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Granulocyte colony-stimulating factor has a sex-dependent positive effect in the maternal immune activation-induced autism model

Ferit Durankuş[1] | **Yakup Albayrak**[2] | **Fırat Erdoğan**[1] | **Neslihan Albayrak**[3] | **Mümin Alper Erdoğan**[4] | **Oytun Erbaş**[5]

[1]Department of Pediatrics, Istanbul Medeniyet University, İstanbul, Turkey

[2]Department of Psychiatry, Faculty of Medicine, Tekirdağ Namık Kemal University, Tekirdağ, Turkey

[3]Department of Cardiology, Çorlu State Hospital, Tekirdağ, Turkey

[4]Department of Physiology, Katip Çelebi University Medical School, İzmir, Turkey

[5]Department of Physiology, Medical School, Demiroğlu Bilim University, İstanbul, Turkey

Correspondence
Yakup Albayrak, Tekirdağ Namık Kemal Üniversitesi Tıp Fakültesi 59100, Süleymanpaşa, Tekirdağ, Turkey.
Email: dr.fuge@hotmail.com

Abstract

Objective: The medical intervention for autism spectrum disorder (ASD) is restricted to ameliorating comorbid situations. Granulocyte colony-stimulating factor (G-CSF) is a growth factor that enhances the proliferation, differentiation, and survival of hematopoietic progenitor cells. In the present study, we aimed to investigate the effects of G-CSF in a maternal immune activation-induced autism model.

Methods: Sixteen female and six male Wistar adult rats were included in the study. After 21 days, 48 littermates (eight male controls, eight female controls, 16 male lipopolysaccharide [LPS]-exposed rats, and 16 female LPS-exposed rats) were divided into groups. Sixteen male LPS-exposed and 16 female LPS-exposed rats were divided into saline and G-CSF treatment groups.

Results: In male rats, the LPS-exposed group was found to have significantly higher levels of TNF-α, IL-2, and IL-17 than the LPS-exposed G-CSF group. Levels of nerve growth factor, brain PSD-95, and brain GAD67 were higher in the LPS-exposed G-CSF group than in the LPS-exposed group in male rats. In female rats, brain NGF levels were similar between groups. There was no difference between groups in terms of brain GAD 67 levels. Brain PSD-95 levels were higher in the control group than in both the LPS-exposed and LPS-exposed G-CSF groups in female rats. Both neuronal CA1 and neuronal CA2 levels were lower, and the GFAP immunostaining index (CA1) and GFAP immunostaining index (CA3) were higher in the LPS-exposed group than in the LPS-exposed G-CSF group in male rats. However, neuronal count CA1 and neuronal count CA3 values were found to be similar between groups in female rats.

Abbreviations: ASD, autism spectrum disorder; BBB, blood–brain barrier; CA, cornu ammonis; GAD 67, glutamate decarboxylase 67; G-CSF, granulocyte colony-stimulating factor; LPS, lipopolysaccharide; OF, open-field; PAL, passive avoidance learning; PSD 95, postsynaptic density protein 95; TNF-α, tumor necrosis factor-α.

Conclusions: The present research is the first to demonstrate the beneficial effects of G-CSF on core symptoms of ASD experimentally depending on male sex. G-CSF can be a good candidate for ameliorating the core symptoms of ASD without serious side effects in males.

KEYWORDS
autism, experimental, granulocyte colony-stimulating factor, treatment

1 | INTRODUCTION

Autism spectrum disorders (ASDs) are a common type of neurodevelopmental disorder that affect cognition and behavior over a wide range depending on the severity of their clinical manifestations (Matson & Goldin, 2014). Sex has a significant role in both the prevalence and clinical characteristics in ASD. Males tend to develop ASD four times more frequently than females, and the onset of ASD was reported to be seen at later ages in female individuals (Chiarotti & Venerosi, 2020). The medical intervention of ASD is restricted to ameliorating comorbid situations, and unfortunately, there is no curative treatment (Chiarotti & Venerosi, 2020).

There are several difficulties for the development of animal autism models. The ASD does not have a biomarker; thus, animal model can be currently defined with a set of core behavioral abnormalities rather than by objective biomarkers. Moreover, two of the core features, deficits in social interaction and in communication, can only be approximated in rodent studies. The interest on possible environmental agents which would be associated with development of ASD increased for last decades (Lawler et al., 2004). Lipopolysaccharide (LPS) is obtained from the walls of gram-negative bacteria and has been demonstrated to be a good candidate for producing infection in animal models (American Psychiatric Association, 2013; Williams et al., 2021). The data have shown that administration of LPS during the perinatal period alters long-term behavioral patterns, including abnormal social communication and disturbed socialization skills (Arsenault et al., 2014; Lawler et al., 2004). Moreover, administration of LPS was shown to induce numerous effects, including increased cell density, neuroinflammatory alterations, and limited dendritic arbors in the hippocampus, which have been found in autism. Electrophysiological recordings reveal reduced synaptic input to CA1 of the hippocampus, heightened excitability of pyramidal neurons, enhanced postsynaptic glutamatergic responses, and impaired NMDA-induced synaptic plasticity (Ashdown et al., 2006).

Filgrastim-granulocyte colony-stimulating factor (G-CSF) is a growth factor (O'Loughlin et al., 2017). It was previously reported that G-CSF could cross the blood–brain barrier (BBB) and had some effects on the central nervous system (CNS) (Patterson, 2011). There have been two previous reports that investigated G-CSF levels in patients with autism. The earlier study investigated the immune response in the brain tissue of patients with ASD.

The authors researched the brain immune response in postmortem brain tissues of patients with ASD and compared them with healthy brain tissue. In this study, Li and coworkers showed that proinflammatory cytokines (TNF-α, IL-6, and GM-CSF), Th1 cytokines (IFN-γ), and chemokines (IL-8) were significantly increased in the brains of ASD patients compared with controls (Wallner et al., 2015). A later study by Ahmad and his coworkers in which they investigated immune factors in patients with ASD by flow cytometric analysis, gene expression, and Western blotting found that patients with ASD showed higher GM-CSF mRNA and protein expression levels than the control group. They concluded that CD45 could play an essential role in the immune abnormalities of ASD (Nervi et al., 2006). In addition to these reports, activation of the G-CSF receptor resulted in stimulating the survival, proliferation, and development of neuronal stem cells (Patterson, 2011). G-CSF is currently used for ischemic stroke in a phase I/II clinical trial. It was reported that treatment with G-CSF promotes somatic growth, prevents brain atrophy, and improves long-term neurological outcomes in a neonatal hypoxic-ischemia model (Schneider et al., 2005). Neuroprotective effects of G-CSF in an experimental model of Parkinson's disease in male mice were demonstrated (Patterson, 2011). G-CSF has been reported to attenuate inflammation and microglial activation in an experimental model of amyotrophic lateral sclerosis (ALS) (Li et al., 2009). G-CSF was shown to have beneficial effects in peripheral nerve injury, in an experimental hypoxia-ischemia model and in traumatic brain injury (Ahmad et al., 2020; Fathali et al., 2010; Pollari et al., 2011).

Although the neuroprotective effects of G-CSF are considered to be well established, no study has investigated the effects of G-CSF in an autism model. The

present study aimed to investigate the effects of G-CSF in a maternal immune activation-induced autism model.

2 | METHODS

2.1 | Animals

Sixteen female (90 ± 4 days old) and six male Wistar adult rats (90 ± 6 days old and 238 ± 10 g, respectively) were included in the study. The experiments performed in this study were carried out according to the rules in the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health (U.S.A.) and approved by the Animal Ethics Committee (T.C. Bilim University Animal Ethics Committee, Ethical number: 19211108).

2.2 | Experimental procedures

Female rats were randomly distributed into two groups: Group A (control, $n = 8$) and Group B (LPS-induced group $n = 8$). All animals were monitored daily for behavior and health conditions throughout the study. Female rats were caged with a fertile male (three female/one male) for 2–3 days during the estrus period. Mating was checked by looking at white vaginal plaque in rats. Then, male rats were removed from the cages. Group A rats were given 0.5 ml % 0.9 NaCl saline (control group) at 10 days of pregnancy. Group B rats were intraperitoneally (i.p.) administered 100 μg/kg LPS (LPS *E. coli* O127: B8, Sigma-Aldrich) to induce autism (LPS-exposed group) at 10 days of pregnancy.

On the day of birth, litter sizes were started as eight pups per dam. The dams were allowed to raise their own litters until weaning on postnatal day 21 (P21).

After 21 days, forty-eight littermates (eight male control, eight female control, 16 male LPS-exposed, and 16 female LPS-exposed) were divided into groups. Sixteen male LPS-exposed and 16 female LPS-exposed rats were divided into saline and G-CSF (Filgrastim) (TEVAGRASTİM®, Teva Company, Israel) treatment groups.

Group 1 male rats ($n = 8$) (male LPS exposed and saline) were given 1 ml/kg/day % 0.9 NaCl saline via i.p. Group 2 male rats ($n = 8$) (male LPS exposed and G-CSF) were given 100 μg/kg/day i.p. G-CSF (Filgrastim) (*Neupogen* 48 MIU/0.5 ml). Group 3 female rats ($n = 8$) (female LPS exposed and saline) were given 1 ml/kg/day % 0.9 NaCl solution via i.p. Group 4 female rats ($n = 8$) (exposed to female LPS and G-CSF) were given 100 μg/kg/day i.p. G-CSF (Filgrastim). To investigate continuous, daily repeated choline chloride treatment, all animals were treated for 45 days (Figure 1).

2.3 | Behavioral tests

2.3.1 | Three-chamber sociability test

The open-field (OF) paradigm is one of the popular behavioral tests to assess locomotion and exploration. Altered OF behavior is relatively simple to observe;

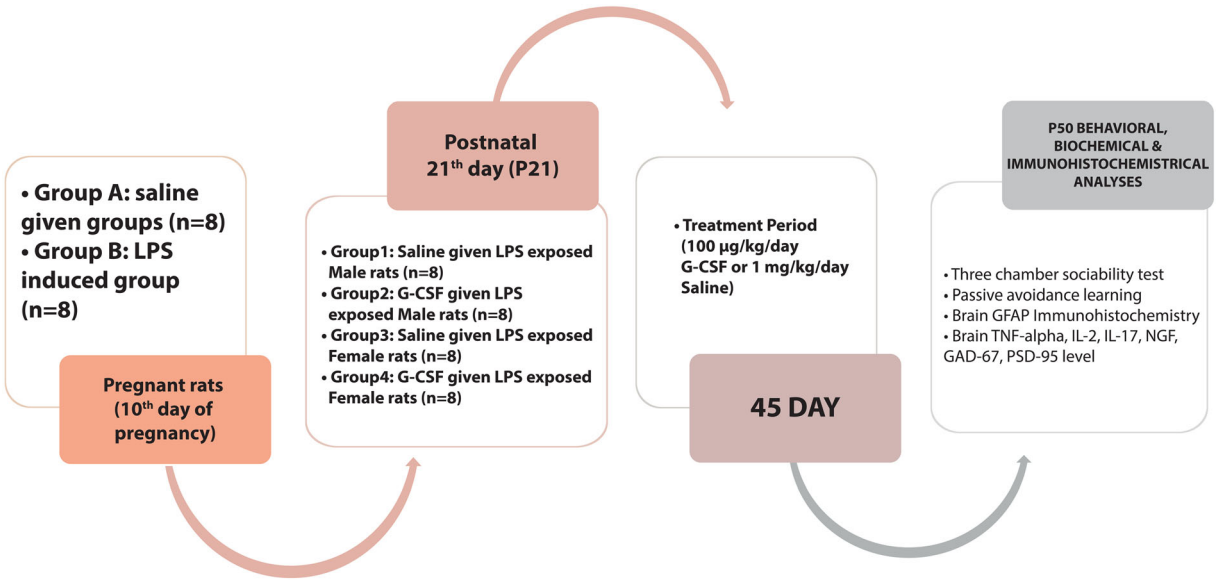


FIGURE 1 Experimental process of study

however, concluding the reasons for the observed changes is a complex task. Generally, there are two factors that determine the behavior in this paradigm: The first is a positive exploratory drive originating from the nature of rodents to explore new environments (for food and shelter), and the second is the animal nature of avoiding open and brightly lit spaces (exposure to predators). The open-field test is considered useful in determining motor stereotyped behavior, repeated autogrooming, and restriction of research activity in autism model. The openfield test was conducted in an open-aired box with dimensions of 50 cm · 50 cm · 40 cm. At the beginning of the test, rats were gently placed in the center of the box and allowed to explore the arena freely for 5 min. Then, each rat was observed for 5 min to evaluate its spontaneous activity level. The total number of ambulation (i.e., the number of floor divisions crossed with four paws) was recorded. The floor of the field was then cleaned between each animal with a 70% alcohol-water solution and dried with paper towel to avoid olfactory cues (Sünnetçi et al., 2021) (Figure 1).

2.3.2 | Open-field

The open-field (OF) paradigm is one of the popular behavioral tests to assess locomotion and exploration. Altered OF behavior is relatively simple to observe; however, concluding the reasons for the observed changes is a complex task. Generally, there are two factors that determine the behavior in this paradigm: The first is a positive exploratory drive originating from the nature of rodents to explore new environments (for food and shelter), and the second is the animal nature of avoiding open and brightly lit spaces (exposure to predators). The open-field test is considered useful in determining motor stereotyped behavior, repeated autogrooming, and restriction of research activity in autism model (Sünnetçi et al., 2021).

2.3.3 | Passive avoidance learning (PAL)

Learning and memory performance of offspring were evaluated by passive avoidance learning (PAL) test as FCA described previously. PAL is comprised of fear-motivated avoidance tasks in which the rat learns to refrain from stepping through a door that seems apparently safer but actually leads into a dark compartment with an electrified grid system that delivers a shock. The PAL box was 20 cm × 20 cm × 20 cm and had both dark and lighted chambers. Normally, when a rat was placed into the lighted compartment, they preferred to enter the dark chamber. After a 10-s habituation period in the lighted compartment, the guillotine door separating the light and dark chambers was opened. When a rat passed into the dark chamber, the door separating the light and dark compartments was closed. Then a 1.5 mA electric shock was delivered over 3 s, and the rat was subsequently removed from the dark chamber and returned to its cage. Twenty-four hours later, the rats were placed into the PAL box again. The duration of time or latency period for the rat to travel from the lighted to the dark chamber was recorded, but a shock was not delivered. The latency period was recorded up to a maximum of 300 s. The time that the rat took to refrain from crossing into the dark chamber served as an index of the rat's memory (Sünnetçi et al., 2021).

Figure 2 presents an illustration of behavioral tests (Figure 2).

2.4 | Hippocampal and cerebellar histopathology

The cornu ammonis (CA) 1 and CA 3 regions of the hippocampus were chosen as the target areas to be examined for hippocampal damage. Briefly, following behavioral tests, animals were euthanized, and their brains were removed and fixed for 3 days in 10% formaldehyde in 0.1 M phosphate-buffered saline (PBS). Then, they were moved into 30% sucrose and stored at 4°C until infiltration was complete.

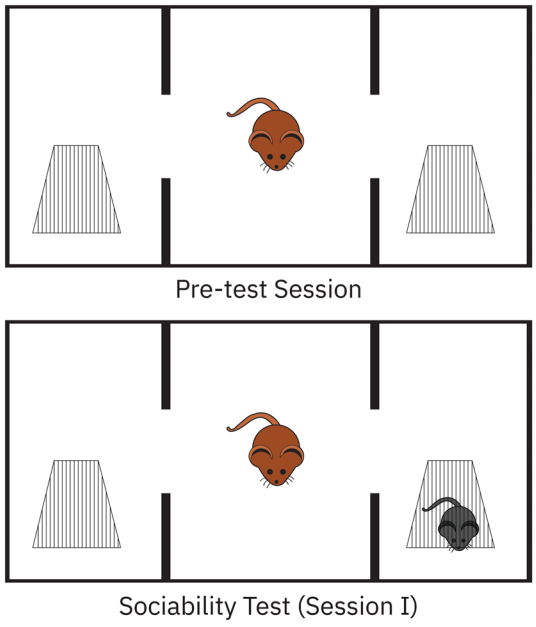


FIGURE 2 Sociability test

2.5 | Tissue biochemical analysis

After decapitation, brains were rapidly removed and stored at −20°C until biochemical analysis. The brain levels of TNF-α, nerve growth factor (NGF), IL-17, GAD 67, and PSD-95 in the brain supernatants were measured using commercially available rat enzyme-linked immunosorbent assay (ELISA) kits. All samples from each animal were measured in duplicate according to the manufacturer's guidelines. A microplate reader was used for the measurement of the absorbances (MultiscanGo, Thermo Fisher Scientific Laboratory Equipment, NH, US).

2.6 | Statistical analysis

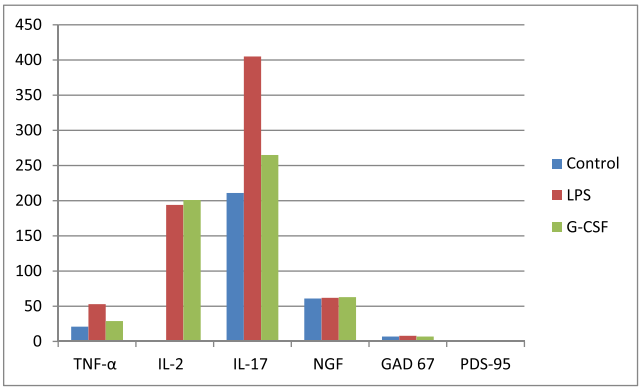
Statistical evaluation was performed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Shapiro–Wilk's W and Levene's tests were used to check the normality and the homogeneity of variance, respectively. The groups were compared with ANOVA test. Post hoc analysis was performed with Tukey test. The results are presented as mean ± standard error of the mean. A value of $p < 0.05$ was considered reflective of statistical significance.

3 | RESULTS

3.1 | Biochemical results

3.1.1 | Female group

Brain TNF-α levels were found to be higher in the LPS-exposed and LPS-exposed G-CSF groups than in the control group ($F = 7.88$; $df = 2$; $p < 0.001$; $p < 0.01$ and $p < 0.01$, respectively). Brain IL-2 levels were higher in the LPS-exposed and LPS-exposed G-CSF groups than in the control group ($F = 11.92$; $df = 2$; $p < 0.001$; $p < 0.001$ and $p < 0.001$, respectively). Brain IL-17 levels were found to be higher in the LPS-exposed and LPS-exposed G-CSF groups than in the control group ($F = 12.66$; $df = 2$; $p < 0.001$; $p < 0.001$ and $p < 0.05$, respectively). Brain IL-17 levels were also higher in the LPS-exposed group than in the LPS-exposed G-CSF group ($F = 6.13$; $df = 2$; $p < 0.001$; $p < 0.05$). Brain NGF levels were similar between groups. Similar to the NGF level, there was no difference between groups in terms of brain GAD 67 levels. Brain PSD-95 levels were higher in the control group than in both the LPS-exposed and LPS-exposed G-CSF groups. The results are presented in Graph 1.



GRAPH 1 Comparison the results of brain biochemical analysis between groups (female group). Control: Control Group, LPS: LPS-Exposed Group, G-CSF: LPS-Exposed and G-CSF Group. Abbreviations: G-CSF, granulocyte colony-stimulating factor; LPS, lipopolysaccharide; PSD 95, postsynaptic density protein 95; TNF-α, tumor necrosis factor-α

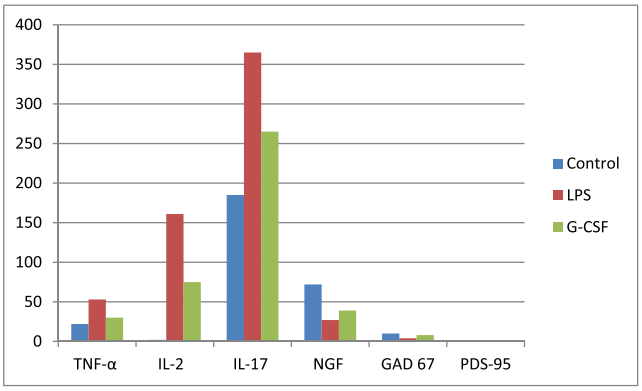
3.1.2 | Male group

Brain TNF-alpha levels were found to be higher in the LPS-exposed group than in the LPS-exposed G-CSF and control groups ($F = 13.12$; $df = 2$; $p < 0.001$; $p < 0.01$ and $p < 0.001$, respectively). The brain TNF alpha values were found to be similar between the LPS-exposed and LPS-exposed G-CSF groups. The brain IL-2 level in the LPS-exposed group was significantly higher than that in the LPS-exposed G-CSF and control groups ($F = 9.72$; $df = 2$; $p < 0.001$; $p < 0.001$ and $p < 0.001$, respectively). The brain IL-17 level in the LPS-exposed group was significantly higher than in the LPS-exposed G-CSF and control groups ($F = 14.01$; $df = 2$; $p < 0.001$; $p < 0.001$ and $p < 0.001$, respectively). Brain NGF levels were higher in the LPS-exposed G-CSF and control groups than in the LPS-exposed group ($F = 11.09$; $df = 2$; $p < 0.001$; $p < 0.05$ and $p < 0.001$, respectively). Brain GAD 67 and PSD-95 levels were found to be significantly higher in the LPS-exposed G-CSF and control groups than in the LPS-exposed groups ($F = 10.79$; $df = 2$; $p < 0.001$; $p < 0.05$ and $p < 0.001$, respectively) (Graph 2).

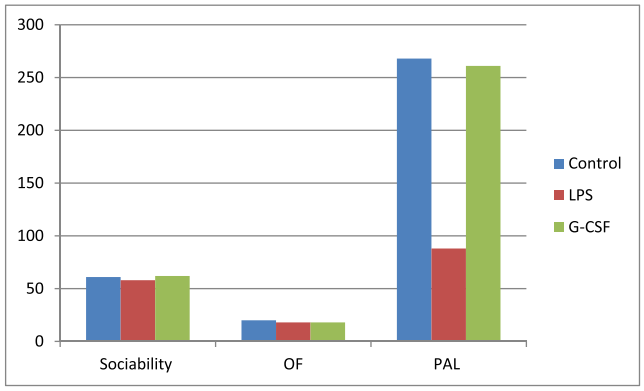
3.2 | Behavioral tests

3.2.1 | Female group

The mean scores on the sociability test (the percentage of time spent with strangers) were found to be similar between groups. The mean scores of the open field test



GRAPH 2 Comparison the results of brain biochemical analysis between groups (male group). Control: Control Group, LPS: LPS-Exposed Group, G-CSF: LPS-Exposed and G-CSF Group. Abbreviations: G-CSF, granulocyte colony-stimulating factor; LPS, lipopolysaccharide; PSD 95, postsynaptic density protein 95; TNF-α, tumor necrosis factor-α

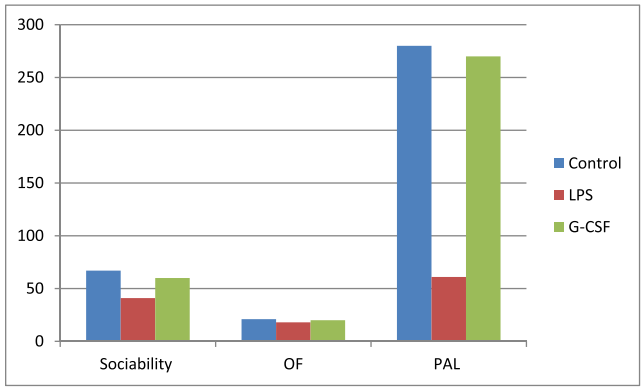


GRAPH 3 Comparison the results of behavioral tests between groups (female group). Control: Control Group, LPS: LPS-Exposed Group, G-CSF: LPS-Exposed and G-CSF Group. Abbreviations: G-CSF, granulocyte colony-stimulating factor; LPS, lipopolysaccharide; PSD 95, postsynaptic density protein 95; TNF-α, tumor necrosis factor-α

(number of ambulations) were also similar between groups. The mean scores of passive avoidance learning (PAL) latency were also found to be higher in the LPS-exposed group than in the LPS-exposed G-CSF and control groups ($F = 9.35$; $df = 2$; $p < 0.001$; $p < 0.01$ and $p < 0.001$, respectively). The results of the behavioral tests are presented in Graph 3.

3.2.2 | Male group

In the sociability test (the percentage of time spent with stranger rats), the mean scores were higher in the LPS-exposed G-CSF and control groups than in the LPS-exposed group ($F = 16.00$; $df = 2$; $p < 0.001$; $p < 0.05$ and $p < 0.01$, respectively). The mean scores of the open field test (number of ambulations) were also similar between groups. The mean scores of passive avoidance learning (PAL) latency were also found to be higher in the LPS-exposed G-CSF and control groups than in the LPS-exposed group ($F = 9.97$; $df = 2$; $p < 0.001$; $p < 0.001$ and $p < 0.001$, respectively). The results of the behavioral tests are presented in Graph 4.



GRAPH 4 Comparison the results of behavioral tests between groups (male group). Control: Control Group, LPS: LPS-Exposed Group, G-CSF: LPS-Exposed and G-CSF Group. Abbreviations: G-CSF, granulocyte colony-stimulating factor; LPS, lipopolysaccharide; PSD 95, postsynaptic density protein 95; TNF-α, tumor necrosis factor-α

3.3 | Hippocampal and cerebellar histopathology

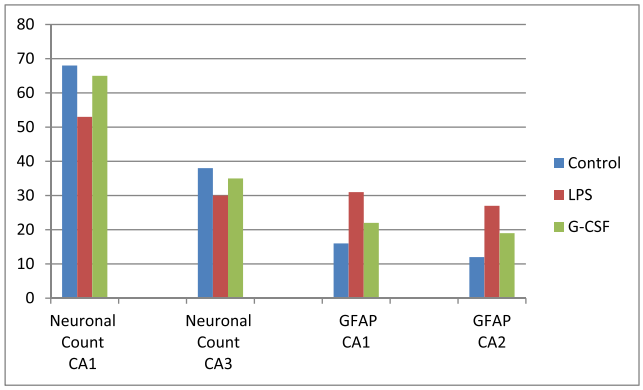
3.3.1 | Female group

Neuronal count CA1 and Neuronal count CA3 values were found to be similar between groups. The GFAP immunostaining index (CA1) was found to be lower in the control group than in the LPS-exposed and LPS-exposed G-CSF groups ($F = 14.22$; $df = 2$; $p < 0.001$; $p < 0.001$ and $p < 0.05$, respectively). The GFAP immunostaining index (CA3) was also lower in the control group than in the LPS-exposed and LPS-exposed G-CSF groups ($F = 8.90$; $df = 2$; $p < 0.001$; $p < 0.01$ and $p < 0.05$, respectively) (Graph 5). The histopathology examinations are shown in Figure 3.

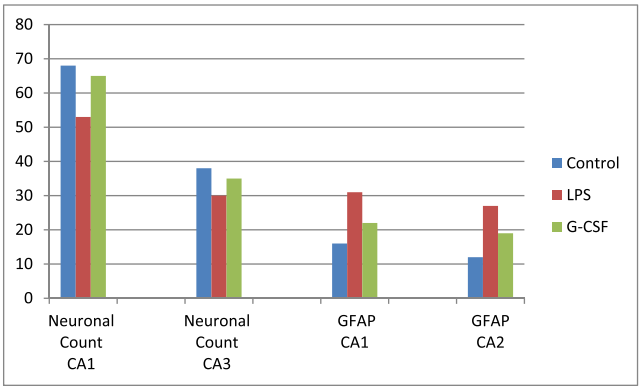
3.3.2 | Male group

Neuronal CA1 counts were significantly higher in the control and LPS-exposed G-CSF groups than in the LPS-exposed group ($F = 14.65$; $df = 2$; $p < 0.001$; $p < 0.01$ and $p < 0.05$, respectively). Neuronal CA3 counts were also

higher in the control and LPS-exposed G-CSF groups than in the LPS-exposed group ($F = 12.22$; $df = 2$; $p < 0.001$; $p < 0.01$ and $p < 0.05$, respectively). The GFAP immunostaining indices (CA1) were found to be lower in the control and LPS-exposed G-CSF groups than in the group ($F = 11.33$; $df = 2$; $p < 0.001$; $p < 0.001$ and $p < 0.05$, respectively). The GFAP immunostaining indices (CA3) were lower in the control and LPS-exposed G-CSF groups than in the LPS-exposed group ($F = 13.12$; $df = 2$; $p < 0.001$; $p < 0.01$ and $p < 0.05$, respectively) (Graph 6). The histopathology examinations are shown in Figure 3.



GRAPH 5 Comparison the results of hippocampus histopathology (female group). Control: Control Group, LPS: LPS-Exposed Group, G-CSF: LPS-Exposed and G-CSF Group. Abbreviations: G-CSF, granulocyte colony-stimulating factor; LPS, lipopolysaccharide; PSD 95, postsynaptic density protein 95; TNF-α, tumor necrosis factor-α



GRAPH 6 Comparison the results of hippocampus histopathology (male group). Control: Control Group, LPS: LPS-Exposed Group, G-CSF: LPS-Exposed and G-CSF Group. Abbreviations: G-CSF, granulocyte colony-stimulating factor; LPS, lipopolysaccharide; PSD 95, postsynaptic density protein 95; TNF-α, tumor necrosis factor-α

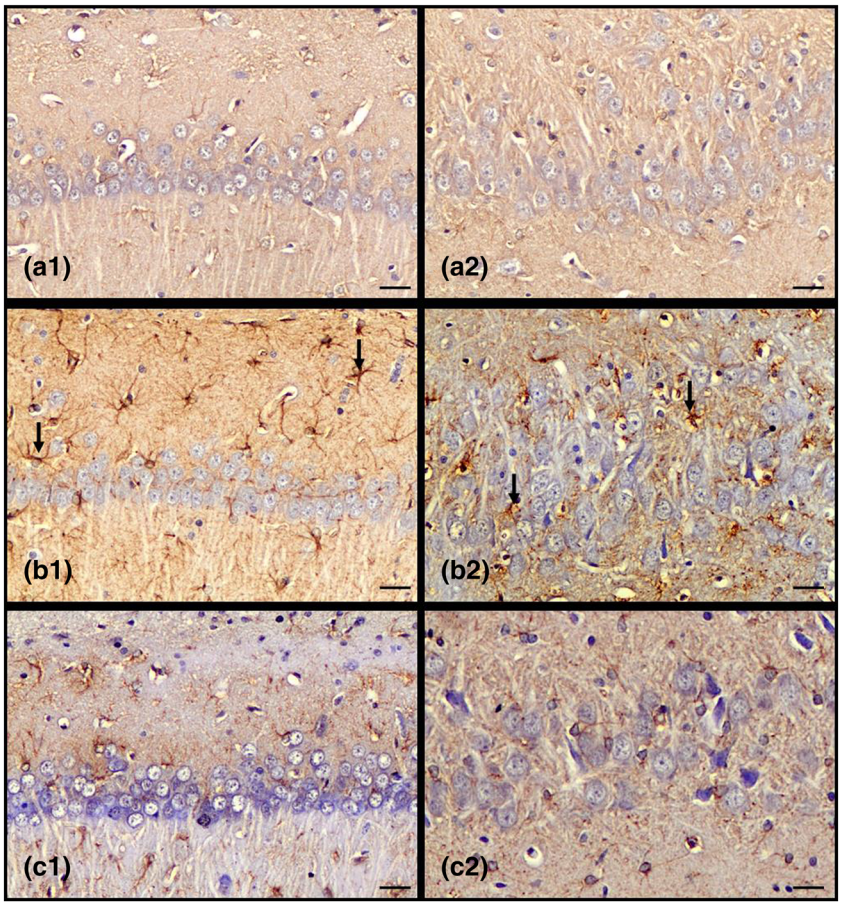


FIGURE 3 CA1 and CA3 of hippocampus × 20 magnification. Astrogliosis was characterized by GFAP immunostaining (Brown staining). A1–A2: Normal control group male rats CA1 and CA3, B1–B2: LPS and saline group male rats have increased glial activity (arrow) CA1 and CA3. C1–C2: LPS and G-CSF (Filgrastim) group male rats have decreased glial activity CA1 and CA3.

4 | DISCUSSION

The treatment of ASD is unfortunately limited because of the insufficient knowledge about its etiology, and there is no cure for ASD even though it affects a large population. The heterogenic nature of ASD is related to genetic background (Song et al., 2016), neuroanatomical substrates and structures (Komine-Kobayashi et al., 2006), and the phenotypic profile (Sünnetçi et al., 2021). This issue is considered a major challenge for highlighting the etiology, diagnosis and treatment of ASD (Sünnetçi et al., 2021). In this context, sex seems to be the major factor in the heterogeneity of ASD.

Preclinical studies showed that prenatal LPS injections caused activation of the immune system and created ASD symptoms (Lotufo Denucci et al., 2021; Pagnozzi et al., 2018; Sullivan et al., 2012). LPS injection was shown to increase the levels of IL-1β, IL-6, and TNF-α in the placenta and amniotic fluid and was also demonstrated to increase TNF-α levels in the fetal brain (Calderoni, 2022). The activation of maternal immunity was shown to induce several histopathological changes in the hippocampus and negatively affect learning and memory (Joel & McCarthy, 2017). IL-17 was reported to have a special role in the maternal immune activation hypothesis (Pagnozzi et al., 2018). The results of the present study support the idea that G-CSF has beneficial effects in terms of ameliorating the core symptoms of ASD in an experimental rat model and in males. Several studies have shown beneficial effects of G-CSF for the improvement of CNS damage in patients who suffer from ALS (Choi et al., 2016). The mechanism of action of G-CSF in ALS patients was reported to be associated with the regulation of TNF-α, IL-17, and NADPH oxidase 2 (NOX2). Thus, fixation of neuroinflammation is considered an essential part of the improvement of neuronal damage in patients with ALS (Fernández de Cossío et al., 2017; Toscano et al., 2021; Urakubo et al., 2001). Regarding the results of the present research, it is plausible to consider that G-CSF can have beneficial effects on the core symptoms of ASD via the improvement of neuroinflammation.

NGF is a well-known neurotrophic factor that plays particular roles in cell death and/or neurodegeneration (Deda et al., 2009; Golan et al., 2005; Ismail et al., 2013). Our previous research also showed that intrauterine LPS exposure decreased NGF levels in rats (Baron et al., 2005). Moreover, G-CSF was reported to inhibit apoptosis and have direct neuroprotective effects (England et al., 2016). In the present study, the brain NGF level was significantly higher in the LPS-exposed G-CSF group than in the LPS-exposed group in male rats. G-CSF seems to have beneficial effects on brain NGF levels and can significantly ameliorate core symptoms in an experimental ASD model depending on sex.

Glutamic acid decarboxylase (GAD) is the key enzyme for the production of γ-aminobutyric acid (GABA) and is critical for GABA activities (Skaper, 2017). It has been demonstrated that reduced GAD67 expression causes deficits in social behavior in an experimental autism model (Allen et al., 2013). PSD-95 facilitates synaptic maturation by recruiting/trafficking both *N*-methyl-D-aspartic acid receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isox-azoleproprionic acid receptors (AMPARs) and plays an important role in the maturation of the postsynaptic membrane (Ciafrè et al., 2020). Previous studies demonstrated that PSD-95 deficiency was associated with psychiatric disorders such as schizophrenia (Lu et al., 2013) and ASD (Oliveira-Junior et al., 2019; Solmaz et al., 2020). It was also reported that PSD-95 knockout rats had significantly impaired social development compared with control subjects (Pitzer et al., 2008). In the present study, we found that brain GAD 67 and PSD-95 levels were higher in the LPS-exposed G-CSF group than in the LPS-exposed group in male rats. Thus, G-CSF could be a good candidate for the improvement of sociality in male patients; however, further experimental and clinical studies are needed to support our preliminary results.

The hippocampus is considered to have an important role in the etiology of ASD (Heckers et al., 2002). It has been reported that hippocampal volume was decreased in patients with ASD who were between 4 and 18 years old compared with their healthy counterparts (Frank et al., 2016; Sandhu et al., 2014). Another study revealed that hippocampal volume was decreased in patients with ASD (Fromer et al., 2014). Concurring evidence shows that ASD may be significantly associated with a disorder in glial cells (Coley & Gao, 2019; Feyder et al., 2010). In particular, GFAP was demonstrated to be expressed at higher levels in the ASD brain than in healthy controls (Gao & Mack, 2021). Our results indicate that G-CSF has beneficial effects on the hippocampus depending on sex. This effect can be associated with the neuroprotective actions of G-CSF.

In this study, the effects of LPS induction and G-CSF treatment on different behavioral and neurochemical outcomes were found to be sex dependent. It is well established that sex hormones affect immune functioning and that the normal process of brain masculinization during very early development is mediated by cells of the innate immune system and several inflammatory molecules (Li et al., 2019). The male brain has been reported to be more vulnerable to inflammatory events in early life (Braden et al., 2017). Hui and his coworkers reported more alterations in microglial distribution and

morphology in male rats than in female rats (Chaddad et al., 2017). However, further studies are needed to clarify the associations between sex and behavioral and neurochemical outcomes.

The present research has several limitations. We could not measure the brain or blood levels of G-CSF in the offspring of rats. We did not measure the body weight of the offspring; thus, we could not provide data on offspring weight.

The present research is the first to demonstrate the beneficial effects of G-CSF on core symptoms of ASD experimentally depending on male sex. Our results indicate that the beneficial effects of G-CSF can be mediated by improving neuroinflammation and neuroprotective effects. G-CSF can be a good candidate for ameliorating the core symptoms of ASD without serious side effects in males. However, further experimental and clinical studies are needed to confirm our preliminary results.

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None.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICS STATEMENT

Present study was approved by T.C. Bilim University Animal Ethics Committee, Ethical number: 19211108.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by OE, FE, FD, and MAE. The first draft of the manuscript was written by YA, FD, and NA, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT

Author elects to not share data.

ORCID

Ferit Durankuş https://orcid.org/0000-0002-3337-8419
Yakup Albayrak https://orcid.org/0000-0002-1926-1695
Fırat Erdoğan https://orcid.org/0000-0003-1123-4729
Neslihan Albayrak https://orcid.org/0000-0002-8490-4722
Mümin Alper Erdoğan https://orcid.org/0000-0003-0048-444X
Oytun Erbaş https://orcid.org/0000-0001-5427-8428

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