



SOCIAL ISOLATION IN JUVENILE PHASE ALTERS THE NORMAL PLASTICITY OF THE SOMATOSENSORY CORTEX IN FEMALE ADOLESCENT RATS

ISOLAMENTO SOCIAL NA FASE JUVENIL ALTERA A PLASTICIDADE NORMAL DO CÓRTEX SOMATOSSENSORIAL EM RATAS ADOLESCENTES

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ABSTRACT: Childhood social isolation results from a lack of contact with peers, and socialization is important in the normal development of social species such as humans and rodents. The aim of this study was to evaluate the effects of social isolation stress in the juvenile phase on the somatosensory cortex, a region related to the perception of tactile stimuli. The cortical thickness and density of dendritic spines in the Upper Lip, Barrel Cortex and Secondary Somatosensory subregions were analyzed. Twenty-seven Wistar rats (*Rattus norvegicus*) were used, organized into Control and Social Isolation Stress groups. Social isolation took place between 21 and 35 days of postnatal life (P21-P35) and euthanasia was carried out at P43. The Nissl and Golgi-Cox histotechniques were used for histological analysis. The results showed neuroanatomical alterations in the group submitted to isolation, where Upper Lip showed less cortical thickness, associated with a lower density of spines. Barrel Cortex showed greater thickness, which may depend on neuronal factors that were not investigated.

KEYWORDS: Cerebral cortex. Cortical thickness. Dendritic spines. Stress.

RESUMO: O isolamento social infantil resulta da falta de contato com congêneres, sendo a socialização importante no desenvolvimento normal das espécies sociais, como o homem e roedores. O objetivo foi avaliar os efeitos do estresse de isolamento social na fase juvenil no córtex somatossensorial, região relacionada à percepção dos estímulos táteis. Analisou-se a espessura cortical e densidade de espinhas dendríticas das subregiões: Upper Lip, Barrel Cortex e Somatossensorial Secundário. Foram utilizadas 27 ratas Wistar (*Rattus norvegicus*) organizadas nos grupos: Controle e Estresse de Isolamento Social. O Isolamento Social ocorreu entre 21 a 35 dias de vida pós-natal (P21-P35) e, em P43, foi realizado eutanásia. Para análises histológicas utilizou-se as histotécnicas Nissl e Golgi-Cox. Os resultados demonstraram alterações neuroanatômicas no grupo submetido ao isolamento, onde Upper Lip apresentou menor espessura cortical, sendo associado a menor densidade de espinhas. Barrel Córtex identificou-se maior espessura, que pode depender de fatores neuronais não investigados.

PALAVRAS-CHAVE: Córtex cerebral. Espessura cortical. Espinhas dendríticas. Estresse.

INTRODUCTION

Childhood stress plays a key role in the onset of various psychiatric disorders, often manifesting with cognitive symptoms that affect overall well-being. Currently, it is estimated that 64% of adolescents suffer from depression or anxiety, with a higher risk for girls (84%) compared to boys (61%).¹ The consequences of the Covid-19 pandemic have further highlighted the negative effects of social restrictions, contributing to a 25% increase in the global prevalence of depression and anxiety.² A growing number of studies suggest a connection between behavioral factors and neuroanatomical changes, with the cerebral cortex emerging as a relevant region for understanding the impact of stress.

The cerebral cortex exhibits significant plasticity throughout life, with heightened malleability during childhood. Cortical volume reduction is a natural process of brain maturation and can be influenced by environmental factors, with childhood being particularly sensitive to stressful environmental stimuli.^{3,4} Such stressors may exert negative effects that vary according to duration, nature, and intensity of the exposure.⁵

Recent magnetic resonance imaging studies have shown that individuals exposed to adverse childhood environments—such as sexual or psychological abuse and parental neglect—present reduced gray matter volume in cortical regions.⁴ Animal models of stress corroborate these findings. For instance, the Maternal Separation (MS) model induces reduced thickness in various cortical areas in adult male Wistar rats.⁷ These neurological alterations are considered predisposing factors for the development of psychiatric disorders, as evidenced by cortical volume reductions observed in individuals with schizophrenia, which are associated with cognitive impairments.⁶

In this context, one of the mechanisms underlying cortical reduction is synaptic pruning, which involves the elimination of dendrites, axonal retraction, and loss of dendritic spines.^{8,9} Microglia are believed to play a central role in synaptic pruning, as they phagocytose and eliminate dendritic elements, thereby modulating neuronal connectivity.¹⁰ This process is essential for synaptic plasticity. The presence of glucocorticoid receptors in microglia increases their responsiveness to psychological and inflammatory stressors, allowing them to respond to hormones through changes in morphology and functional profile. These changes can lead to deficits in the synaptic pruning mechanism and subsequent behavioral effects.¹⁰

An increasing body of research using animal models has demonstrated stress-induced alterations in synaptic pruning. Rats exposed to Social Isolation Stress (IS) exhibited reduced dendritic spine density and decreased complexity of dendritic arborization in the medial prefrontal cortex (mPFC).¹¹ In the somatosensory cortex (SSC), MS stress led to changes in dendritic spine density that varied depending on the age and specifics of the separation protocol.^{12,13}

The SSC is recognized for processing sensory perception and is functionally linked to motor and cognitive areas. Its specialization is crucial for modulating perception and influencing behavior.¹⁴ Tactile stimuli are understood to activate the somatosensory region. In rats, the somatotopic map designates the head as the body part with the highest receptor density and the largest cortical processing area.¹⁵

Rats develop complex social relationships shaped by hierarchical dominance and mating partnerships.¹⁶ Several sensory areas are involved in social interaction, particularly those receiving input from the facial region, a critical area for environmental recognition and the formation of social bonds.¹⁶ There are two main primary regions within this cortex: the Upper Lip (UpL) and Barrel Cortex (BC). The UpL receives tactile stimuli from the upper lips and nearby vibrissae, while the BC is dedicated to processing inputs from vibrissae. Each vibrissa is mapped to structures known as "barrels," reflecting a highly ordered somatotopic organization.¹⁷

As social animals, rodents naturally require interaction, particularly during early life when tactile facial stimulation is intense.¹⁶ Given that deprivation of recreational social interactions in juvenile rats results in impairments in normal cortical development and may alter neuronal pruning patterns,¹⁸ this research focused on the somatosensory cortex. Therefore, the aim of this study was to investigate the effects of the SI model experienced during the juvenile phase, specifically between postnatal days 21 and 35 (P21–P35), in female rats. Based on the hypothesis that IS would induce morphological impairments in brain structures associated with sensory functions, changes were identified in the cortical thickness of facial somatosensory areas, such as the BC and UpL, along with impacts on dendritic spine density.

METHODS

ANIMALS

A total of 27 female Wistar rats (*Rattus norvegicus*) were used, originating from pregnant females obtained from the Central Animal Facility of the State University of Maringá. The pups were born and maintained under standard housing conditions at the Sectoral Animal Facility of the Department of Pharmaceutical Sciences at the same university, with a 12-hour light/dark cycle, temperature maintained at 22 ± 2.5 °C, and *ad libitum* access to food (Nuvilab-nuvital) and water. After weaning, the litters were randomly assigned to two groups: Control (C) and Social Isolation Stress (SI). All procedures were approved by the Institutional Animal Care and Use Committee of the State University of Maringá (CEUA/UEM/ nº 9639231121).

METHODOLOGY

Between postnatal days P21–P35, SI group animals were individually housed in polypropylene cages (30 × 20 × 13 cm) under standard vivarium conditions and handled only for routine cage cleaning (once per week). While exposed to olfactory, visual, and auditory stimuli from the environment, the animals were deprived of physical (social) contact, thus preventing the development of juvenile-stage social interactions. The control group (C) was housed in groups of three per polypropylene cage (30 × 40 × 15 cm), under the same standard vivarium conditions, with cage cleaning performed three times per week (Figure 1).

From P36 to P42, animals were subjected to behavioral testing, which is not addressed in this study. On P43, animals were euthanized under anesthesia (ketamine 80 mg/kg + xylazine 10 mg/kg + fentanyl 0.03 mg/kg) and perfused transcardially with room-temperature 0.9% buffered saline solution (phosphate buffer, pH 7.4, 0.1 M) for 8 minutes. Following postfixation and cryoprotection (30% sucrose), brains were frozen in liquid nitrogen at –25 °C and sectioned in a cryostat. 11 brains were serially sectioned at 100 µm thickness and processed using the Golgi-Cox technique¹⁹ for dendritic spine quantification. The remaining 16 brains were semi-serially sectioned at 40 µm thickness, with 200 µm intervals between selected bregma levels, and processed with Nissl staining for cortical thickness analysis.

CORTICAL THICKNESS ANALYSIS

In the parietal cortex, the following somatosensory cortex regions were analyzed (Figure 2): (a) the primary somatosensory cortex region of the Upper Lip (UpL), corresponding to bregma levels 1.32

mm, 1.08 mm, and 0.96 mm; (b) the primary Barrel Cortex (BC), at bregma levels -2.76 mm and -3.00 mm.²⁰ Quantification was performed using an Olympus BX40 light microscope equipped with a $1000\ \mu\text{m}$ graded eyepiece ruler. Both hemispheres were measured.

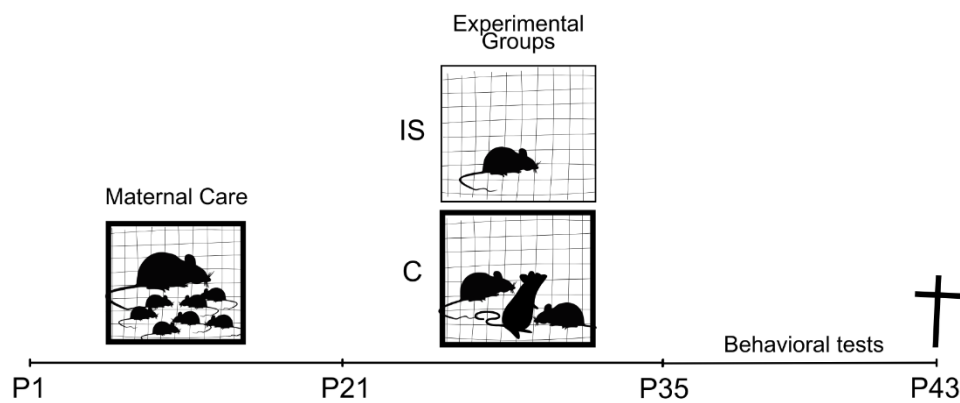


Figure 1. Experimental Design. Source: The authors.

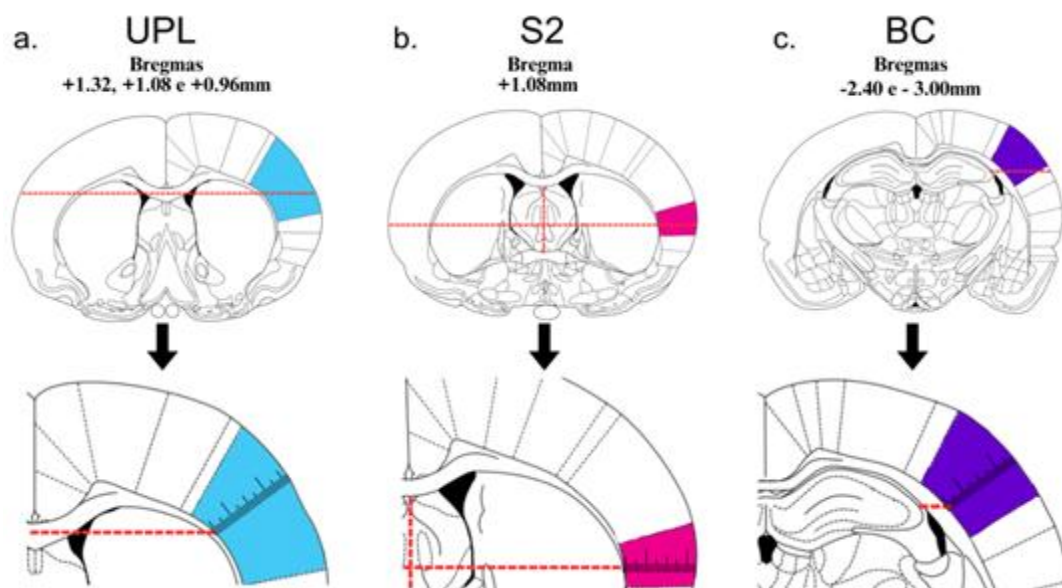


Figure 2. Representation of the bregmas used with the location and position of the ruler to quantify the thickness of the primary sensory cortex UpL (a), cortex S2 (b), BC (c). Source: The authors.

DENDRITIC SPINES QUANTIFICATION

For dendritic spine quantification, brain sections processed with the Golgi-Cox staining technique were used. Images of pyramidal neurons were captured using a microscope with an image acquisition system (Image ProPlus software), based on the following criteria: (I) clearly defined pyramidal neuron morphology, (II) a single, apical dendrite oriented perpendicular to the pial surface, (III) proper staining of the soma and dendrites, (IV) isolated neurons with no overlap with adjacent cells, and (V) neurons located in layers II–III of the cortex.

For each pyramidal neuron, three dendritic segments were selected from the apical region and three from the basal region, in both the UpL area (bregma $+1.32$ mm and $+0.96$ mm) and BC (bregma -2.40 mm and -3.00 mm). In each segment, the number of visible spines was counted over a $20\ \mu\text{m}$ length using a $100\times$ objective lens. Between three and five neurons were selected per cerebral hemisphere.

STATISTICAL ANALYSIS

Data were analyzed using GraphPad Prism 8 software. Normal distribution (Kolmogorov–Smirnov test) and homoscedasticity (F-test) were assessed. When assumptions were met, cortical thickness data were subjected to parametric analysis (t-test); otherwise, non-parametric analysis (Mann–Whitney test) was used. For dendritic spine density analysis, interactions between group and hemisphere were assessed using a two-way ANOVA (Group C vs. IS and Right vs. Left Hemisphere), followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$. Parametric results are presented as standard deviation, and non-parametric results as standard error.

RESULTS

SOMATOSENSORY CORTEX THICKNESS

The effect of social isolation was assessed through changes in cortical thickness in a region-dependent manner. In the Upper Lip (UpL) area, a reduction in cortical thickness was observed in one of the analyzed sections (bregma 0.96 mm) ($t_{(13)} = 2,725$; $p = 0,017$) (Figure 3c). Conversely, in the Barrel Cortex (BC), an increase in cortical thickness was detected at bregma -3.00 mm ($t_{(11)} = 3.013$; $p = 0.011$) (Figure 3f). No alterations were found in the secondary cortex (Figure 3h).

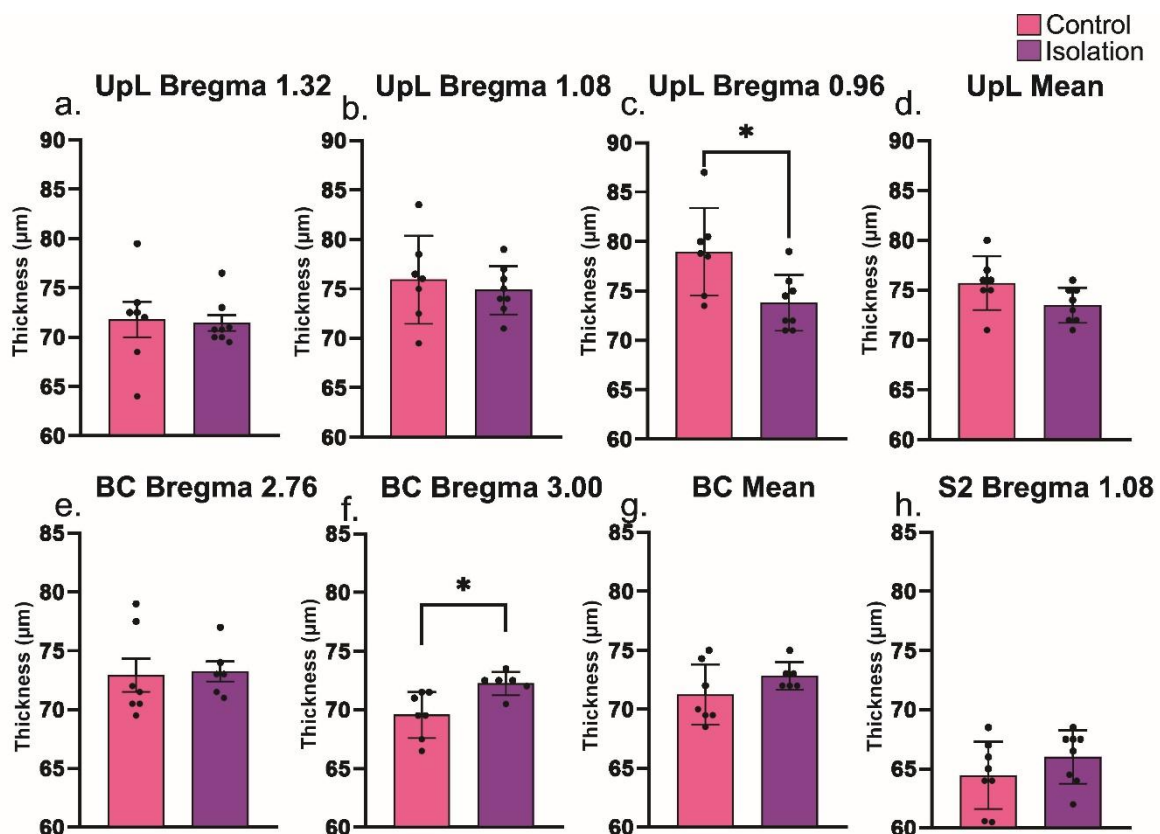


Figure 3. Effects of IS on the thickness of the Somatosensory Cortex. The primary UpL area and corresponding bregma levels: 1.32 mm (a), 1.08 mm (b), 0.96 mm (c), and the mean across sections (d). The BC area and corresponding bregma levels: -2.76 mm (e), -3.00 mm (f), and the mean (g), as well as the thickness of the S2 cortex (h). Data are presented as mean $\pm$ standard deviation/standard error. * $p < 0.05$. Source: The authors.

APICAL DENDRITIC SPINES DENSITY

Two-way ANOVA analysis revealed a significant effect for the group factor ($F_{(1, 16)} = 5,106$, $p = 0,038$), indicating that social isolation impacted apical dendritic spine density in the UpL cortex (Figure 4a). No significant effects were observed for the side factor ($F_{(1, 16)} = 0,002$, $p = 0,964$) or for the interaction between group and side ($F_{(1, 16)} = 0,073$, $p = 0,79$). These results suggest that socially isolated animals exhibited altered apical dendritic spine density in the UpL cortex, with overall differences captured by the statistical model. Despite the main effect of group, post hoc tests did not reveal statistically significant differences between specific comparisons driving this effect.

BASILAR DENDRITIC SPINES DENSITY

Similar to the findings for apical dendritic spines, basilar dendritic spine density also showed a significant effect for the group factor as revealed by Two-way ANOVA ($F_{(1, 16)} = 6,222$, $p = 0,023$), indicating that social isolation impacted basilar dendritic spine density in the UpL cortex (Figure 4c). No significant effects were observed for the side factor ($F_{(1, 16)} = 0,559$, $p = 0,864$) or for the interaction between group and side ($F_{(1, 16)} = 0,03$, $p = 0,79$). These results suggest that socially isolated animals experienced alterations in basilar dendritic spine density in the UpL cortex, with general differences detected by the statistical model. As with the analysis of apical spine density, post hoc tests did not identify which specific comparisons accounted for the significant group effect.

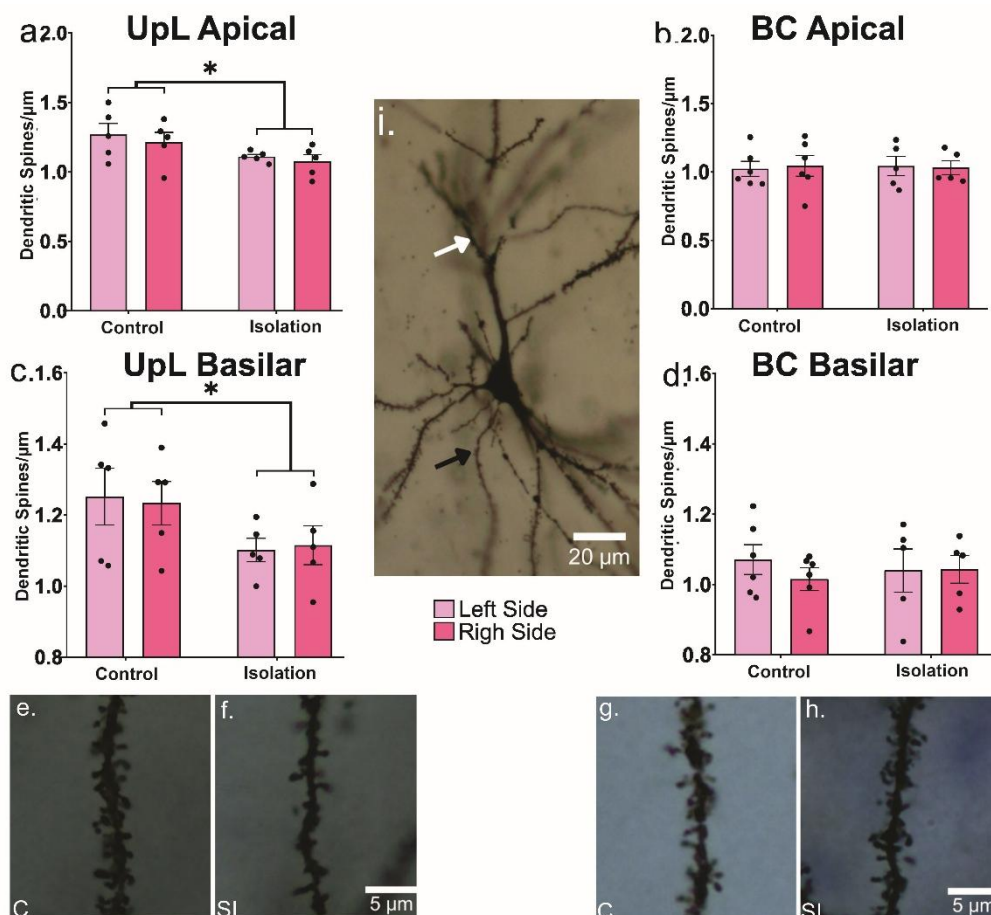


Figure 4. Effects of EIS on dendritic spine density (Control $n = 6$; EIS $n = 5$), in the apical (a) and (b) dendrites of pyramidal neurons, and in the basal dendrites (c) and (d), with a reduction observed in the IS group. Representation of the analyzed dendritic segments in the UpL region (e) and (f), and in BC (g) and (h), imaged at 100x magnification, and of the pyramidal neuron (i) at 20x magnification. The white arrow indicates apical dendrites, and the black arrow indicates basal dendrites.

Source: The authors.

Table 1. Summary of the results obtained from morphological analyses of cortical thickness and dendritic spine density. The arrows show the direction of significant effect of the experience (social isolation vs control, $p < 0.05$) whereas dash (-) indicates nonsignificant effects.

Cortical Thickness	Result
UpL 1.32mm	-
UpL 1.08mm	-
UpL 0.96mm	↓
UpL Média	-
BC -2.76mm	-
BC -3.00mm	↑
BC Mean	-
S2 -0.12	-
S2 -0.48mm	-
S2 Mean	-
SD Density	Result
Apical UpL DS	↓
Apical BC DS	-
Basilar UpL DS	↓
Basilar BC DS	-

Source: The authors.

DISCUSSION

The present study investigated the effects of Isolation Stress (IS) during the juvenile phase on the thickness of the somatosensory cortex and dendritic spine density in adolescent female rats. A detailed analysis of specific sections of the cerebral cortex revealed a reduction in the thickness and spine density of the primary UpL cortex as an effect of IS. The primary somatosensory cortex receives input from stimulated peripheral areas, such as stimulation of the lips and vibrissae processed by the UpL. The secondary cortex, in turn, receives input from the primary cortex.¹⁴ Notably, cortical plasticity can be driven by both genetic and environmental factors, with stimulation impacting corresponding cortical activity.

Accordingly, the findings of this study, which indicate a reduction in cortical thickness and spine density in the region, are consistent with the experience of social isolation. Reduced contact with conspecifics during the juvenile period resulted in less stimulation of the region, consequently leading to a reduction in dendritic spines. As for the decreased cortical thickness, it is likely that the spine reduction contributed to this outcome; however, other mechanisms may be involved, such as a marked reduction in glial cells.

The BC is responsible for processing vibrissae and maps them in such that they share cortical representations, enabling a coordinated response to tactile stimuli.¹⁷ The variation in size affects their function: shorter ones are sensitive to the detection of textures and nearby details, while longer detect stimuli from greater distances.¹⁷ Vibrissae serve an exploratory function, and electrophysiological studies have shown that facial proximity increases BC activation, with an average of 44% in neuronal activity in males and 19% in females.²¹ Exploration, as a form of sensory training, can lead to plastic changes in BC, increasing the vibrissae representation area. This suggests that social experience may shape how rodents perceive and socially interact, underscoring the role of cortical plasticity in adapting to different social and environmental contexts.

As previously mentioned, responses to stress vary according to the nature of the stressor, age, and intensity.⁵ MRI studies in earthquakes survivors who developed Post-Traumatic Stress Disorder (PTSD) revealed have shown an increase in the volume of the cortical region in the parietal lobe and the superior temporal gyrus.²² The explanation for this paradoxical increase remains a subject of speculation,

with some authors suggesting that the increase is a process that precedes the reduction in cortical volume, a phenomenon also observed in patients with schizophrenia.²²

The intriguing result, reduction in one region and an increase in another, may be associated with interference in cortical remodeling and synaptic pruning, which is not a global and uniform mechanism when it comes to brain areas. Pathological reduction could be associated to two mechanisms: an anomalous process in cortical development, that is, a loss of control over cortical reduction, as seen in some neurodevelopmental disorders such as schizophrenia and bipolar disorder^{6,23}; or, atrophy due to disuse from lack of stimulation, which naturally occurs during aging and in comorbid conditions that may be prevented through physical exercise.²⁴ In the present case, a stressor leads to sensory deprivation during the cortical remodeling phase, and further studies would be necessary to understand the reasons behind these regional differences.

Similar to the finding of cortical reduction in UpL, stress models have pointed to interference of stress in cortical development. Restraint stress in adult mice caused decreased dendritic complexity and spine density in mPFC.²⁵ Rats subjected to maternal separation showed reductions in various cortical areas in adulthood, such as the prefrontal, retrosplenial, and motor cortex.⁷ are consistent with neurological alterations observed in some neurodevelopmental disorders, such as schizophrenia, which is characterized by reduced cortical volume in cognitively important regions.⁶

The absence of studies highlighting morphometric changes following stress during the juvenile phase underscores the relevance of the present work. The response observed in the BC, where cortical thickness increased, cannot be directly compared to models used in other studies, which examined different life stages, such as adulthood or early childhood.^{11,12} Although no significant differences were found in spine density in BC layers II and III, the delimitation of the analyzed layers is a critical consideration when investigating this cortical region. BC layer activation varies according to the type of vibrissa stimulated principal or adjacent and the stimulus context, whether in an enriched or restricted environment.¹⁷ Therefore, it is possible that other layers of this cortex may show increased spine density.

Dendritic spine plasticity is dependent on the brain region and the type of environmental stimulation. For instance, adult female rats exposed to stress via the Elevated Open Platform apparatus from P40 to P51 showed increase in dendritic spine density in the mPFC²⁶, as did male and female mice isolated from P21 to P61, who exhibited increased dendritic spine density in adulthood in the same region.²⁷ This increase in neuronal structure—both dendrites and spines—is thought to be pathological, with stress altering the normal pruning process that led to this increase. Therefore, the increase should not be interpreted as beneficial.

When toxic, stress may enhance microglial activity related to the phagocytosis of pre- and postsynaptic neural elements. Since microglia are the primary cells involved in neuronal pruning, this effect could alter synaptic density.¹⁰ It is possible that the UpL region experienced microglial activation and, therefore, interference in the pruning process. However, this parameter was not investigated in the present study.

In addition to microglial hyperactivation caused by stress, the deprivation of environmental and social experiences can also negatively affect spine density. In contrast to social isolation, environmental enrichment offers a variety of experiences through the combination of physical exercise, social interactions, and learning. Rats exposed to an acoustically enriched environment demonstrate greater synaptic complexity with increased dendritic spine density in the auditory cortex.²⁸ Stark (2023) also highlights the importance of typical childhood play resulting from social interaction, showing how deprivation of social activities influences synaptic pruning in both male and female juvenile rats, leading to reduced dendritic spine density in the prefrontal cortex.¹⁸

Although few studies have evaluated the effects of IS during childhood, existing ones show that isolation during childhood and adolescence causes alterations in dendritic arborization and dendritic spine density in mPFC of adult male rats.¹¹ These findings are consistent with postmortem studies of individuals diagnosed with schizophrenia, who exhibited reduced spine density in cortical regions.²⁹ In the somatosensory cortex, the effects of maternal separation have been investigated in rodents. In rats separated for 1 hour per day during various periods between P3 and P16, spine density increased.¹² In mice separated from P2 to P14 and evaluated in adulthood, spine density in layers II and III decreased.¹³

This study focused on the analysis of global parameters to assess cortical plasticity in response to stress, raising the hypothesis that additional factors at the cellular and molecular levels may also be involved in this process. It is widely accepted that various mechanisms contribute to reduced cortical thickness, including dendritic arbor complexity, dendrite length, and the number of neurons. Additionally, neuronal and synaptic pruning processes have also been identified as potential contributors to this reduction.

CONCLUSION

The findings of this study indicate that psychological stress resulting from social isolation during the juvenile period can induce both macrostructural and microstructural alterations in the somatosensory cortex of adolescent female rats. Specifically, the Upper Lip (UpL) region exhibited a reduction in cortical thickness accompanied by a decrease in dendritic spine density. In contrast, the Barrel Cortex (BC) region demonstrated an unexpected increase in cortical thickness, without significant changes in dendritic spine density.

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