Journal of Affective Disorders Reports 12 (2023) 100462

Contents lists available at ScienceDirect

Journal of Affective Disorders Reports

journal homepage: www.sciencedirect.com/journal/journal-of-affective-disorders-reports

Research Paper

Microglial activation mediates maternal separation-induced depressive-like behavior in rats: A neurodevelopmental depression model

Cangul Yurtdas [a], Hacer Zortul [a], Bayram Yilmaz [b], Feyza Aricioglu [a,*]

[a] Marmara University, Institute of Health Sciences, Department of Pharmacology, Turkey
[b] Yeditepe University, Department of Physiology, Istanbul, Turkey

A R T I C L E I N F O

Keywords:
Maternal separation
Depression
Imipramine
NLRP3
ASC
Caspase-1

A B S T R A C T

Background: : Maternal Separation (MS) is an early life stress which has been associated with neurological, behavioral, chemical and endocrinological changes in adult life in rats and both short term and long-term mental health disturbances, notably depression in human. This study was planned as a neurodevelopmental depression model, to investigate the possible role of the NLRP3 pathway.

Material and Method: : Rat pups were separated from their mothers on their second postnatal day (PND) for 4 h and were placed back in their home cage every day for 21 days. On PND23 they were randomly assigned to either the MS or the MS + imipramine group (receiving 30 mg/kg/day i.p. imipramine for 15 days). Depression-like behaviors were assessed by open field, sucrose preference, forced swimming and elevated plus maze tests. Prefrontal cortex regions were dissected to evaluate NLRP3, ASC and caspase-1 mRNA expressions.

Results: : Significant decrease of total activity, sucrose preference as an anhedonia indicator, longer immobilization time in the forced swim test, increased time and the number of entries into the closed arms in elevated plus maze were found in the MS group compared to Control group, and these effects were reversed by imipramine treatment. NLRP3, ASC and caspase-1 mRNA expressions were increased with MS and this increase was suppressed with imipramine treatment.

Conclusion: : MS causes depressive-like behaviors in rats and these behavioral changes may be related to the NLRP3 pathway. Imipramine treatment can prevent not only depression-like behaviors, but also neuroinflammation by suppressing NLRP3-mediated mechanisms in the neurodevelopmental depression process.

Introduction

Depression is a very common psychiatric disorder with many symptoms, such as depressed mood, anhedonia, irritability, impaired concentration, sleep disturbances, and loss of appetite and weight loss. For many years studies have been conducted to understand the pathogenesis of depression. Although the monoamine hypothesis is generally accepted, the pathogenesis of depression cannot be explained only on the basis of this hypothesis, since drugs acting via this mechanism do not provide adequate treatment response in all patients (Yilmaz et al., 1996; Kessler RC, 2012; Menken et al., 2000; Murray and Lopez, 1997; Culpepper 2010; Cetin and Aricioglu, 2020).

More recent findings have demonstrated involvement of neuronal circuits, neurotrophic factors, inflammation, and neuroendocrine systems in the pathogenesis of depression. Stressful life experiences are also being recognized as important factors for the induction and maintenance of depression (Kutlu et al., 2010; Sahin et al., 2016; Ferrari et al., 2018; Réus et al., 2015).

For many years research efforts have been directed toward understanding the neurobiology of depression. Although the most common and oldest view is the monoamine hypothesis, the neurobiology of depression cannot be explained solely on the basis of this hypothesis. The most important indicator of this contention is that drugs that are widely used in clinical practice, and that utilize this mechanism, cannot provide satisfactory treatment response in all patients. Recent findings indicate that neuronal circuits, neurotrophic factors, the neuroendocrine system and neuroinflammation play critical roles in the neurobiology of depression. Therefore, identification of cellular and molecular mechanisms that can be targeted to diagnose, effectively treat, and prevent depression is of great importance for public health. Studies in this area have confirmed that the overproduction of cytokines is the key mechanism responsible for neuroinflammation, and this is an essential factor

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* Corresponding author.
E-mail addresses: feyza.aricioglu@gmail.com, faricioglu@marmara.edu.tr (F. Aricioglu).

https://doi.org/10.1016/j.jadr.2023.100462
Received 4 December 2022; Accepted 3 January 2023

contributing to the etiopathology of depression (Maes, 2011; Maes et al., 2011; Dowlati et al., 2010). Therefore, it is of paramount importance for public health that cellular and molecular mechanisms, which can be targeted to prevent and ultimately treat depression effectively, must be identified.

Our knowledge regarding the molecular mechanisms underlying the relationship and correlation between depression and neuroinflammation is still limited. Interleukin-1 beta (IL-1β) is one of the many proinflammatory cytokines, and has been proven to participate in the brain inflammatory responses. Recent research suggests that both stress and IL-1β are responsible for the decline in hippocampal neurogenesis, which in turn, is associated with the development of depressive-like behaviors. It has also been suggested that IL-1β plays a key role in the pathogenesis of depression (Lin et al., 2015; Matcham et al., 2013; Kim et al., 2022).

Evidence also suggests that depression is likely to be a type of microglial disease. Microglia make up approximately 10% of the entire brain, and they are the main line of innate immune defense in the brain (Yirmiya et al., 2015). Microglial cells contribute to the normal development and regulation of ongoing structural and functional processes of individual synapses to neural circuits and ultimately to behavior. They are activated during pathological conditions, such as neurodegeneration, inflammatory, and neuroprotective processes, which can have a detrimental impact on brain cells, including neurons and glial cells. There are patent recognition receptors in the membrane of microglial cells, which are known to be protective against factors that threaten the organism, which can be also stimulated by severe stressors. Activation of these receptors leads to activation of an intracellular pathway through node-like receptors in the microglia cell (Spellman et al., 2020).

NOD-like receptor proteins (NLRP), a member of the pattern recognition receptors of the innate immune system, have been the focus of central nervous system (CNS) pathologies including depression. Although many subtypes of NLRP receptors have been identified (NLRP1, NLRP3, NLRP6, NLRC4, NLRC5, and AIM2 inflammasome) among others, NLRP3 is the best characterized member of the NLRP family and is mainly present in microglia. NLRP3 inflammasomes consist of the apoptosis-associated speck-like protein containing a CARD (ASC) and the effector protein caspase-1. NLRP3 inflammasomes are normally inactive unless they encounter danger/pathogen associated stimuli (Di Virgilio F., 2013)

NLRP3 play a particular role in the initiation of IL-1β and IL-18 mediated inflammatory responses. This multi-protein platform formation leads to the autocatalysis and activation of caspase-1, which, in turn, converts IL-1β and IL-18 into their respective biological active forms prior to secretion. Newer studies have indicated that the NLRP3 inflammasome is involved in stress induced depression and, therefore, may be a potential target for the treatment of depression (Sahin and Aricioglu, 2013; Sahin et al., 2016). Once NLRP is activated, it causes release of caspase-1, and triggers the production of active forms of potent proinflammatory cytokines, such as IL-1β and IL-18, and their release. (Guarda and So, 2010; Iwata et al., 2016; Fleshner et al., 2017).

We have previously shown that neuroinflammation can cause depression-like behaviors in experimental animal models, and one of the main sources of neuroinflammation is the pathological activation of microglia cells. Studies on the mechanisms for the causes and consequences of pathological activation of microglia cells have shown that nod-like receptor activation, which is involved in the early step of immune system activation, may be important. Similarly, NLRP3 inflammatory activation and subsequent cytokine responses are increased in experimental models of depression. (Kaufmann et al., 2017; Haapakoski et al., 2016; Velasquez and Rappaport, 2016). Although many subtypes of NLRP receptors have been identified, NLRP3 is the best characterized member of NLRP family and is mainly present in microglia (Kummer et al., 2007; Kigerl et al., 2014; Song and Li., 2018; Zhang et al., 2015; Alcocer-Gómez et al., 2016).

Many studies have evaluated the effect of MS in different postpartum periods. To our knowledge, no study has explored the potential role of microglial activation through the NLRP3 pathway, in a certain brain region in the MS model, as an early indicator of microglial activation, associated with depression-like behaviors. As stated above, we sought to investigate whether early life stress, such as MS, induces neuroinflammation by means of NLRP3. Accordingly, we investigated whether NLRP3, ASC and caspase-1 levels accompany behavioral changes in MS, which is a neurodevelopmental experimental model, and the possible effect of imipramine, a classic antidepressant, on the NLRP3 pathway.

Material and method

Animals

Pregnant Wistar Albino rats were obtained from Yeditepe University Experimental Research Center (Istanbul, Turkey). The animals were housed under a 12:12 h light/dark cycle with appropriate temperature (22–25 °C) and humidity (55–65%). Procedures involving animal subjects were approved by the Institutional Animal Care and Ethics Committee of Yeditepe University (Ethics Code No. 2017/613).

The following MS paradigm was used in this study. Four pregnant female rats, purchased from Mother rats, were housed at 22–24 °C and 12 h light/12 h dark cycle, without food or water limitation. Following birth, pups excluding the control group were kept at room temperature of 31 ± 0,5 °C. The study protocol was approved by the Laboratory Animals Ethics Committee and performed in accordance with the guidelines of the Ministry of Food, Agriculture, and Livestock. Pups were separated from their mothers for 4 h every day, between the hours of 9 a.m. and 12 p.m. from postnatal day (PND) 2 to 14. During separation, MS pups were placed in a clean cage. The day of pups' birth was considered as PND0. All the offspring (male and female) of each mother were kept in a separate cage covered with fresh bed in a separate room from the mother's room with standard laboratory conditions.

Imipramine hydrochloride (Sigma) was used by dissolving in saline and prepared just before injection every day which was administered for 14 days at PND53–67. It was applied once a day and at the same time every day. The volume of all injections was adjusted to be 0.1 ml / 100 g and administered intraperitoneally.

Experimental groups

Pups were exposed to maternal separation stress for 21 days, starting PND 1. Following weaning in PND 23, all pups were separated as MS and control groups and separated again as male and female (Farrell et al., 2016). Only female pups were used in order to avoid gender-related outcomes. Female pups in the MS group were placed in individual cages where the control group were placed collectively.

Groups defined as

Group 1: Control group ($n = 9$): Female pups living with the mother and housed in a cage collectively. Saline was applied at the same time, duration, and volume as the treatment groups.

Group 2: MS group ($n = 8$): Female pups experiencing MS stress and housed in individual cages. Saline was administered at the same time, duration, and volume as the treatment groups. Group 3: MS + Imipramine group ($n = 6$): Female pups experiencing MS stress and housed in individual cages. Imipramine was applied as 30 mg/kg, as i. p. and between PND 53–67.

MS model creation

MS is selected as an early life stress-induced depression model. In PND 1–21, any external event that will adversely affect the mother-offspring relationship, may negatively affect CNS development and stress-related axes of the rat, which can cause psychiatric diseases, such as depression and anxiety, in adulthood. Based on this information, early adverse life models are used in rats as a depression model. In the literature, the results of MS stress and behavioral tests have been examined on different days and for different periods of time. Based on these results, separation for 21 days (PND 1–23) and for 4 h a day were evaluated as ideal conditions in terms of adaptation, to establish the effect on neurodevelopment, as well as to allow real-life projections. The duration and length used in model formation were chosen in accordance with the literature (Farrell et al., 2016). Pups were isolated between PND 23–53 (single caged) and imipramine group received treatment between PND 53–67. Model and control groups only received saline in order to control for injection stress. Behavioral tests were performed immediately after the completion of treatment.

Behavioral tests

Open field test

The OFT was performed to investigate anxiety-like/depression-like behavior and locomotor activity. The pups were taken from their home cage and placed individually in a well illuminated (300 lux) open area. Testing was performed for 5 min in a transparent acrylic cage divided into 16 squares. Rats were put into the middle of a cage and allowed to move freely, and their movement was recorded. The cage was thoroughly cleaned with 70% ethanol and then dried between each animal in order to prevent influence of the odor of the previous one (Brudzynski and Krol, 1997).

Forced swimming test

Forced swimming test was performed to investigate depression-like behavior and despair. On PND 71, the rats were taken from their home cage and placed individually in a glass cylinder too high for the rat to escape, and too deep not to sink, and was filled with water at 24 ± 2 °C. Rats were allowed to swim for 15 min the day before the test to become accustomed to the tank. During the test, the total duration of immobility was recorded for the last 5 min. The duration of immobility was defined as floating or only making slight movements to keep the head above the water (Porsolt et al., 1977; Petit-Demouliere et al., 2005).

Elevated plus maze test

The elevated plus maze test consists of four arms; two closed arms have high walls and the other arms are open. The maze was elevated 50 cm above the floor. On PND 70, rats were placed in the center of the maze facing an open arm, and allowed free access to four arms for 5 min. The surface of the platform was cleaned with a 70% ethanol solution. The number of entries and time spent on closed arms were measured (Treit et al., 1993).

Sucrose preference test

This test was used to evaluated anhedonia on PND 68. Two-bottle choice procedure is used to calculate total water and sucrose consumption based on their preference. Rats were used to drink water in two bottles, five days prior to the test, and they were deprived of food and water for the last 12 h. Rats were given two bottles for an hour, one containing a 1% sucrose solution, and one containing water. Bottles were weighed before and after the test and the percent of sucrose preference was calculated as: {sucrose consumption (ml)/(sucrose + water consumption)} x 100 formula (Eagle et al., 2016).

Molecular analysis real-time polymerase chain reaction (PCR) analysis

Frozen prefrontal cortex tissue was homogenized and total RNA was extracted using a commercial RNAzol RT isolation kit (Molecular Research Center, Inc., Cincinnati, OH, USA). RNA concentrations were spectrophotometrically determined. The purification and the concentration of 1 μl RNA samples were assessed by 260/280 and 260/230 ratios. Complementary DNA (cDNA) synthesis from RNA samples was performed with a commercial cDNA synthesis kit (Jena Bioscience, Jena, Germany; Cat no. PCR511). Two microliters of the cDNA sample were used along with qPCR GreenMaster kit (Jena Bioscience; Cat no: PCR306) for real-time reverse transcription PCR. The primers used in the study were obtained from DNA Technology (Moscow, Russia) were;

Gene	Forward primer	Reverse primer
NLRP3	CCATGAGCTCCCTTAAGCTG	TTGCACAGGATCTTGCAGAC
NLRP1	GTTGCAAGTCCCTTCAGCTC	CATCTCTGTTTCCGAGCACA
Caspase-1	GCTTGAAAGACAAGCCCAAG	CCTTTCAGTGGTTGGCATCT
ASC	GCAATGTGCTGACTGAAGGA	TGTTCCAGGTCTGTCACCAA

Beta-actin (*β-actin*) was used as an internal control (housekeeping) gene. The cycle of threshold (CT) of investigated primers was determined and normalized to housekeeping gene, *β-actin*. Relative quantitation was calculated with 2ˆ-(ddCT) method and data are presented as relative changes from the control group.

Statistical analysis

GraphPad Prism Program (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analyses. Results were expressed as mean ± standard error of mean. One-way analysis of variance (ANOVA) was used for statistical comparisons between groups. The Tukey's test was used for post hoc analysis. A p value less than 0.05 was considered as a value of significance.

Results

Three independent groups of animals were used to evaluate the effect of MS on neuroinflammation in rats. The timeline is shown in Fig. 1.

The effect of MS on body weight

Body weight measurements were made starting from the 23rd day (PND 23), the day of weaning, on the 53rd day (starting day of the injection-PND 53) and the 67th day (the last day of injection-PND 67). No statistical significance was found when the groups were compared with their baseline values, the control group, and the MS group (Table 1).

Results of behavioral tests

In the open field test, in which the sum of rearing and line crossing numbers of rats was evaluated as total activity, there was a significant decrease in the MS group compared to the control group ($p<0.001$). In rats treated with imipramine total activity was increased (NS) compared to the MS group that did not receive treatment, but saline (Fig. 2).

In the sucrose preference test, in which the sucrose preference of rats was evaluated, there was a significant decrease in the MS group compared to the control group ($p<0.001$). Sucrose preference increased significantly in rats receiving imipramine ($p<0.001$) treatment compared to the MS group which received saline (Fig. 3).

The Forced Swimming Test is used extensively for the evaluation of antidepressant activity. In this test, as expected, MS remarkably increased the immobility time ($p<0.001$). In rats treated with imipramine this period was significantly shorter ($p<0.001$) than in the MS group (Fig. 4).

In the elevated plus maze test, we observed a significant increase in the portion of time spent in the closed arms and total entries into closed arms due to MS procedure ($p<0.001$). After IMI administration, MS-induced increase in closed arms (Fig. 5A). and total entries into closed arms was significantly reversed ($p<0.001$) (Fig. 5B).

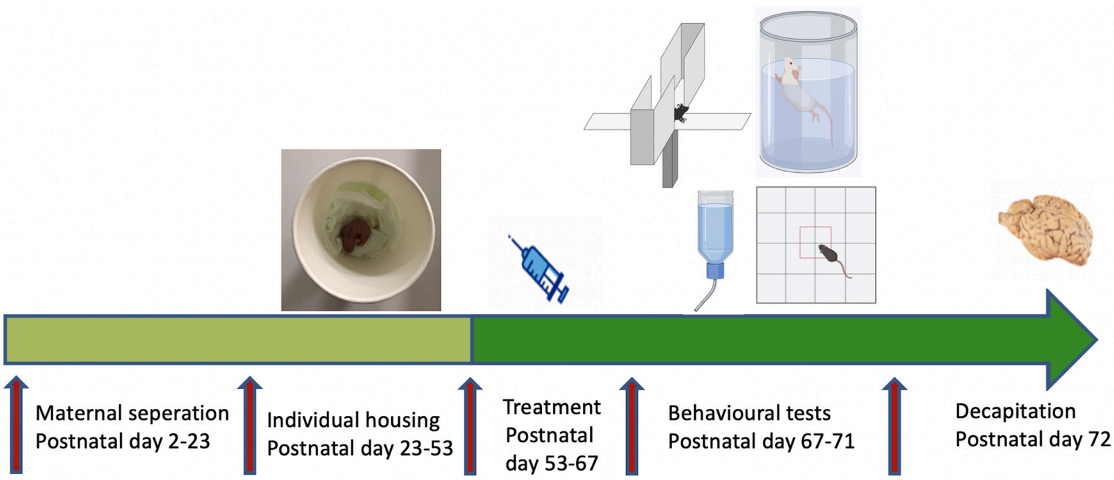

Fig. 1. Scheme of the study protocol.

Table 1

Body weight measurements. Maternal separation (MS), imipramine (IMI).

Group	PND 23 (grams)	PND 53 (grams)	PND 67 (grams)
Control	29.12±2.9	123.51±9.7	157.87±15.0
MS	26.18±3.1	132.66±10.2	176.08±14.7
MS+IMI	25.76±2.1	123.76±12.0	138.46±12.9

Results of molecular analysis relative mRNA expressions

After the behavioral experiments were completed, all rats on PND 72 were decapitated and prefrontal cortex regions were dissected on ice. They were stored at −18° centigrade until they were used to perform mRNA expression. Fluorescence-based real-time reverse transcription PCR (RT-PCR) is widely used for the measurement of steady-state mRNA levels. The expression of NLRP3, ASC and cleaved caspase-1 were

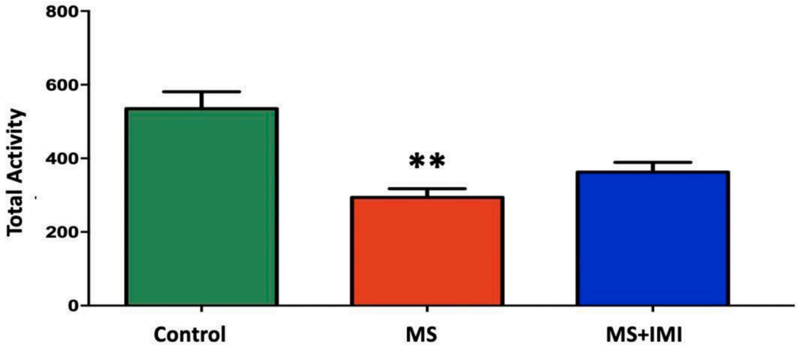

Fig. 2. Total activity in open field test. Data are presented as mean ± standard error of mean. Statistical analysis was performed with one-way ANOVA and post-hoc Tukey test was applied ($n = 69$/group). $^{**}p<0.01$; compared to the control group. Maternal separation (MS), imipramine (IMI).

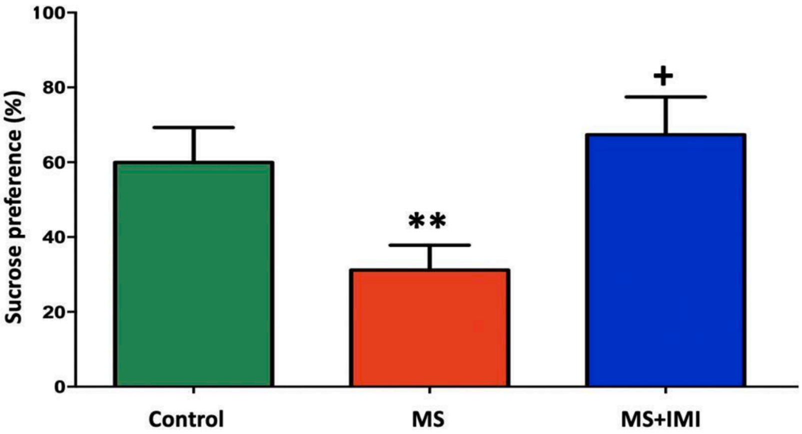

Fig. 3. Results of sucrose preferences test. Data are presented as mean ± standard error of mean.
Statistical analysis was performed with one-way ANOVA and post-hoc Tukey test was applied ($n = 69$/group). $^{**}p<0.01$; compared to the control group, $+p<0.05$; compared to the MS group. Maternal separation (MS), imipramine (IMI).

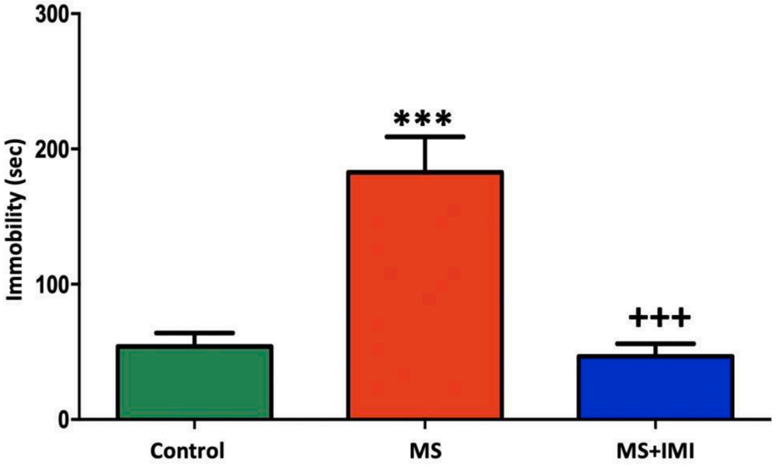

Fig. 4. Results of Forced Swimming Test. Data are presented as mean ± standard error of mean.
Statistical analysis was performed with one-way ANOVA and post-hoc Tukey test was applied (n = 69/group). ***$p<0.001$; compared to the control group, +++$p<0.01$; compared to the MS group. Maternal separation (MS), imipramine (IMI).

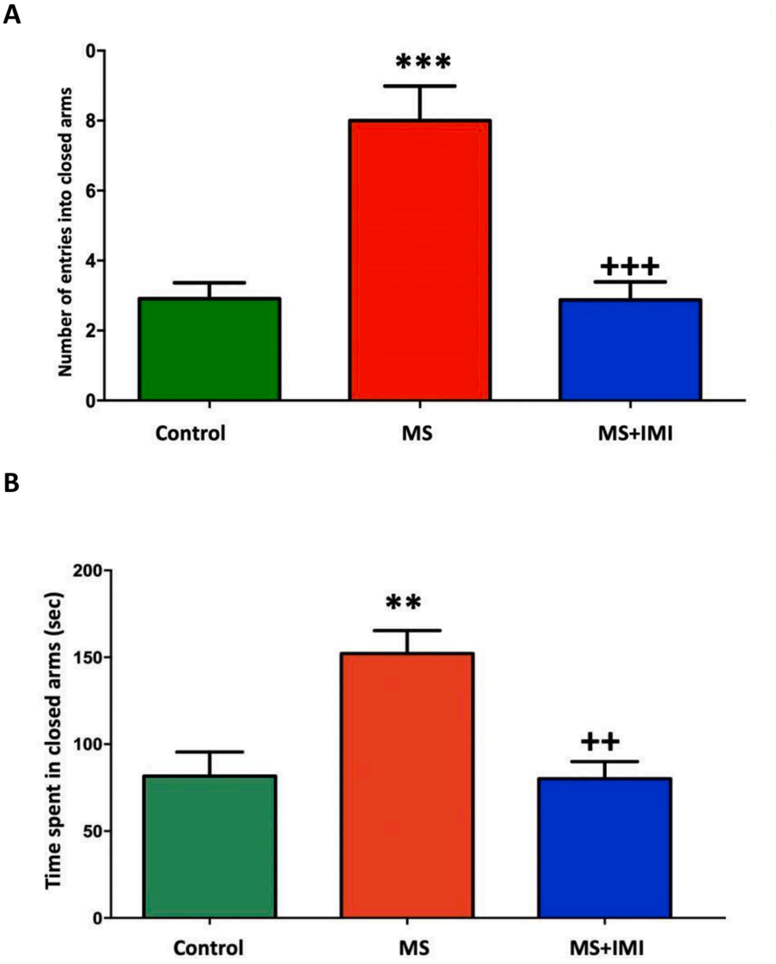

Fig. 5. Results of elevated plus maze test. Data are presented as mean ± standard error of mean.
Statistical analysis was performed with one-way ANOVA and post-hoc Tukey test was applied (n = 69/group). ***$p<0.001$;**p<0.01 compared to the control group, +++$p<0.01$;++p<0.01 compared to the MS group. Maternal separation (MS), imipramine (IMI).

examined.

The NLRP3 inflammasome, an inflammatory signaling molecular complex, plays a crucial role in depression. Therefore, possible involvement of NLRP3 and an effect of imipramine on neuroinflammation were investigated in MS-rats. As shown in Fig. 6, there is a significant increase of NLRP3 in the prefrontal cortex of MS rat pups. After imipramine treatment, the levels of NLRP3, were downregulated.

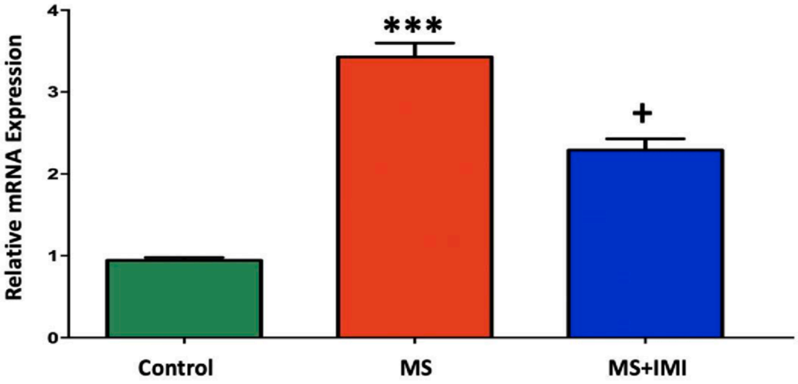

Fig. 6. Relative mRNA expression of NLRP3 in prefrontal cortex. Data are presented as mean ± standard error of mean. Statistical analysis was performed with one-way ANOVA and post-hoc Tukey test was applied ($n = 6$–9/group). ***$p<0.001$; compared to the control group, +$p<0.05$; compared to the MS group. Maternal separation (MS), imipramine (IMI).

The inflammasome is a multi-protein complex that recruits pro-caspase-1 via ASC (the adaptor molecule apoptosis associated speck-like protein containing a CARD) and then proceeds to cleave the pre-cursor cytokines, such as pro-IL-1β and pro-IL-18 into mature forms. Upon activation, the inflammasome also promotes an inflammatory form of cell death named pyroptosis. In the evaluation of ASC, a cytokine protease involved in cell apoptosis, there was a significant increase in the MS group compared to the control group ($p<0.001$). In rats treated with imipramine ($p<0.001$), ASC values were significantly reduced compared to the MS group (Fig. 7).

In the evaluation of cleaved caspase-1, a cytokine protease involved in cell apoptosis, there was a significant increase in the MS group compared to the control group ($p<0.001$). In rats treated with imipramine ($p<0.001$), caspase-1 values were significantly reduced compared to the MS group (Fig. 8).

As we expected, in the prefrontal cortex of the MS group there was a significant increase of caspase-1 activity which has a catalytic activity indicated autoactivation of caspase-1 within the NLRP3 inflammasome. Therefore, MS stress caused an activation of the multiprotein complex with all components of NLRP3 inflammasome.

Discussion

Stressful life experiences are important contributory factors for the onset and maintenance of depression. Although psychiatric disorders usually appear in adulthood, research has shown that adverse conditions which occur early in life, such as in infancy and childhood, lead to deterioration of mental health in later life. For the offspring, especially in the early postnatal period, the mother is the most important element for nutrition, protection and neurodevelopment. Based on this information, various early life animal stress models have been proposed to investigate the impact of adverse early life events, such as maternal separation (MS), in adulthood (Howard et al., 2011; Targum and Nemeroff, 2019).

MS is a widely used neurodevelopmental experimental animal model. In this model, it has been confirmed that early stress affects brain development, growth, and stress-related axes, leading to depression like behaviors in adulthood (Vetulani, 2013). Research has confirmed exposure to adverse conditions in early life, such as infancy and childhood, lead to impairments in later life. For offspring, especially in the early postnatal period, the mother is the most important element for nutrients, protection, and neurodevelopment (Nishi, 2020; Bolton et al., 2017). Based on this information, a variety of early life animal stress models are proposed to investigate the effect of adverse early life events on adulthood such as maternal separation (MS) and handling (Cirulli et al., 2007; Mete et al., 2012; Chen and Baram, 2016; Fareri and Tottenham, 2016; Hanson et al., 2015a, 2015b).

The MS procedure used in this study is widely used for basic research in the pathophysiology of human depression. The MS model is characterized by deficits in behavioral tests, which we confirmed for rat pups with the tests we performed in the current study. In the first part of the study, behavioral tests, such as open field, sucrose preference, elevated plus maze and forced swimming tests were performed to ensure that depression-like behaviors were observed in the offspring during the

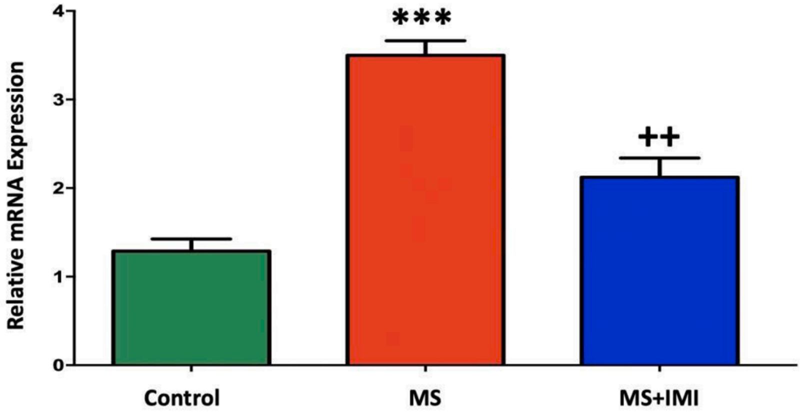

Fig. 7. Relative mRNA expression of ASC in prefrontal cortex. Data are presented as mean ± standard error of mean. Statistical analysis was performed with one-way ANOVA and post-hoc Tukey test was applied ($n = 6$–9/group). ***$p<0.001$; compared to the control group, ++$p<0.01$; compared to the MS group. Maternal separation (MS), imipramine (IMI).

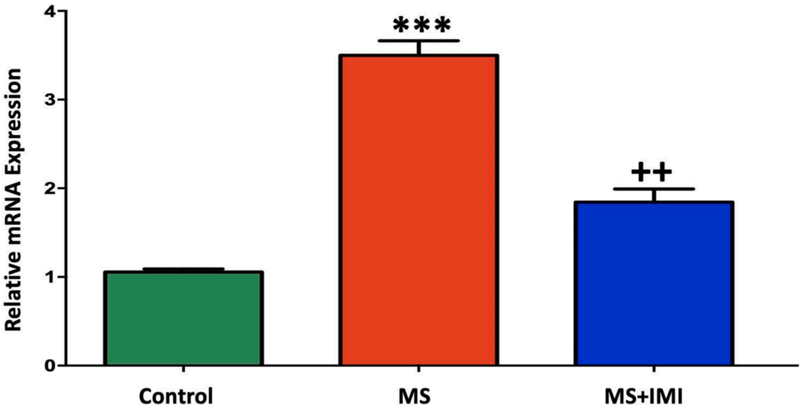

Fig. 8. Relative mRNA expression of caspase-1 in prefrontal cortex. Data are presented as mean ±standard error of mean. Statistical analysis was performed with one-way ANOVA and post-hoc Tukey test was applied ($n = 6–9$/group). ***$p<0.001$; compared to the control group, ++$p<0.01$; compared to the MS group. Stress of early separation from mother; MS, imipramine; IMI.

chosen time interval.

Rats in the MS model became more immobile and struggled less during the forced swimming test, exhibited decreases in total activity in the open field test, and spent more time in the closed arms during the elevated plus maze test. In addition, we aimed to reverse the above deficits by using imipramine, which is a widely used prototype of antidepressant. Imipramine effectively ameliorated MS-related behavioral changes in the forced swimming and elevated plus maze tests at a dose that did not cause any impairment in locomotor activity in open field test, as expected. Thus, we secured validation of the MS model by comparing it with the control and treatment groups in the time period we chose to evaluate.

In the open field test, there was a decrease in rearing and line crossing behaviors and thus in total activity in the MS group. The decrease in the number of rearing and crossing lines reached values close to the control group with imipramine treatment. This showed that the decrease in general mobility, which is considered as depression-like behavior, is also valid for MS, which is a neurodevelopmental model. It has been suggested that imipramine, which has no effect on motor activity per se, may also be effective in the developmental process. The behaviors observed in the MS group are similar to the study of Jin et al. (2018). In their studies, two experimental groups exposed to the stress of separation from the mother for 3 h and 15 min a day were used. Therefore, although exposure to the stress of separation from the mother was for a shorter period of time than in our study, it was shown that the activity decreased in the open field test.

In the elevated plus maze test, there was an increase in the number of entries into closed arms and a prolonged time spent in the closed arm in the MS group compared to the control and imipramine-treated groups. This result shows that imipramine treatment reduces depression like symptoms caused by MS stress. In a study by Liu et al. (2018), the MS group exposed to the stress of separation from the mother entered into the closed arms more and spent more time in the closed arms compared to the control group. In the study of Kalinichev et al. (2002), no difference was observed between the model group exposed to separation stress and the control group in terms of total time spent in open arms, but the number of times the model group entered closed arms was higher than the control group. In the study of Huot et al. (2001), the time spent in the closed arms of the model group was higher than the control group. Considering these findings, the results of the current study are in line with the literature.

Anhedonia is characterized by a reduced sensitivity to natural rewards like weak sucrose solutions, which is present in animal models of early-life stress (Houwing et al., 2019). Consistent with the literature, our MS group of rats also displayed anhedonia and preferred less sucrose solution compared to controls, and this effect was reversed with imipramine treatment.

There is a large body of literature describing that the neurodevelopmental process is vulnerable to alterations (Rice and Barone 2000). It has been suggested that perturbations observed in the adult brain are a product of an early neurodevelopmental disturbance (Rice and Barone, 2000; Volpe 2000). It has been suggested that trauma at an early time point, hinders normal neurodevelopment of regions involved in emotional function, thereby increasing the risk of developing the depressive phenotype later in life (Ansorge et al., 2007). Accordingly, the main aim of this study was to examine the neurobiology of the depressive syndrome in the developmental process. On the other hand, there are many recent studies pointing out that depression is a neuroinflammatory disease, and that the main reason for this is that microglia cells contribute to the production of proinflammatory cytokines. Within the scope of this information, the NLRP3 inflammasome pathway, which is the most important pathway in microglia cells, has been clarified in recent years. Our current knowledge suggests that activation of the NLRP3 inflammasome pathway may be an early key mechanism that determines the subsequent increase of proinflammatory cytokines.

The NLRP3 inflammasome complex is a molecular mechanism that translates psychological stressful stimuli into inflammatory responses. Recent studies have demonstrated that NLRP3 activation is the key factor in the pathogenesis of depressive disorders (Kim et al., 2016). In the CNS, NLRP3 was found in microglia cells (Wang et al., 2015). As the most studied inflammasome, the NLRP3 inflammasome contains an NLRP3 that recruits the ASC adapter protein through its PYD domain, followed by caspase-1 binding to the ASC through its CARD domain (Lamkanfi and Dixit, 2014). The assembly of NLRP3 complex is responsible for the proteolytic cleavage of active and secreted forms driving proinflammatory responses that culminate in cellular damage (Gurung et al., 2015). To our knowledge, there is no other study in the literature examining NLRP3 expression in the prefrontal cortex and related ASC and caspase expressions in the MS model, and ours is the first study in which microglia activation in the MS model was examined through the NLRP pathway.

Based on this information, we sought an answer to the question of whether inflammasome pathway plays a role in maternal separation and the neurodevelopmental period. For this reason, we sought to investigate the NLRP3, caspase-1 and ASC mRNA expressions that act as a bridge in this complex in the prefrontal cortex of rats using the MS model. Based on the fact that insufficient clinical response/resistance to classical antidepressants remains a major problem in at least some of clinical cases, we examined the effect of imipramine, which we frequently used in our previous studies on depression.

Repeated treatment with imipramine significantly normalized the behavioral deficits of stressed rats, indicated by spending less time immobile and more time struggling. In the open field test, imipramine treatment increased total activity which was significant suppressed by MS. We found that imipramine administration completely inhibited the increase and activation of microglia in the prefrontal cortex and reduced MS-induced depressive-like behaviors in the forced swim and open field tests. Furthermore, we found that the above-mentioned antidepressant effect of imipramine maybe associated with decreased NLRP3 inflammasome activity. Imipramine treatment significantly inhibited MS-induced NLRP3 inflammasome mRNA expression. Consistent with NLRP3 expression, as expected, other components of the inflammasome complex, ASC and caspase-1, and mRNA expressions significantly normalized after imipramine treatment. Chronic imipramine treatment remarkably prevented/reversed increases in ASC and caspase1 expression seen in the MS group.

In conclusion, the results of this study demonstrate that NLRP3 inflammation is involved in MS induced depressive-like behavior in rat offspring. NLRP3 inflammation may be a central mediator between immune activation and the neurodevelopment of depression. The current study is actually a pilot study planned to answer the question of whether the NLRP3 pathway plays a role in the MS model in rat pups. Therefore, it is important to pursue further studies that will explore the underlying mechanism(s) in greater detail. It is possible that NLRP3 inflammation may be a more specific target for the development of new pharmacological agents for depression treatments in the near future. Although these are promising findings regarding the role of the NLRP3 pathway in the process of neurodevelopmental depression, measurement of proinflammatory cytokine levels, and the evaluation of other components in the NLRP3 pathway will provide a better understanding of the neurobiology of neurodevelopmentally induced depression. The presented model of MS may ultimately allow early assessment of the child's vulnerability to develop depressive disorder at some point in their lifetime. Additionally, the demonstrated effectiveness of antidepressant drug intervention may allow arrest of negative mental health sequelae for the child, if such pharmacotherapeutic intervention is undertaken early.

Author statement

The authors confirm that the submitted review article is original work and it has not been published elsewhere not is it being considered for publication in another journal.

Part of this manuscrip formed the basis of a thesis of gaduate students Cangul Yurtdas and Hacer Zortul and was patially supported by the Scientific Research Project Units of Marmara University, Turkey (Grant number: SAG-B-230119–0016 and SAG-C-YLP-150218-0035).

Conflict of interests

The authors declare that they have no conflict of interest.

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