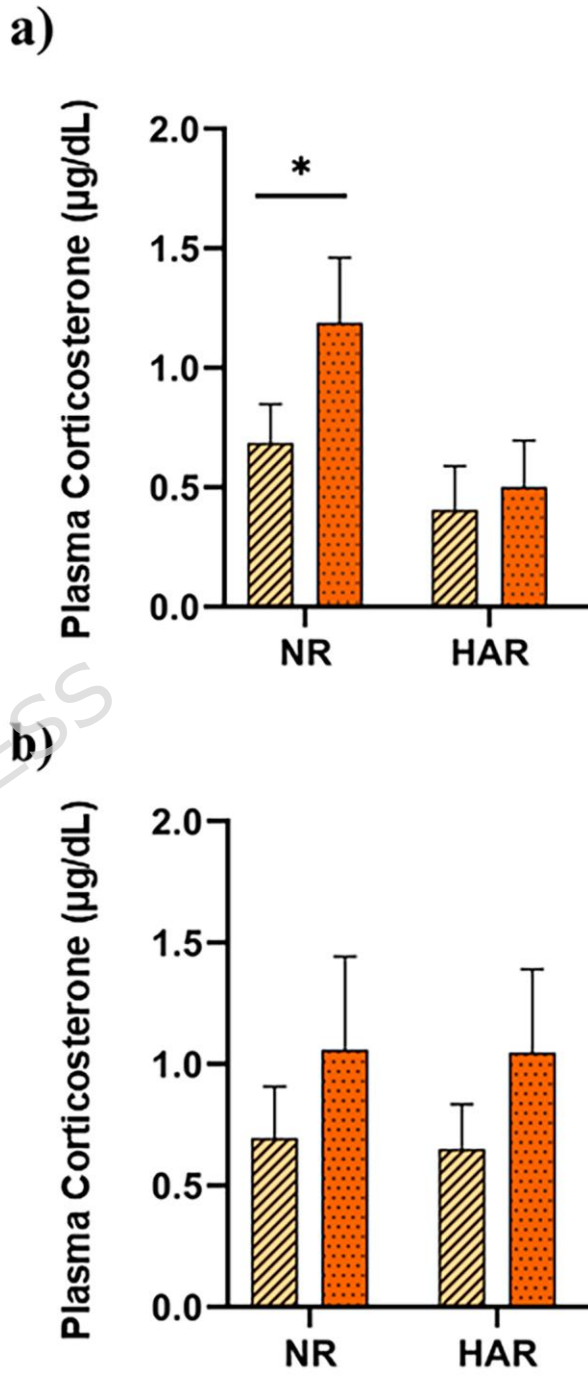
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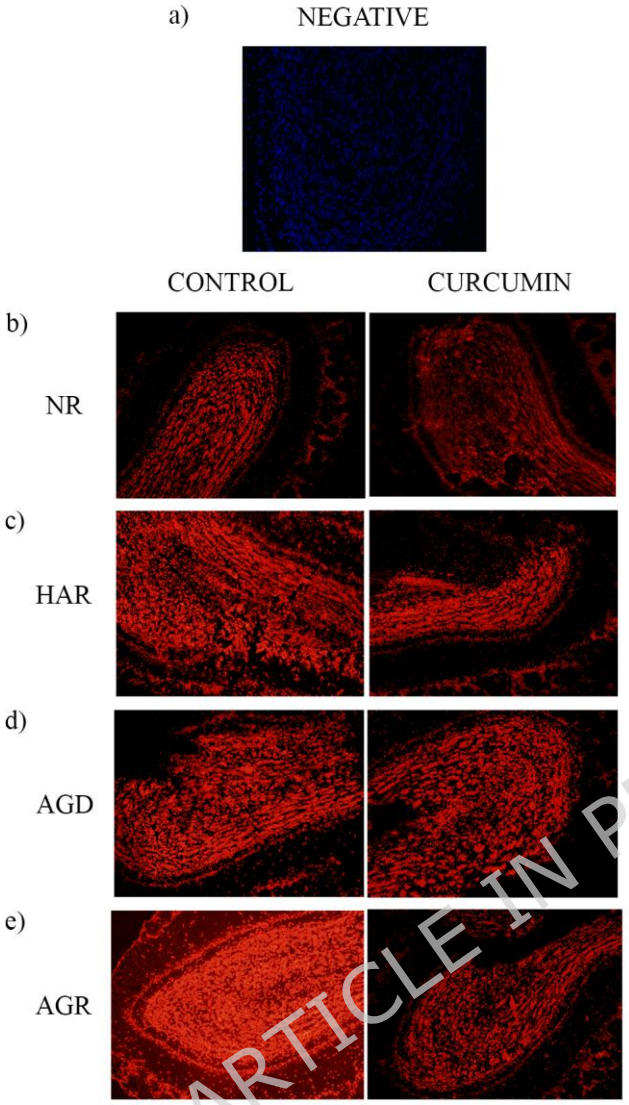
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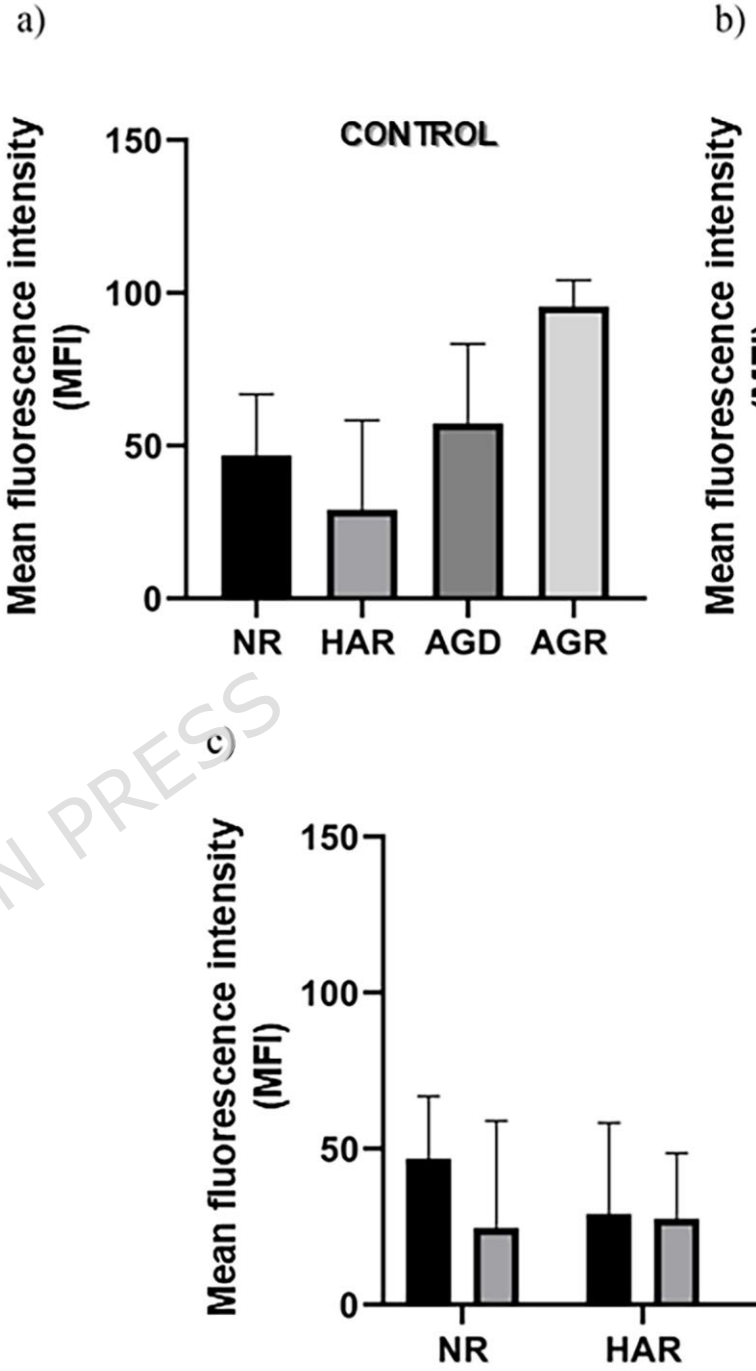
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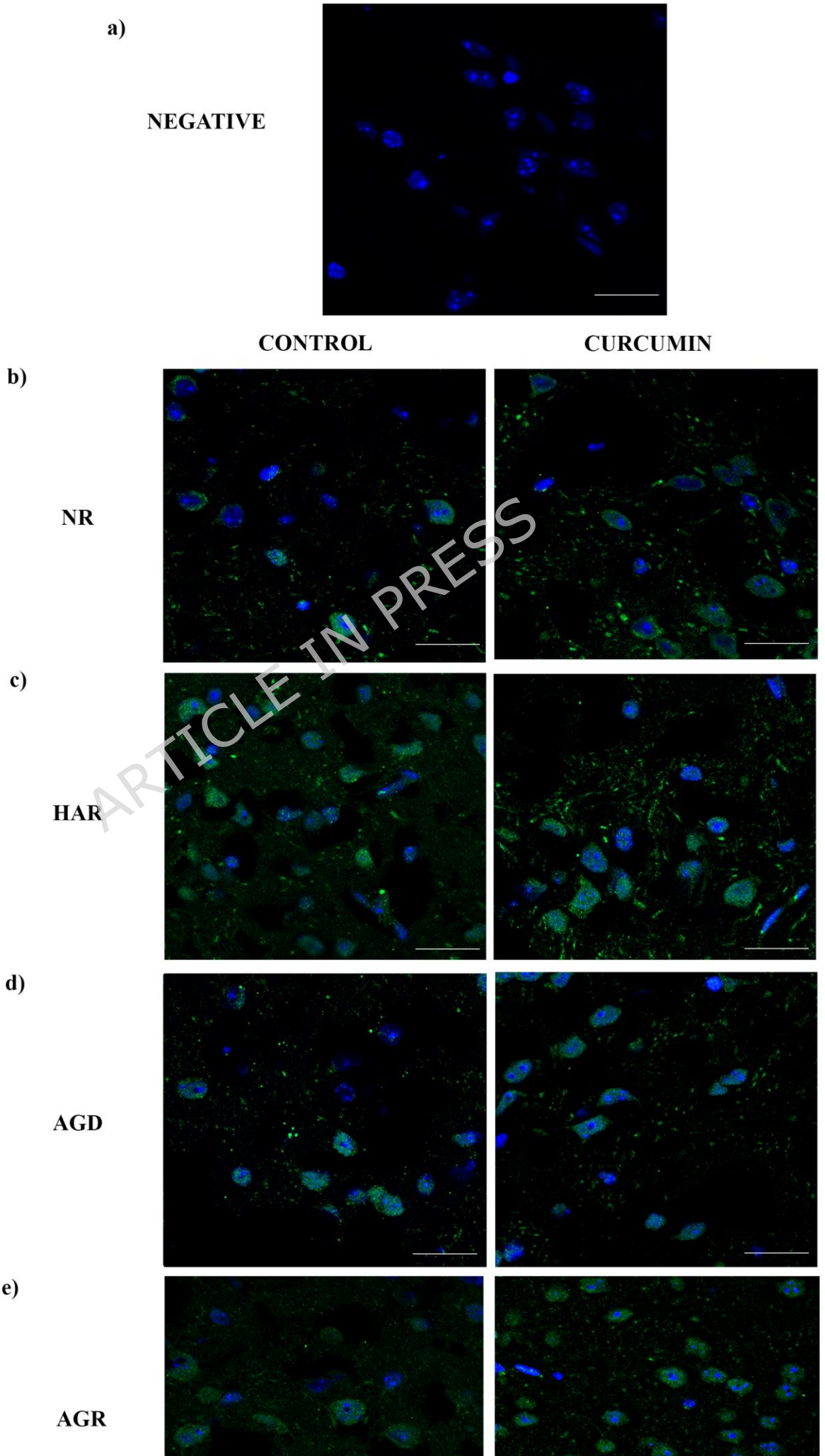
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