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Anxiolytic and Anti-Inflammatory Effects of Agomelatine in a LPS-Induced In vivo Model

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ABSTRACT: Neuroinflammation, which results from alterations in the immune system, plays a central role in the pathophysiology of major depressive disorder by inducing neurotoxic effects in brain regions involved in emotion regulation. Agomelatine (AGO), a novel antidepressant, may influence emotional regulation by modulating the critical mechanisms of the interaction between autophagy and neuroinflammation. Lipopolysaccharide (LPS) is an endotoxin that causes depression-like behavior by increasing microglial activation and inflammatory response in the brain. To evaluate the anxiolytic and anti-inflammatory effects of agomelatine, a rat model of depression was induced with LPS. A total of 24 male rats were used in this study, divided into three experimental groups (n=8): control, LPS, and LPS+AGO. Rats in the LPS and LPS+AGO groups received AGO (40 mg/kg, intragastrically) and LPS (0.5 mg/kg, i.p.) for the first 10 days, respectively. AGO was administered daily to the LPS+AGO group for of the 21-day study period. Behavioral assessments were conducted using the open field test. At the conclusion of the experiment, IL-1β and IL-10 levels were measured in the hippocampus, prefrontal cortex, and serum samples using ELISA. According to these results, AGO was found to significantly increase IL-10 levels, particularly in the prefrontal cortex, while decreasing IL-1β levels in the hippocampus. These results confirmed that agomelatine has anxiolytic and anti-inflammatory effects.

KEYWORDS: Lipopolysaccharide; agomelatine; neuroinflammation; open field test; elisa analysis.

1. INTRODUCTION

In recent years, neuroinflammation has been regarded as an active cellular and molecular process that links immune activation to alterations in brain function. Microglial cells, the resident immune cells of the brain, act as primary mediators of this response [1]. Once activated, they release a variety of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6), which can disturb synaptic homeostasis and neural connectivity [2]. Persistent or dysregulated microglial activation can induce neuronal dysfunction through several mechanisms, including oxidative stress, excitotoxicity, and disruption of neurotrophic signaling. These alterations may contribute to maladaptive changes in neural circuits responsible for emotion, cognition, and stress reactivity [1]. In addition to traditional neurotransmitter-based explanations, accumulating evidence indicates that immune and inflammatory mechanisms make a substantial contribution to the pathophysiology of anxiety [3]. Moreover, interactions between the immune and nervous systems have been shown to compromise the functional integrity of key brain regions involved in emotional regulation and behavioral control, including the amygdala, hippocampus, and prefrontal cortex [4].

Agomelatine (AGO) is a unique antidepressant with a dual mechanism of action, acting as an agonist at melatonin MT1 and MT2 receptors and an antagonist at serotonin 5-HT2C receptors [5]. Through this combination, the drug not only restores circadian rhythm disturbances but also enhances monoaminergic neurotransmission in key limbic and cortical regions. These neurochemical effects contribute to improved emotional regulation and stress resilience, processes frequently disrupted in anxiety-related conditions [6].

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Beyond its classical receptor-mediated actions, recent studies have highlighted the anti-inflammatory and neuroprotective properties of AGO [7]. It has been shown to attenuate microglial activation, reduce the production of pro-inflammatory cytokines, and restore oxidative balance within the central nervous system [8]. Furthermore, AGO has been reported to enhance brain-derived neurotrophic factor (BDNF) expression and support neuronal survival under inflammatory conditions [9]. By modulating these convergent pathways—circadian, serotonergic, and inflammatory—AGO may exert a protective influence against neuroinflammation-induced behavioral and cognitive alterations.

Lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria, is widely used as a well-established in vivo model of neuroinflammation [10]. By activating Toll-like receptor 4 (TLR4), systemic administration of LPS triggers peripheral immune responses that extend to the brain, leading to microglial activation, cytokine release, and inflammation-associated behavioral alterations [11]. As an initial step toward understanding, this study examined the potential effects of AGO on anxiety-like behavioral outcomes and neurochemical changes in the brains of rats exposed to LPS-induced neuroinflammation.

2. RESULTS

2.1. Agomelatine effects on the open field test

The open-field test (OFT) was conducted to determine the efficacy of AGO in mitigating anxiety-like behavior elicited by LPS, with analyses focusing on locomotor activity to distinguish anxiolytic effects from nonspecific changes in motor function (Fig. 1). The first parameter evaluated in the OFT is the number of square crossing (Fig. 1a). According to the results of this test, animals in the LPS group showed significantly more mobility compared to the LPS+AGO group (***p<0.001). Analysis of latency to enter center showed that the LPS group took longer to enter the center than the control and LPS+AGO groups (***p<0.001), whereas analysis of time spent in the central zone indicated that the LPS group spent less time in the center than the other groups (***p<0.001, Fig. 1b and 1c). The number of rearings and grooming, indicators of anxiety behavior in animals, are shown in Fig. 1d and 1e. The number of rearings in the LPS group was increased compared to the control group (*p<0.05). The number of grooming in the LPS group was significantly higher than in both the control and LPS+AGO groups (**p<0.01).

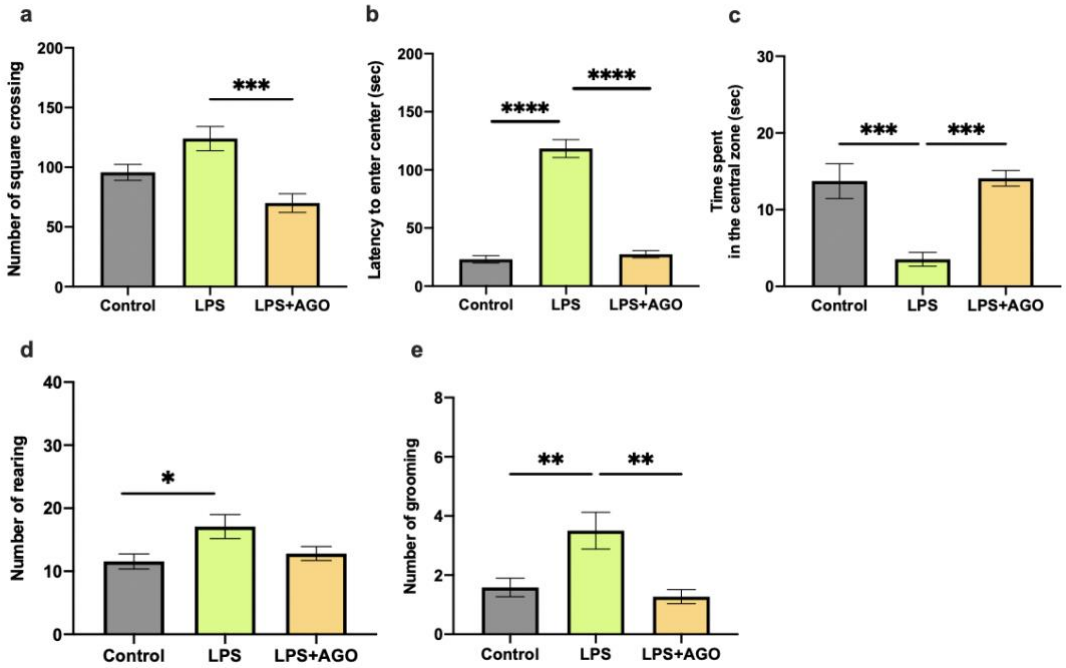


Figure 1. Agomelatine effects anxiety-like behaviour in the open field test. The test parameters: a) Number of square crossings, b) Latency to enter center, c) Time spent in the central zone, d) Number of rearing, e) Number of grooming. One-way ANOVA was used the analyze statistical differences, and data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001,****p<0.0001, LPS vs control and LPS+AGO groups.

2.2. ELISA analysis

Protein expression levels of IL-10 and IL-1β in the prefrontal cortex (PC), hippocampus (HC), and serum were determined by ELISA (Fig. 2.). In the PC, IL-10 levels were significantly higher in the control (**p<0.01) and LPS+AGO (*p<0.05) groups than in the LPS group. In the HC, IL-10 levels were significantly higher in the control group than in the LPS group (**p<0.01). Serum IL-10 results were similar to those in the HC. Additionally, no significant difference in IL-10 levels was observed between the LPS and LPS+AGO groups in either HC or serum (Fig. 2a). As shown in Figure 2, LPS administration significantly increased IL-1β levels in the PC and HC relative to the control group (*p<0.05). AGO administration significantly reduced LPS-induced IL-1B levels in HC (*p<0.05). However, no significant variations in the IL-1β levels were detected in PC, HC, and serum samples in all groups.

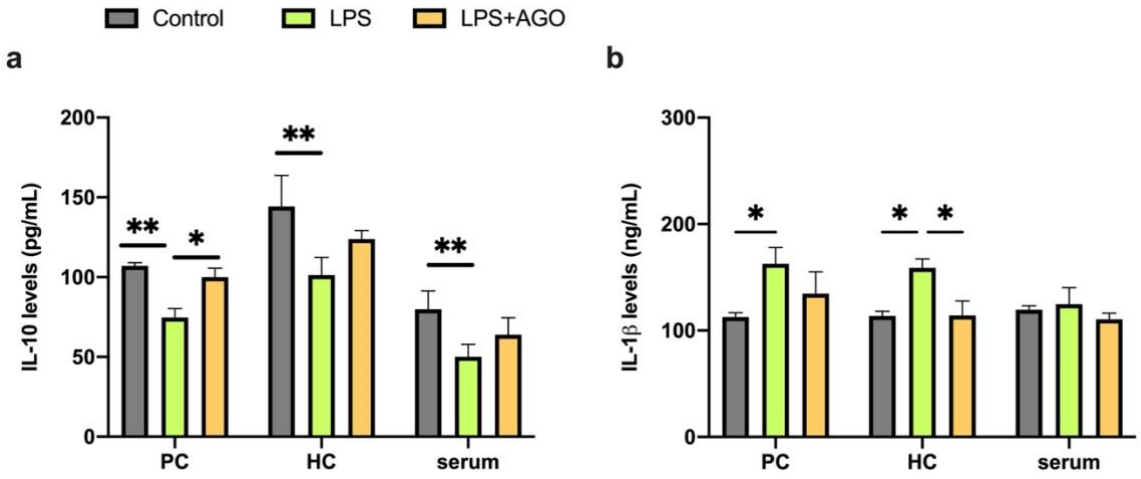


Figure 2. Effects of agomelatine on (a) IL-10 and (b) IL-1β levels, quantified by ELISA in the prefrontal cortex, hippocampus, and serum. Data are mean ± SEM. p<0.05 was considered significant. One-way ANOVA was used to analyze statistical differences between groups and was expressed as follows (*p<0.05, **p<0.01).

3. DISCUSSION

In the present study, anxiety-like behavior in LPS-induced rats was evaluated using the open field test following AGO treatment. In parallel, IL-10 and IL-1β levels were quantified in the prefrontal cortex, hippocampus, and serum to characterize the neuroinflammatory response. Activation of microglia and astrocytes, accompanied by excessive production of cytokines can alter synaptic transmission and impair neuroplasticity, ultimately leading to anxiety-like behavioral outcomes [12]. LPS acts as a potent activator of the innate immune system through TLR4 signaling, leading to peripheral cytokine release and subsequent transmission of inflammatory signals to the brain. Previous experimental studies have consistently demonstrated that systemic administration of LPS induces anxiety-like behaviors in rodents, often accompanied by robust neuroinflammatory responses [13].

Behavioral studies employing paradigms such as the open field, elevated plus maze, and light–dark box tests have reported that LPS-treated animals exhibit reduced exploratory activity and avoidance of open or illuminated areas, reflecting heightened anxiety levels [14]. In the open field test, several parameters are used to infer anxiety-related behavior. A reduction in the number of square crossings, time spent in the central zone, and rearing frequency, along with an increased latency to enter the center, are indicative of reduced exploratory drive and heightened anxiety. In addition, alterations in grooming behavior may reflect changes in emotional reactivity or stress-coping responses. Collectively, these measures provide a reliable behavioral index of anxiety-like responses following systemic LPS administration. In line with previous findings, our study demonstrated that LPS-induced rats exhibited increased numbers of square crossings and prolonged latency to enter the central zone, accompanied by a reduction in the time spent in the center when compared with the control group. These behavioral alterations are interpreted as indicators of anxiety-like behavior. AGO treatment effectively reversed these behavioral alterations, as reflected by reduced latency to enter the

center, increased time spent in the central zone, and restoration of exploratory activity. These data imply that AGO alleviates LPS-induced anxiety-like behavior, potentially through its modulatory effects on neuroinflammatory processes. Given its dual pharmacological profile as a melatonin receptor agonist and 5-HT_2C receptor antagonist, AGO may attenuate microglial activation and cytokine release, thereby normalizing neural circuit function and emotional regulation.

At the neurochemical level, LPS exposure has been associated with elevated cytokine concentrations in the prefrontal cortex, hippocampus, and amygdala, regions crucial for emotional regulation [15]. These neuroimmune alterations are thought to impair synaptic plasticity and neuronal communication, thereby mediating the behavioral manifestations of anxiety [16]. Previous studies have consistently reported that systemic LPS administration elevates IL-1β levels while suppressing IL-10 in both central and peripheral tissues, reflecting a shift toward a pro-inflammatory state. Increased IL-1β expression has been observed in the prefrontal cortex and hippocampus, where it disrupts synaptic plasticity and contributes to anxiety- and depression-like behaviors [17]. Conversely, reduced IL-10—an anti-inflammatory cytokine—has been associated with impaired immune regulation and prolonged microglial activation [18]. Neuroinflammation has been reported to decrease anti-inflammatory marker levels, particularly in the PFC, while concomitantly increasing pro-inflammatory markers. Our findings are consistent with these reports and align closely with the existing literature [19]. Serum cytokine alterations parallel these central changes, indicating systemic propagation of inflammation. In our study, LPS administration resulted in a significant increase in IL-1β and a concomitant decrease in IL-10 levels in both the prefrontal cortex and hippocampus, indicating a pronounced pro-inflammatory shift within these brain regions. This pattern is consistent with previous reports showing that LPS-induced activation of microglia and TLR4-mediated signaling elevates IL-1β while suppressing anti-inflammatory mediators such as IL-10. In the AGO-treated group, IL-10 levels in the prefrontal cortex increased while IL-1β levels decreased compared with the LPS group, suggesting a partial restoration of the pro-/anti-inflammatory balance. Similarly, AGO administration reduced IL-1β concentrations in the hippocampus, indicating an attenuation of the local inflammatory response within this region. However, no significant changes were observed in serum cytokine levels, implying that AGO's anti-inflammatory actions were more pronounced at the central rather than peripheral level. These findings support that AGO may exert its behavioral and neuroprotective effects primarily through modulation of neuroinflammatory signaling within specific brain regions involved in emotional regulation.

4. CONCLUSION

In conclusion, our findings suggest that AGO has the potential to modulate anxiety-like behaviors associated with neuroinflammation through its effects on underlying neurochemical and immune processes. By restoring the balance between pro- and anti-inflammatory cytokines within key brain regions such as the prefrontal cortex and hippocampus, AGO may attenuate the behavioral and molecular consequences of LPS-induced neuroinflammation. These results highlight AGO's promise as a candidate for targeting inflammation-related emotional disturbances. However, further studies are warranted to elucidate the precise mechanisms by which AGO interacts with neuroimmune pathways and to confirm its therapeutic potential in broader neuroinflammatory models.

5. MATERIALS AND METHODS

5.1. Materials

Lipopolysaccharides (LPS; Escherichia coli, serotype O127:B8) was received from Sigma-Aldrich (cat no: L3129) and for prepare the solution dissolved with saline. AGO is purchased from TCI America. IL-1β and IL-10 ELISA kits were all purchased from Bioassay Technology Laboratory, Korain Biotech Co., Ltd (Shanghai, China).

5.2. Animals

For in vivo studies, 36 male Sprague-Dawley rats, approximately 9 weeks old and weighing 200–250 grams, were obtained from the Marmara University Experimental Animals Application and Research Center (DEHAMER). Ethics committee approval (65.2023mar) was received from the Marmara University Experimental Animals Local Ethics Committee for the entire experimental process. Laboratory conditions were checked daily by the researchers, ensuring a 12-hour light/12-hour dark cycle and an ambient temperature of 22 ± 2 °C. The animals had ad-libitum waterand food intake, and also were housed with a

maximum of four rats per cage. The experiment lasted 21 days, and at the end of the experiment the animals were sacrificated by guillotine under thiopental anesthesia.

5.3. Animal model

A 1-week adaptation period was provided for all rats before the experiment began. For 3 days before induction, rats were given daily intraperitoneal (i.p.) saline (1 ml/kg) to accustom them to injection stress. Lipopolysaccharide (LPS; 0.5 mg/kg, Escherichia coli, serotype 0127:B8), dissolved in saline, was administered i.p. for 10 consecutive days to induce neuronal inflammation and anxiety in rats. The injection volume was 1 ml/kg in all conditions.

5.4. Drug administration

AGO treatment (40 mg/kg) was administered intragastrically once daily, 30 minutes before LPS administration throughout the study. AGO was administered at the specified dose, suspended in distilled water. The rats were randomly divided into three groups (N = 8): (a) control (non-LPS administrated group), (b) LPS (0.5 mg/kg daily), and (c) LPS treated with AGO group (LPS+AGO).

5.5. Open field test

Prior to testing, rats were acclimated in a quiet, undisturbed room. The open-field test apparatus consisted of a rectangular platform (100 × 100 × 40 cm; length × width × wall height). Each rat was placed individually in the arena and video-recorded for 5 min. The arena was cleaned with ethanol between subjects and allowed to air-dry completely to minimize residual odor cues. Videos were analyzed offline to quantify standard exploratory/anxiety-related measures, including the number of square crossing, latency to enter center, time spent in the central zone, number of rearing, and grooming. Scoring was performed for all animals under identical conditions.

5.6. ELISA analysis

IL-1β and IL-10 levels were measured in hippocampus, prefrontal cortex, and serum samples using a rat ELISA kit according to the manufacturer's instructions.

5.7. Statistical analysis

GraphPad Prism 9 analysis program was used for statistical analyses. One-way ANOVA and Tukey's multiple comparison test were used in the analysis of all tests. All values are expressed as mean ± SEM.

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REFERENCES

[1] Colonna M and Butovsky O. Microglia Function in the Central Nervous System During Health and Neurodegeneration. Annu Rev Immunol. 2017; 35: 441-468. https://doi.org/10.1146/annurev-immunol-051116-052358

[2] Glass CK, Saijo K, Winner B, Marchetto MC, and Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell. 2010; 140(6): 918-34. https://doi.org/10.1016/j.cell.2010.02.016

[3] Guo B, Zhang M, Hao W, Wang Y, Zhang T, and Liu C. Neuroinflammation mechanisms of neuromodulation therapies for anxiety and depression. Translational Psychiatry. 2023; 13(1): 5. https://doi.org/10.1038/s41398-022-02297-y

[4] Dantzer R, O'Connor JC, Freund GG, Johnson RW, and Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008; 9(1): 46-56. https://doi.org/10.1038/nrn2297

[5] Barbosa-Méndez S, Perez-Sánchez G, and Salazar-Juárez A. Agomelatine decreases cocaine-induced locomotor sensitisation and dopamine release in rats. World J Biol Psychiatry. 2023; 24(5): 400-413. https://doi.org/10.1080/15622975.2022.2123954

[6] Conboy L, Tanrikut C, Zoladz PR, Campbell AM, Park CR, Gabriel C, Mocaer E, Sandi C, and Diamond DM. The antidepressant agomelatine blocks the adverse effects of stress on memory and enables spatial learning to rapidly increase neural cell adhesion molecule (NCAM) expression in the hippocampus of rats. Int J Neuropsychopharmacol. 2009; 12(3): 329-41. https://doi.org/10.1017/S1461145708009255

[7] Savran M, Aslankoc R, Ozmen O, Erzurumlu Y, Savas HB, Temel EN, Kosar PA, and Boztepe S. Agomelatine could prevent brain and cerebellum injury against LPS-induced neuroinflammation in rats. Cytokine. 2020; 127: 154957. https://doi.org/10.1016/j.cyto.2019.154957

[8] Chumboatong W, Khamchai S, Tocharus C, Govitrapong P, and Tocharus J. Agomelatine Exerts an Anti-inflammatory Effect by Inhibiting Microglial Activation Through TLR4/NLRP3 Pathway in pMCAO Rats. Neurotox Res. 2022; 40(1): 259-266. https://doi.org/10.1007/s12640-021-00447-6

[9] Lu Y, Ho CS, McIntyre RS, Wang W, and Ho RC. Agomelatine-induced modulation of brain-derived neurotrophic factor (BDNF) in the rat hippocampus. Life Sci. 2018; 210: 177-184. https://doi.org/10.1016/j.lfs.2018.09.003

[10] Mendonça IP, Paiva IHR, Duarte-Silva EP, Melo MG, Silva RSD, Oliveira WH, Costa B, and Peixoto CA. Metformin and fluoxetine improve depressive-like behavior in a murine model of Parkinsońs disease through the modulation of neuroinflammation, neurogenesis and neuroplasticity. Int Immunopharmacol. 2022; 102: 108415. https://doi.org/10.1016/j.intimp.2021.108415

[11] Zhang J, Zhang N, Lei J, Jing B, Li M, Tian H, Xue B, and Li X. Fluoxetine shows neuroprotective effects against LPS-induced neuroinflammation via the Notch signaling pathway. Int Immunopharmacol. 2022; 113(Pt A): 109417. https://doi.org/10.1016/j.intimp.2022.109417

[12] Wang YL, Han QQ, Gong WQ, Pan DH, Wang LZ, Hu W, Yang M, Li B, Yu J, and Liu Q. Microglial activation mediates chronic mild stress-induced depressive- and anxiety-like behavior in adult rats. J Neuroinflammation. 2018; 15(1): 21. https://doi.org/10.1186/s12974-018-1054-3

[13] Aranđelović J, Ivanović J, Batinić B, Mirković K, Matović BD, and Savić MM. Sucrose binge-eating and increased anxiety-like behavior in Sprague-Dawley rats exposed to repeated LPS administration followed by chronic mild unpredictable stress. Sci Rep. 2024; 14(1): 22569. https://doi.org/10.1038/s41598-024-72450-8

[14] Zhang N, Gao X, Li D, Xu L, Zhou G, Xu M, Peng L, Sun G, Pan F, Li Y, Ren R, Huang R, Yang Y, and Wang Z. Sleep deprivation-induced anxiety-like behaviors are associated with alterations in the gut microbiota and metabolites. Microbiol Spectr. 2024; 12(4): e0143723. https://doi.org/10.1128/spectrum.01437-23

[15] Cai L, Gong Q, Qi L, Xu T, Suo Q, Li X, Wang W, Jing Y, Yang D, Xu Z, Yuan F, Tang Y, Yang G, Ding J, Chen H, and Tian H. ACT001 attenuates microglia-mediated neuroinflammation after traumatic brain injury via inhibiting AKT/NFκB/NLRP3 pathway. Cell Commun Signal. 2022; 20(1): 56. https://doi.org/10.1186/s12964-022-00862-y

[16] Goulart da Silva M, Daros GC, Santos FP, Yonamine M, and de Bitencourt RM. Antidepressant and anxiolytic-like effects of ayahuasca in rats subjected to LPS-induced neuroinflammation. Behav Brain Res. 2022; 434: 114007. https://doi.org/10.1016/j.bbr.2022.114007

[17] Lan T, Wu Y, Zhang Y, Li S, Zhu Z, Wang L, Mao X, Li Y, Fan C, Wang W, and Yu SY. Agomelatine rescues lipopolysaccharide-induced neural injury and depression-like behaviors via suppression of the Gαi-2-PKA-ASK1 signaling pathway. J Neuroinflammation. 2022; 19(1): 117. https://doi.org/10.1186/s12974-022-02479-x

[18] Kakhki FSH, Asghari A, Bardaghi Z, Anaeigoudari A, Beheshti F, Salmani H, and Hosseini M. The Antidiabetic Drug Metformin Attenuated Depressive and Anxiety-like Behaviors and Oxidative Stress in the Brain in a Rodent Model of Inflammation Induced by Lipopolysaccharide in Male Rats. Endocr Metab Immune Disord Drug Targets. 2024; 24(13): 1525-1537. https://doi.org/10.2174/0118715303275039231228065050

[19] Algaidi SA. Chronic stress-induced neuroplasticity in the prefrontal cortex: Structural, functional, and molecular mechanisms from development to aging. Brain Research. 2025; 1841: 149461. https://doi.org/10.1016/j.brainres.2025.149461