Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8648
Doi: 10.4025/actascihealthsci.v46i1.65855

Short-term physical and psychological stress did not cause lasting changes in the integrity of the brain white matter of male rats

Letícia Alexandrino Barilli*, Gustavo Henrique Pereira, João Pablo Dias and Silvana Regina de Melo

Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringa, Parnaá, Brazil. *Author for correspondence. E-mail: leticiaabarilli@hotmail.com

ABSTRACT. The effect of juvenile stress on brain morphology, and especially white matter, is poorly understood. The present study aimed to evaluate the effects of two models of stress, physical and psychological, in the juvenile phase of male rats and their long-term impact on the integrity of the brain white matter. Morphological analysis was based on two major pathways of brain connection and myelin concentration, corpus callosum (CC) and fornix. Animals were randomly assigned to three groups: Control (C), Immobilization Stress (IS), and Predator Exposure Stress (PES). The stress procedures occurred for three consecutive days from d95 of postnatal life (P25 to P27). For long-term evaluation, in adulthood (P90-P95), the brains were collected, fixed, and processed by the Klüver-Barrera technique. The collected material was evaluated using image capture and analysis in the ImageJ Software. Both models of stress studied produced no changes in body and brain weight, and all regions analyzed (genu, body, and splenium of the corpus callosum and the fornix) showed no changes in optical integrity. Thus, this study suggests that short-term juvenile stress does not cause lasting morphological effects on white matter structure, and this adaptation, in which neither reductive nor protective changes occurred, can be considered a positive adaptation.

Keywords: predator stress; immobilization; fornix; corpus callosum; childhood.

Received on November29, 2022.
Accepted on May25, 2023.

Introduction

Two bundles of white matter connect different brain regions: the corpus callosum (CC) communicates the two cerebral hemispheres (Reyes-Haro, Mora-Loyola, Soria-Ortiz, & García-Colunga, 2013) while the fornix mainly connects the hippocampus to the hypothalamus (Senova, Fomenko, Gondard, & Lozano, 2019) and other regions such as the thalamus, amygdaloid body, and prefrontal cortex, contributing to form the Papez circuit (Oikawa, Sasaki, Tamakawa, & Kamei, 2001). Among these circuits, the hippocampus-hypothalamus connection stands out, where the hypothalamic region in question is the medial mammillary nucleus, and this connection relates the fornix with episodic memory (Vann & Aggleton, 2004).

The CC, with about 200 million nerve fibers, represents a large portion of the white matter of the telencephalon of rodents (Baynes, 2002), and is anatomically divided into genu, body, and splenium (Reyes-Haro et al., 2013). In rats, myelination of CC nerve fibers begins around P15, and the number of myelinated axons in the splenium increases from P25 to 60 (Kim & Juraska, 1997). And yet, the increase in the corpus callosum area with development is a consequence of the increase in myelination and not the increase in the number of axons. It is also pointed out that each region of the CC is related to the respective neighboring areas and the development of the areas is regional. For example, a study with male and female rats demonstrated that the development of the splenium, an area mainly related to the interhemispheric transmission of visual information, persists until adulthood. That area increased by 14.5% in males and 5% in females between P60 and P180 due to the continuous myelination process (Nuñez, Nelson, Pych, Kim, & Juraska, 2000).

After completing its development, the CC is still susceptible to morphological and functional changes, and it has been shown that the neuroplasticity of the CC depends on the environment. In the splenium of rats, there is an alteration of oligodendrocytes where the number of myelinated axons was increased as a result of the enriched environment (Juraska & Kopcik, 1988). And environments with negative experiences also impact this region. Clinical studies report smaller CC volumes in children exposed to stress early in life (De Bellis

et al.,1999; Teicher et al., 2003), and a reductive effect was also evidenced in the CC volume in non-human primates exposed to stress early in life (Sánchez, Hearn, Do, Rilling, & Herndon, 1998; Jackowski et al., 2011). Such volume reductions would be correlated with cognitive deficits in non-human primates (Sánchez et al., 1998), as well as other abnormalities in morphology and at the molecular level in the white matter, which have been related to neuropsychiatric disorders (Fields, 2008; Bernstein, Steiner, & Bogerts, 2009).

The fornix is believed to play a key role in cognition, especially learning, due to connections between limbic areas. And it is suggested that the degeneration of the fornix bundles may precede abnormality in the hippocampus, which may interfere with the dysfunction of the area, and lead to cognitive impairment as well as hippocampal atrophy (Oishi & Lyketsos, 2016). If fornix degeneration can lead to cognitive impairment, our question is whether stress leads to excessive stimulation and affects the structure of the fornix, that is, whether there would be hypermyelination of the fornix as a result of stress. In addition, there are reports that the fornix abnormality may be associated with mental disorders such as schizophrenia and pre-psychosis. A study on medication administration in individuals with schizophrenia showed that the N-acetylcysteine drug helped to prevent the integrity of the fornix in these patients, decreasing the fractional anisotropy (Klauser et al., 2018).

Studies on white matter in humans using techniques such as DTI (Diffusion Tensor) have brought important information about this brain region. With DTI, it is possible to describe the direction of movement of water molecules inside tissues (Witwer et al., 2002). In structures such as white matter tracts, the diffusion of molecules will be more restricted perpendicular than parallel to the microstructure boundaries, which is called anisotropic diffusion (Sundgren et al., 2004). Thus, this information generates a schematic map of the white matter tracts, in which the color intensity is directly proportional to the fractional anisotropy (FA) (Melhem et al., 2002). Another way of analyzing the white matter for post-mortem tissues is through the disclosure technique, Klüver-Barrera combined with the image capture program that is capable of analyzing the optical density (OD) of myelin. OD corresponds to image brightness on a standardized gray scale ranging from 0 to 255 with 8-bit pixels, where 0 corresponds to maximum white and 255 to maximum black. Thus, this technique has been used to determine the distribution of cerebral white matter in different brain diseases. This assessment is clinically important, as studies have shown that individuals who suffered chronic stress and developed post-traumatic stress disorder (PTSD) had a lower anisotropy (FA) index in different brain regions. And studies in transgenic rodents for the FKBP51 protein showed increased fractional anisotropy in several brain regions including the fornix region (Engelhardt, Boulat, Czisch, & Schmidt, 2020).

Since the development of the CC is a long process and its structure can be affected by experiences, our question is whether stress during childhood causes structural changes in the CC. Furthermore, if the fornix is related to episodic memory, and in individuals with PTSD one of the sequels is the difficulty in reducing traumatic memories, our hypothesis is that childhood stress causes anatomical changes in this region. As it is well established that the brain response to stress depends on the type of stress (Isgor, Kabbaj, Akil, & Watson, 2004), duration of exposure to the stressor (Luine, Villegas, Martinez, & McEwen, 1996), age of exposure (Stylianakis, Harmon-Jones, Richardson, & Baker, 2018), and animal sex (Melo, Antoniazzi, Hossain, & Kolb, 2018), our study sought to investigate the effects of short-term juvenile stress on the optical density of referenced brain regions, the CC and the fornix. Our aim was to check for differences in the long-term morphological responses to two stress models (physical and psychological), analyzing the integrity of the white matter, in the CC in different regions, genu, body, splenium, and the fornix.

Material and methods

Animals

Male Wistar rats (n = 15) were acquired from the Central Animal Facility of the State University of Maringá, and randomly assigned to experimental groups: Immobilization Stress (IS, n = 5), Predator Exposure Stress (PES, n = 5), and Control (n = 5) and kept in a sectoral animal house under standard temperature-controlled conditions (22°C ± 1°C) in a 12/12h light/dark cycle (lights on at 7h00), chow (Nutrilab-CR1, Nuvital Nutrients, Curitiba, PR, Brazil) and water provided ad libitum. Experiments were carried out in accordance with the experimental procedures approved by the local Animal Research Ethics Committee (CEUA) (CEUA authorization 499.305.061.7) of the State University of Maringá.

Immobilization stress: considered a physical stressor (Watanabe, Gould, & McEwen, 1992), it was applied between postnatal days 25 and 27 (P25-P27), in which the animals were placed in a plastic restraint tube (10

cm long, 4 cm in diameter) for three periods of 30 minutes, separated by intervals of 15 minutes for 3 days. During the restraint sessions, rats were placed in a room adjacent to their bioterium and every day, at the end of the stress session, they were returned to their original cages.

Predator stress: considered psychological stress (Blanchard et al., 1998), it was carried out between P25-P27, and consisted of two separate adjacent boxes, the rat box, a square of transparent propylene (15 cm long X 27 cm wide x 21 cm height) with holes in the walls. The cat box is a square with wire mesh walls (80 cm long X 80 cm wide x 60 cm high). Young animals were placed individually in the rat box for two 10-minute periods separated by 5-minute intervals for 3 days. Three adult female cats were used as a predator stimulus, one per day, which was kept during the days of the experiment inside the cat box. Both stress procedures were performed under white light, between 7 and 17h00, and after each stress session, the devices (tubes and rat box) were sterilized with a 70% ethanol solution. The control group received only human interventions related to cage cleaning. After stress procedures, rats were left undisturbed until perfusion.

Perfusion and staining

In adulthood, P90 to P95, animals were anesthetized (Thiopental 100 mg + Lidocaine 10 mg kg^{-1}, i.p., 0.1 mL $100g^{-1}$), after which they were weighed and intracardially perfused with 0.9% buffered saline solution (phosphate buffer pH 7.4, 0.1M) and 4% buffered paraformaldehyde solution (phosphate buffer pH 7.4; 0.1M). The brain was removed, and the regions of the olfactory bulb, optic nerves, and spinal cord were sectioned, and post-fixed in the same 4% paraformaldehyde solution, dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Sections were cut in the coronal plane with a thickness of 16 µm and stained using the Klüver-Barrera technique and contrasted with cresyl violet.

White matter integrity analysis

For CC, the following regions were analyzed: anterior (Genu = Bregma 2.28 to 1.56 mm), central region (Body= Bregma -1.44 to -4.96 mm), and posterior region (Splenium = Bregma -5.04 to -5.16 mm), according to Paxinos and Watson (1998). Nine fields per animal were considered, obtained from 3 semi-serial sections, with 3 random fields from each section. For analysis of the fornix, the regions of the right and left fornix were captured, corresponding to Bregma -0.36 and Bregma -0.60 (Paxinos & Watson, 1998). For quantitative analysis, CC and fornix images were captured with a 40X objective, using a high-resolution camera (3CCD Pro-series), attached to the microscope, and exported to a computer using the ImagePro Plus software. These images were quantified using the ImageJ software, where the optical density was analyzed.

Statistical analysis

The results were evaluated using the Graph Prism 8 software, with One-Way analysis (One-way ANOVA) and Tukey's posthoc test for comparison between groups. A significance level of 5% ($p < 0.05$) was adopted, and data were expressed in graphs as mean ± standard deviation.

Results

Body and brain weight

Statistical analysis of both variables was not significant between groups C, IS, and PES. For body weight (GC: 297.20 ± 13.48 g; IS: 308.70 ± 20.48 g; PES: 318.00 ± 31.90 g; $F_{2,39} = 2.813$, $p = 0.723$), and for brain weight (GC: 1.88 ± 0.10 g; IS: 1.87 ± 0.08 g; PES: 1.84 ± 0.16 g; $F_{2,39} = 0.2998$, p = 0, 7427) (Figure 1)

Although it is a general physiological measure, both body weight and brain weight represent an important parameter, and in a previous analysis, with the same animals, the adrenal gland also did not show any difference in weight or morphology in the cortex and adrenal medulla (Kato, Melo, Dada, & Barbosa, 2020). Together, these data suggest that from a physiological point of view, the models adopted were not toxic to the point of altering measures such as weight, and are in agreement with Saber, Abd El Aleem, Aziz, & Ibrahim (2019), who report that the body weight of rats subjected to immobilization stress is related to the chronicity of the model. In addition, there is a tendency to regain weight, and in our study, the recovery period may have been one of the mechanisms. Nevertheless, we failed to check the effects of body weight soon after stress, and even the responses after a short recovery interval.

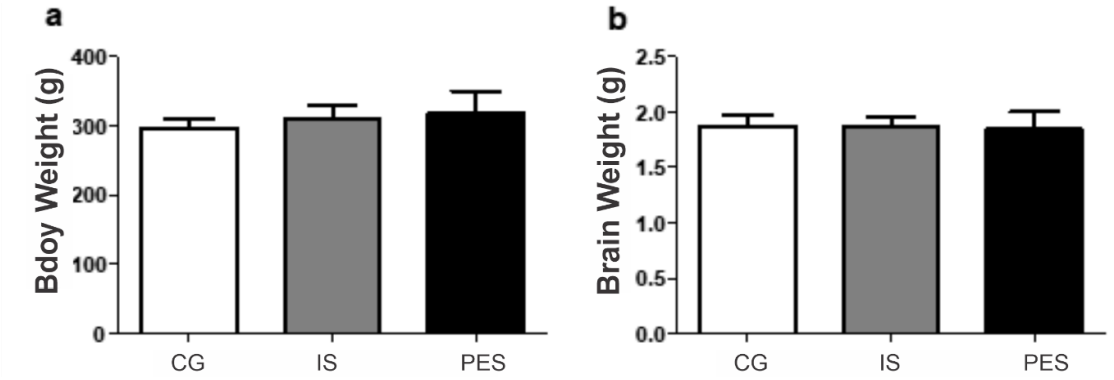

Figure 1. Body (a) and brain (b) weight of animals (each group, n = 14), expressed as mean ± standard deviation. Control Group (CG); Immobilization Stress (IS), and Predator Exposure Stress (PES).

CC white matter integrity

The optical density of the analyzed regions was not significantly different between the Control, Physical Stress, and Psychological Stress groups for the Genu, Body, and Splenium. For the Genu region, we verified [F(2,11) = 0.79472; p = 0.476], Body [F(2,11) = 2.2337; p = 0.153] and Splenium [F (2,11) = 1.5212; p = 0.261] (Figure 2 b).

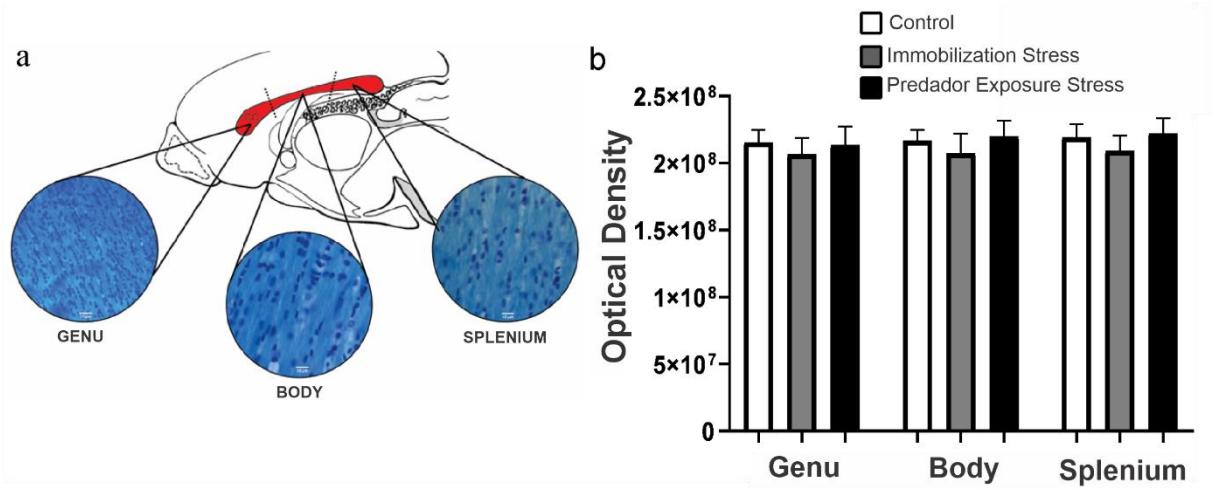

Figure 2. a. CC areas analyzed in sections stained by the Klüver-Barrera technique. b. Optical density of the Genu, Body, and Splenium of the corpus callosum in the Control, Immobilization Stress, and Predator Exposure Stress groups expressed as mean ± standard deviation. Scale bar: 10 µm (a).

In the fornix, we observed no significant difference in optical density between the experimental groups, C (right, 3100.8 ± 89.1871; left, 2971.6 ± 48.6432), IS (right, 2919.25 ± 129.6376; left, 2936.75 ± 75.9511), and PES (right, 2918.8 ± 217.904; left, 3038.4 ± 170.4047) (Figure 3 e).

Thus, both models of stress, immobilization, and exposure to predators, did not produce alterations in the integrity of the white matter in both analyzed regions of the CC, genu, body, and splenium. This result is consistent with previous analyses carried out by our study group, where we found that both the number of glial nuclei and the thickness of the central CC region were not affected by the same models (Lima et al., 2022, Melo et al., 2022).

Clinical studies that point to CC volume reduction were carried out with individuals who suffered various forms of abuse (De Bellis et al., 1999, Teicher et al., 2003), suggesting that childhood is a sensitive period, and the corpus callosum can be shaped by chronic stress. A study with rats under immobilization stress of long frequency and intensity (28 days/4h daily) reported a structural change in myelin, including distortion, disintegration, a reduction in the forebrain (telencephalon + diencephalon), and a decrease in MBP (Myelin Basic Protein) (Thamizhoviya & Vanisree, 2019).

Probably the impact of the stress on the cerebral white matter also depends on the intensity and duration of the stress. And a study carried out with offspring of pregnant rats exposed to stress showed a time-dependent effect on the structure and amount of myelin, where immobilization (3 sessions/45 min daily) for 14 days in the final period of pregnancy caused changes in myelin in the hippocampus, but for 7 days, it did not provoke changes (Xu et al., 2013).

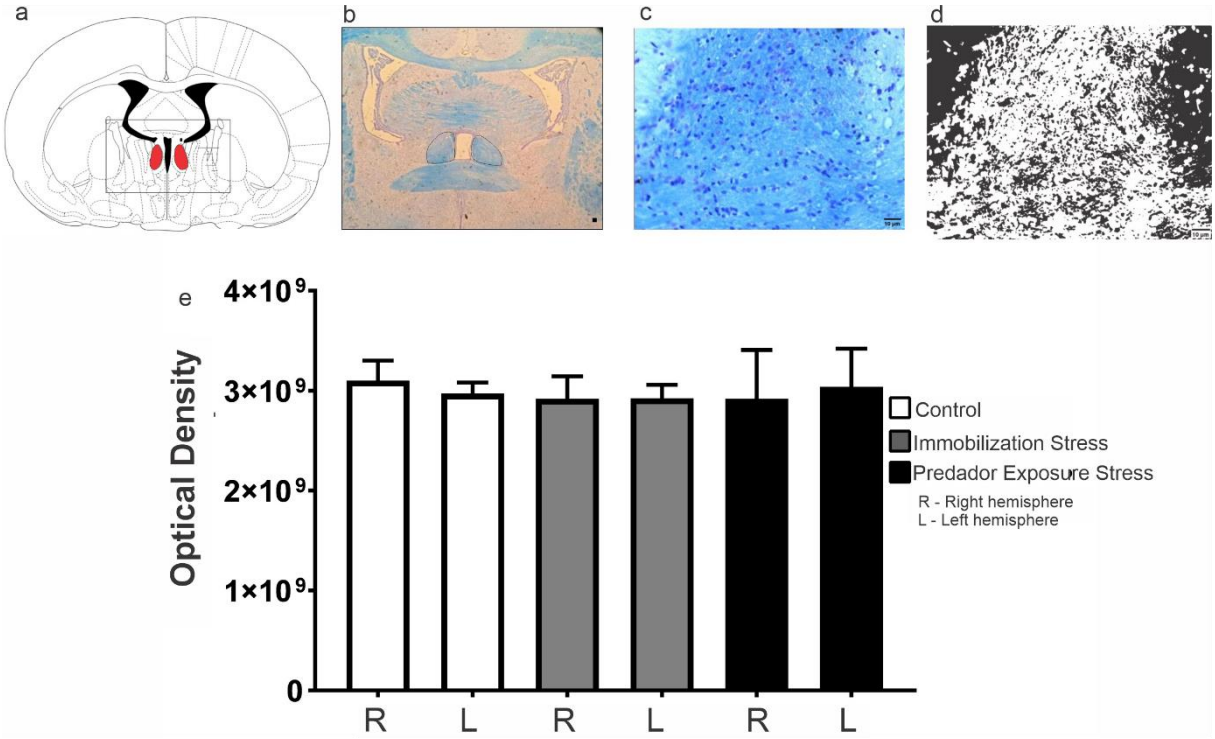

Figure 3. (a) Representative drawing with the fornix region. (b) Photomicrograph in the 4x objective with the Kluver-Barrera technique, representing the analyzed region, (c) and showing the glial nuclei and the myelinated area. (d) Fornix optical density expressed as mean ± standard deviation of the experimental groups. Scale bar: 10 µm (b); 10 µm (c).

In our study, the 67-day interval between exposure to the stressor and analysis may have been a determining factor in recovery. The importance of the recovery period to restore normal values of glial cell count has been demonstrated. In rats exposed to acute stress (immobilization + exposure to predator), females showed a reduction in oligodendrocytes in the hippocampus and prefrontal cortex, after a short recovery period (12 days). However, in the long term (67 days), a reduction in the amygdala body was noted, in both sexes (Breton et al., 2021), but the same model with greater frequency and intensity (14 days/4 hr daily) led to a decrease of glial cells in the hippocampus of mice (Kurokawa et al., 2020). Another study with mice demonstrated that after 21 days of recovery, the increase in the interfibrillar space, occupied by oligodendrocytes and observed shortly after stress, returned to the level of control animals (Miyata et al., 2011).

Myelination is related to the maturation of brain regions and behavior, in laboratory rats in their juvenile phase (P22 - P34) (Sengupta, 2013), the myelination process is still incomplete and this is a moment considered sensitive due to the intense neuroplasticity (Kolb & Gibb, 2011). And the analysis of the integrity of the fornix, in adult life, can give clues about the myelination process after childhood stress. In the present study, the integrity of the white matter of the fornix and corpus callosum was evaluated by optical density. In our analysis, the degree of image darkening reflects areas that contain less myelin. We evaluated the effect of two models of stress in the juvenile phase and found that both models, physical and psychological, did not cause any changes in the analyzed parameter.

White matter is an important component of the brain, and it is possible to measure its density in studies in humans and animals using fractional anisotropy. Magnetic resonance images and computer programs indicate the density and myelination of the fibers that make up the white matter of the brain. Kircanski et al. (2019) found an increase in fractional anisotropy in adolescents, at the beginning of puberty, showing the implication of stress on the limbic system of these individuals. This last comparison allows to state that, depending on the type and intensity of the stressor during the juvenile phase, it can cause damage to the white matter of the brain.

The results presented here, added to the previous ones, both morphological in the corpus callosum and fornix, and behavioral (Melo et al., 2022) suggest that both the physical stress model of immobilization for 1:30 min and the psychological stress of exposure to the cat for 20 min for 3 days during the juvenile phase were innocuous in the long term. And considered here as a short-term or short-term model. Under the morphological aspect, in adult life, the morphological structure of the corpus callosum and fornix were not

affected. However, we cannot say that there was a change and recovery during the 67-day interval, or if there was no change since we did not perform a short-term analysis for comparisons.

Our study demonstrated no changes in the optical density of the CC or fornix regions, suggesting that the integrity of the white matter in these regions was not affected by the stress models. In line with this microscopic data, both body and brain weight were also not impacted by stress models, physical (immobilization) and psychological (exposure to predators).

Conclusion

Our study allows to conclude that short-frequency stress models cause no long-term morphological changes in the corpus callosum and fornix, as revealed by the preservation of the integrity of the white matter in these regions. But it is necessary to investigate whether there was damage after the stressful event and whether there was recovery, or whether the model was not able to cause morphological damage.

Acknowledgements

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and *Fundação Araucária* for supporting this study.

References

Baynes, K. (2002). Corpus Callosum. In V. S. Ramachandran, *Encyclopedia of the Human Brain* (2nd ed.). Amsterdã, NL: Elsevier.

Bernstein, H.G., Steiner, J., & Bogerts, B. (2009). Glial cells in schizophrenia: pathophysiological significance and possible consequences for therapy. *Expert Review of Neurotherapeutics, 9*(7), 1059-107. DOI: https://doi.org/10.1586/ern.09.59.

Blanchard, R. J., Nikulina, J. N., Sakai, R. R., & McKittrick, C., McEwen, B., & Blanchard, D. C. (1998). Behavioral and endocrine change following chronic predatory stress. *Physiology &. Behavior, 63*(4), 561-569. DOI: https://doi.org/10.1016/s0031-9384(97)00508-8.

Breton, J. M., Barraza, M., Hu, K.Y., Frias, S. J., Long, K. L. P., & Kaufer, D. (2021). Juvenile exposure to acute traumatic stress leads to long-lasting alterations in grey matter myelination in adult female but not male rats. *Neurobiology of Stress, 14*, 100319. DOI: https://doi.org/10.1016/j.ynstr.2021.100319.

De Bellis, M. D., Keshavan, M. S., Clark, D. B., Casey, B. J., Giedd, J. N., Boring, A. M., ... Ryan, N. D. (1999). Developmental traumatology. part II: brain development. *Biological Psychiatry*, *45*(10), 1271-1284. DOI: https://doi.org/10.1016/s0006-3223(99)00045-1.

Engelhardt, C., Boulat, B., Czisch, M., & Schmidt, M. V. (2021). Lack of FKBP51 shapes brain structure and connectivity in male mice. *Journal of Magnetic Resonance Imaging (JMRI), 53*(5), 1358-1365. DOI: https://doi.org/10.1002/jmri.27439.

Fields, R. D. (2008). White matter in learning, cognition and psychiatric disorders. *Trends in Neurosciences*, *31*(7), 361-370. DOI: https://doi.org/10.1016/j.tins.2008.04.001.

Isgor, C., Kabbaj, M., Akil, H., & Watson, S. J. (2004). Delayed effects of chronic variable stress during peripubertal-juvenile period on hippocampal morphology and on cognitive and stress axis functions in rats. *Hippocampus, 14*(5), 636-648. DOI: https://doi.org/10.1002/hipo.10207.

Jackowski, A., Perera, T. D., Abdallah, C. G., Garrido, G., Tang C. Y., Martinez J., ... Kaufman J. (2011). Early-life stress corpus callosum development, hippocampal volumetrics, and anxious behavior in male nonhuman primates. *Psychiatry Research: Neuroimaging, 192*(1), 37-44. DOI: https://doi.org/10.1016/j.pscychresns.2010.11.006.

Juraska, J. M., & Kopcik, J. R. (1988). Sex and environmental influences on the size and ultrastructure of the rat corpus callosum. *Brain Research, 450*(1-2), 1-8. DOI: https://doi.org/10.1016/0006-8993(88)91538-7.

Kato, K. T.,Melo, S. R., Dada, M. E. G., & Barbosa, C. P. (2020). Efeitos do estresse físico e psicológico juvenil sobre a glândula suprarrenal em ratos adultos. *Saúde e Pesquisa*, *13*(1), 53-61. https://doi.org/10.17765/2176-9206.2020v13n1p53-61

Kim, J. H., & Juraska, J. M., (1997). Sex differences in the development of axon number in the splenium of the rat corpus callosum from postnatal day 15 though 60. *Developmental Brain Research*, *102*(1), 77-85. DOI: https://doi.org/10.1016/s0165-3806(97)00080-1.

Kircanski, K., Sisk, L. M., Ho, T. C., Humphreys, K. L., King, L. S., Colich, N. L., ... Gotlib, I. H. (2019). Early life stress, cortisol, frontolimbic connectivity, and depressive symptoms during puberty. *Development and Psychopathology,31*(3), 1011-1022. DOI: https://doi.org/10.1017/S0954579419000555.

Klauser, P., Xin, L., Fournier, M., Griffa, A., Cleusix, M., Jenni, R., ... Do K. Q. (2018). N-acetylcysteine add-on treatment leads to an improvement of fornix white matter integrity in early psychosis: a double-blind randomized placebo-controlled trial. *Translational Psychiatry, 8*(220). DOI: https://doi.org/10.1038/s41398-018-0266-8.

Kolb, B., & Gibb, R. (2011). Brain plasticity and behaviour in the developing brain. *Journal of the Canadian Academy of Child and Adolescent Psychiatry, 20*(4), 265- 76.

Kurokawa, K., Tsuji, M., Takahashi, K., Miyagawa, K., Mochida-Saito, A., & Takeda, H. (2020). Leukemia inhibitory factor participates in the formation of stress adaptation via hippocampal myelination in mice. *Neuroscience, 446*, 1-13. DOI: https://doi.org/10.1016/j.neuroscience.2020.08.030.

Lima, V. F., Kato, K. T., Barilli, L. A., & Melo, S. R. (2022). Estresse juvenil de curta frequência e intensidade não afeta a substância branca cerebral de ratos. *Revista Saúde e Pesquisa,15*(2), 1-12. DOI: https://doi.org/10.17765/2176-9206.2022v15n2.e10469.

Luine, V., Villegas, M., Martinez, C., & McEwen, B. S. (1994). Repeated stress causes reversible impairments of spatial memory performance. *Brain Research*, *639*(1), 167–170. https://doi.org/10.1016/0006-8993(94)91778-7

Melhem, E. R., Mori, S., Mukundan, G., Kraut, M. A., Pomper, M. G., & Zijl, P. C. van (2002). Diffusion tensor MR imaging of the brain and white matter tractography. *American Journal of Roentgenology ,178*(1), 3-16. DOI: https://doi.org/10.2214/ajr.178.1.1780003.

Melo, S. R., Antoniazzi, C. T. D., Hossain, S., & Kolb, B. (2018). Neonatal stress has a long-lasting sex-dependent effect on anxiety-like behavior and neuronal morphology in the prefrontal cortex and hippocampus. *Developmental Neuroscience,40*(2), 93-103. DOI: https://doi.org/10.1159/000486619.

Melo, S. R., Gremaschi, L. B., Blanco, L. F. M. S. B., Orathes, B. M., Tarosso, I. V. A., & Bernardi, T. C. (2022). Short juvenile stress has no long-lasting effects on anxiety-like behavior, object recognition memory, or gross brain morphology but affects dendritic spines in the hippocampus in male rats. *Developmental Neuroscience,44*(6), 466-477. DOI: https://doi.org/10.1159/000523955.

Miyata S., Koyama Y., Takemoto K., Yoshikawa K., Ishikawa T., Taniguchi M., ... Tohyama M. (2011). Plasma corticosterone activates SGK1 and induces morphological changes in oligodendrocytes in corpus callosum. *PLoS One, 6*(5), 19589. DOI: https://doi.org/10.1371/journal.pone.0019859.

Nuñez, J. L., Nelson, J., Pych, J. C., Kim, J. H., & Juraska, J. M., (2000). Myelination in the splenium of the corpus callosum in adult male and female rats. *Developmental Brain Research, 120*(1), 87-90. DOI: https://doi.org/10.1016/s0165-3806(99)00193-5.

Oikawa, H., Sasaki, M., Tamakawa, Y., & Kamei, A., (2001). The circuit of Papez in mesial temporal sclerosis: MRI. *Neuroradiology, 43*, 205-210. DOI: hthttps://doi.org/10.1007/s002340000463.

Oishi, K., & Lyketsos, C. G. (2016). Editorial: Alzheimer's disease and the Fornix. *Frontiers in Aging Neuroscience, 8*, 149. DOI: https://doi.org/10.3389/fnagi.2016.00149.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (5th ed.). San Diego, CA: Academic Press.

Reyes-Haro, D., Mora-Loyola, E., Soria-Ortiz, B., & García-Colunga, J. (2013). Regional density of glial cells in the rat corpus callosum. *Biological Research, 46*(1), 27-32. DOI: https://doi.org/10.4067/S0716-97602013000100004.

Saber, E. A., Abd El Aleem, M. M., Aziz, N. M., & Ibrahim, R. A. (2019). Physiological and structural changes of the lung tissue in male albino rat exposed to immobilization stress. *Journal of Cellular Physiology, 234*(6), 9168-9183. DOI: https://doi.org/10.1002/jcp.27594.

Sánchez, M. M., Hearn, E. F., Do, D., Rilling, J. K., & Herndon, J. G. (1998). Differential rearing affects corpus callosum size and cognitive function of rhesus monkeys. *Brain Research, 812*(1-2), 38-49. DOI: https://doi.org/10.1016/s0006-8993(98)00857-9.

Sengupta, P. (2013). The laboratory rat: relating its age with human's. *International Journal of Preventive Medicine, 4*(6), 624-630.

Senova, S., Fomenko, A., Gondard, E., & Lozano, A. M. (2020). Anatomy and function of the fornix in the context of its potential as a therapeutic target. *Journal of Neurology, Neurosurgery and Psychiatry, 91*(5), 547-559. DOI: https://doi.org/10.1136/jnnp-2019-322375.

Stylianakis, A. A., Harmon-Jones, S. K., Richardson, R., & Baker, K. D. (2018). Differences in the persistence of spatial memory deficits induced by a chronic stressor in adolescents compared to juveniles. *Developmental Psychobiology, 60*(7), 805-813. DOI: https://doi.org/10.1002/dev.21750.

Sundgren, P. C., Dong, Q., Gómez-Hassan, D., Mukherji, S. K., Maly, P., & Welsh, R. (2004). Diffusion tensor imaging of the brain: review of clinical applications. *Neuroradiology, 46*, 339-50. DOI: https://doi.org/10.1007/s00234-003-1114-x.

Teicher, M. H., Andersen, S. L., Polcari, A., Anderson, C. M., Navalta, C. P., & Kim, D. M. (2003). The Neurobiological consequences of early stress and childhood maltreatment. *Neuroscience & Biobehavioral Review, 27*(1-2), 33-44. DOI: https://doi.org/10.1016/s0149-7634(03)00007-1.

Thamizhoviya, G., & Vanisree, A. J. (2019). Enriched environment modulates behavior, myelination and augments molecules governing the plasticity in the forebrain region of rats exposed to chronic immobilization stress. *Metabolic Brain Disease, 34*, 875-887. DOI: https://doi.org/10.1007/s11011-018-0370-8.

Vann, A. D., & Aggleton, J. P. (2004). The mammillary bodies:two memory systems in one? *Nature Reviews Neuroscience, 5*(1), 35-44. DOI: https://doi.org/10.1038/nrn1299.

Watanabe, Y., Gould, E., & McEwen, B. (1992). Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Research, 588*(2), 341-345. DOI: https://doi.org/10.1016/0006-8993(92)91597-8.

Witwer, B. P., Moftakhar, R., Hasan, K. M., Deshmukh, P., Haughton, V., Field, A., ... Badie B. (2002). Diffusion-tensor imaging of white matter tracts in patients with cerebral neoplasm. *Journal of Neurosurgery, 97*(3), 568-575. DOI: https://doi.org/10.3171/jns.2002.97.3.0568.

Xu, J., Yang, B., Yan, C., Hu, H., Cai, S., Liu, J., ... Shen, X. (2013). Effects of duration and timing of prenatal stress on hippocampal myelination and synaptophysin expression. *Brain Research, 1527*, 57-66. DOI: https://doi.org/10.1016/j.brainres.2013.06.025.