Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Inhibition of the TNF-α/IDO1 Axis Restores Brain Serotonin and Mitigates Aggression-like Phenotypes in BCG-inoculated Mice

Pooja R. Patil
SVKM NMIMS Global University
Dhiraj D. Vispute
SVKM NMIMS Global University
Sameer N. Goyal
SVKM NMIMS Global University
Sanjay N. Awathale
sanjay.awathale@svkm.ac.in

SVKM NMIMS Global University
Kartik T. Nakhate
SVKM NMIMS Global University

Research Article

Keywords: Aggression, TNF-α, IDO1, BCG, Etanercept, Minocycline

Posted Date: March 25th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9174921/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Aggression is linked to systemic inflammation and serotonergic dysfunction. Tumor necrosis factor-alpha (TNF-α), a key pro-inflammatory cytokine, disrupts serotonin synthesis by upregulating indoleamine 2,3-dioxygenase 1 (IDO1), which diverts tryptophan metabolism away from serotonin production. This study investigated whether inhibiting the TNF-α/IDO1 signaling pathway could reduce aggressive behavior. Aggression-like behaviors were induced in mice using Bacillus Calmette–Guérin (BCG), known to elevate systemic TNF-α and reduce brain serotonin. Behavioral assessments included the Social Aggression Test (SAT), Rod Biting Test (RBT), and locomotor activity. Biochemical analyses of TNF-α (plasma and amygdala) and serotonin (amygdala) were conducted using ELISA and HPLC. Treatment groups received either the TNF-α inhibitor etanercept, the IDO1 inhibitor minocycline, or both. BCG-inoculated mice exhibited increased aggression and reduced serotonin levels, alongside elevated TNF-α in the amygdala. Etanercept significantly reduced aggressive behavior and restored serotonin levels. Co-treatment with minocycline further enhanced these effects. Locomotor activity remained unchanged across all groups. TNF-α inhibition mitigates aggression by restoring serotonin levels, likely through suppression of IDO1 activity. Co-inhibition of IDO1 enhances this effect, highlighting the TNF-α/IDO1 pathway as a promising target for managing aggression.

1. Introduction

Aggression, characterized as hostile, injurious and destructive behavior, contributes to acts of violence that, according to the WHO, result in approximately 4.4 million deaths globally each year (Halvachizadeh et al. 2025). Mental disorders such as intermittent explosive disorder (IED) involve sudden, recurrent episodes of impulsive and disproportionate aggressive behavior in response to minor daily provocations (Liu and Yin 2024). Moreover, aggression can be triggered by a range of factors, including circadian rhythm disturbances, stress, hormonal fluctuations, experiences of victory or defeat, and comorbidity with certain psychological disorders (Lischinsky and Lin 2020). The aggressive behavior is modulated by a broad neural circuit involving several brain regions, including the amygdala (Rosell and Siever 2015; Blair 2016). Central serotonergic regulation of aggressive behavior is well documented, with the *serotonin deficiency hypothesis* of human aggression remaining the most widely supported hypothesis after decades of empirical testing (Duke et al. 2013). A polymorphism within an intron of the tryptophan hydroxylase (TPH) gene, which encodes the rate-limiting enzyme in serotonin synthesis, has been linked to aggressive behavior (Manuck et al. 1999). The efficacy of fluoxetine in treating IED by enhancing serotonergic neurotransmission supports the involvement of serotonin deficiency in the disorder (Liu and Yin 2024). The dorsal raphe nucleus is a primary source of serotonergic projections to several brain regions, including the prefrontal cortex, amygdala, hippocampus, striatum, and hypothalamus (Ren et al. 2019). Since serotonin promotes inhibition of the prefrontal cortex, reduced serotonergic activity increases the likelihood of aggression (Siever 2008). Notably, individuals exhibiting violent aggressive behavior have been found to show deficits in serotonergic transmission within the prefrontal cortex (Meyer et al. 2008). Further, the reduced serotonin levels in the prefrontal cortex-amygdala circuitry have been implicated in impulsive aggression (Passamonti et al. 2012).

Aggression has been strongly linked to the inflammatory state in the body. Individuals with IED show elevated plasma inflammatory markers that correlate with aggression (Coccaro et al. 2014). Anger and interpersonal conflict can elevate cytokines such as TNF-α, especially in individuals displaying high hostility (Kiecolt-Glaser et al. 2005). Studies report a positive correlation between LPS-stimulated monocyte TNF-α expression and aggression in both males and females (Suarez et al. 2004). Similarly, T-cell-derived TNF-α levels are higher in individuals with greater hostility (Mommersteeg et al. 2008). Animal studies further support the immune system's involvement in aggression, as male mice lacking TNF receptors (TNF-R1 and TNF-R2) show reduced aggressive behavior in the resident-intruder test (Patel et al. 2010). Consistent with this, elevated TNF-α levels have been observed in highly aggressive individuals (Takahashi 2024). These data highlight the contributory role of inflammatory cytokines, particularly TNF-α, in aggressive behavior. It is important to note that peripheral TNF-α crosses the intact blood-brain barrier (BBB) through receptor-mediated transport mechanisms, which are upregulated under inflammatory conditions (Pan and Kastin 2007). In the brain, TNF-α activates the enzyme indoleamine 2,3 dioxygenase 1 (IDO1), which catabolizes tryptophan (serotonin precursor) into kynurenine, resulting in serotonin deficiency (Deore et al. 2024a; Sarangi 2024). These findings suggest that targeting TNF-α/IDO1 signaling can restore brain serotonin contents and suppress aggressive behavior.

Our recent findings suggest a critical role of the amygdala in the regulation of aggressive behavior (Chaudhari et al. 2025a). In restrained animals, we observed elevated levels of pro-inflammatory cytokines, including TNF-α, as well as a reduction in serotonin levels within the amygdala. These neurochemical changes indicate that the amygdala may be a key neural substrate mediating aggression under stress-related conditions. However, the role of peripheral immune activation in modulating central mechanisms of aggression has not been fully explored. In this context, Bacillus Calmette–Guérin (BCG), a well-established immune activator and model for chronic immune stimulation, offers a valuable tool to investigate how peripheral immune challenges influence brain function and behavior. While BCG has been extensively studied in the context of depression-like behavior, its effect on aggression remains poorly characterized. Therefore, examining BCG-induced changes in aggressive behavior could provide novel insights into the neuroimmune pathways, particularly within the amygdala, that mediate aggression in response to immune stressors.

In the present study, we aimed to evaluate the effects of TNF-α and IDO1 inhibition in a mouse model of BCG-induced aggressive-like behavior. Inoculation of BCG in mice has been shown to elevate plasma TNF-α levels, increase brain IDO1 expression, and decrease serotonin levels (Deore et al. 2024a). BCG-treated mice received TNF-α inhibitor etanercept, either alone or in combination with the IDO1 inhibitor minocycline. Aggressive behavior was assessed using the social aggression test (SAT), which involved monitoring attacks on an unfamiliar intruder, and the rod biting test (RBT), where restrained mice were observed for rod-directed biting behavior. Following behavioral testing, the concentration of the plasma and amygdalar TNF-α levels was measured using ELISA, and serotonin content in the amygdala was quantified using high-performance liquid chromatography (HPLC). Our findings revealed that BCG induced aggressive behavior, elevated TNF-α, and reduced brain serotonin levels. These behavioral and

biochemical changes were attenuated by etanercept and further enhanced by co-administration with minocycline.

2. Materials and Methods

2.1. Animals

Adult male Swiss Albino mice weighing about 30 g were housed in polypropylene cages at constant room temperature (25 ± 1°C), and relative humidity (50–70%), and fed on a 12:12 h light-dark cycle. All behavioral tests were performed between 9:00 a.m. and 2:00 p.m. Chow food pellets (Nutrivet Life Sciences, Pune, India) and water were given *ad libitum*. All experimental protocols were sanctioned by the Institutional Animal Ethics Committee (IAEC) of SVKM's Institute of Pharmacy, Dhule, Maharashtra, India (SVKM-IOP/IAEC/2024/December/05).

2.2. Drug preparations

Commercial BCG preparation (Onco-BCG®, Serum Institute of India Pvt. Ltd., India) was used for the induction of aggressive-like phenotypes in mice. The vial contents were suspended in 4 mL saline to achieve a concentration of 10^9 CFU/mL (Vijaya Kumar et al. 2014). Commercially available injections of IDO1 inhibitor minocycline (Divaine®, Cipla Pharmaceuticals Ltd., India) and TNF-α inhibitor etanercept (Etacept®, Cipla Pharmaceuticals Ltd., India) were used. Lyophilized powder of etanercept was reconstituted with water for injection.

2.3. Experimental design

The mice were assigned randomly to various groups (n = 6). BCG was inoculated (0.225 mL/mouse) via the intraperitoneal (i.p.) route on day 1 of the study, and housed individually. Then the animals received various treatments from days 15–21 as stated below. Alongside, saline was administered to the control group.

Group I : Control

Group II : BCG (Disease control)

Group III : BCG → Etanercept (1.5 mg/kg, s.c.)

Group IV : BCG → Etanercept (3 mg/kg, s.c.)

Group V : BCG → Minocycline (25 mg/kg, i.p.)

Group VI : BCG → Minocycline (25 mg/kg, i.p.) + Etanercept (1.5 mg/kg, s.c.)

Group VII : BCG → Minocycline (25 mg/kg, i.p.) + Etanercept (3 mg/kg, s.c.)

On day 21, animals were subjected to behavioral studies followed by biochemical analysis of blood and brain samples. The experimental protocol is outlined in **Fig. 1**.

2.4. Behavioral Assessments

Social Aggression Test (SAT):

Attacking behavior towards an unfamiliar partner permits the measurement of reactive aggression. In this test, unfamiliar male mice were introduced into the home cages of the test mice. The unfamiliar partners were at least weighing 5 g less than the test animals (e.g., 25 g vs 30 g). The reactive aggressive behavior, i.e., the physical attack exhibited by the test mouse against an unfamiliar partner mouse, was monitored for 5 minutes. Once the experiment finished, the unfamiliar mouse was returned to its home cage (Kim et al. 2017). The interaction video was recorded from above using cameras, and the quantification of aggressive biting behavior was assessed.

Rod Biting Test (RBT):

The restraint-induced aggressive behavior in rodents has been validated in our laboratory (Chaudhari et al. 2025a). In this test, mouse was placed in the in-house fabricated restrainer for 5 minutes. Subsequently, a removable metal rod (4 cm in length and 5 mm in diameter) was inserted at the end of the restrainer, positioned near the animal's nose. The rod was consisted of two parallel metals connected to an electrical circuit and a counter. When animal bites the rod, the electric circuit completed and number of bites were recorded. The number of rod bites by the restrained mouse was counted for 3 minutes and used as an index of IED (Kuchiiwa and Kuchiiwa 2014).

Open field test (OFT)

To test the effect of various treatments on locomotor activity, the OFT was conducted based on protocols from earlier research (Deore et al. 2024b; Awathale et al. 2025; Shirasath et al. 2025). The OFT apparatus (50 × 50 × 20 cm) was equipped with invisible vertical and parallel infrared (IR) beams connected to an actimeter system. Each mouse was individually placed at the center of the arena and observed for 5 minutes. Locomotor activity was automatically recorded by counting the number of IR beam interruptions, expressed as actimeter counts. To eliminate residual odors, the arena was cleaned with 70% alcohol after each trial.

Blood sample collection

Immediately after RBT, all the mice from control, diseased and treatment groups (Groups I to VII) were deeply anesthetized by overdose of thiopentone sodium (75 mg/kg; i.p.). The blood samples were collected by retro-orbital puncture in EDTA tubes. Blood samples were centrifuged at 10000xg for 15 min at 4°C. The plasma supernatant was collected, and the levels of TNF-α were determined using ELISA kits.

2.5. Preparation tissue homogenate

After completion of the behavioral assessment and blood withdrawal, mice from various experimental groups [(saline, BCG, BCG + etanercept (3 mg/kg) and BCG + etanercept (3 mg/kg) + minocycline (25 mg/kg)] were sacrificed using thiopentone sodium (75 mg/kg, ip), the brains were isolated and kept at -80°C. Amygdala from both hemispheres were separated using a biopsy punch (2 mm diameter). Tissues were homogenized in 500 µl 0.1 N perchloric acid and centrifuged at 10000×g for 5 min at 4°C. Tissue samples were taken from coronal brain sections spanning bregma coordinates − 2.12 mm to -2.80 mm, based on the rat brain atlas by Paxinos and Watson (1998), to maintain anatomical consistency among all subjects. The collected tissue was then homogenized in ice-cold PBS at pH 7.5. The resulting homogenate was transferred to Eppendorf tubes and centrifuged at 10000xg at 0°C for 10 minutes. The supernatant was then collected and stored at -20°C for further analysis. (Deore et al. 2024b; Pardeshi et al. 2025; Awathale et al. 2025; Chaudhari et al. 2025a). Half of the supernatant was utilized for ELISA, and the remaining was used for HPLC analysis.

2.6. Determination of concentration of TNF-α in the plasma and the amygdala

For the determination of plasma TNF-α, 100 µL of the plasma samples and standards were added to a 96-well plate in triplicate. The total protein content in the supernatant of the amygdala was measured using the bicinchoninic acid (BCA) assay. Protein concentrations were then adjusted to 1 µg/µL using PBS. Each tissue sample (100 µL, equivalent to 100 µg of protein) was loaded in triplicate into a 96-well plate along with the standards. The concentrations of TNF-α in the plasma and amygdala were quantified following the manufacturer's instructions mentioned in the ELISA kits (Cat No. ELK1396, ELK Biotechnology Co. Ltd., USA) (Pardeshi et al. 2025; Chaudhari et al. 2025b).

2.7. Measurement of serotonin in the amygdala by using high-performance liquid chromatography (HPLC)

The method of HPLC was adopted from our previous studies (Deore et al. 2024a; Pardeshi et al. 2025; Chaudhari et al. 2025a). The supernatant was removed and filtered through a nylon filter (22 µm), again centrifuged at 12,000 rpm for 5 min at 4°C (O'Connor et al. 2009). The supernatant was removed and diluted with 0.02 N perchloric acid at a 1:100 ratio (v:v). These samples were injected into the HPLC system for the determination of serotonin (O'Connor et al. 2009; Awathale et al. 2020). Serotonin levels in the tissue homogenate were analyzed by HPLC using a photodiode array detector, and the isocratic reverse phase-HPLC was performed on Sunfire C18 column (250 mm × 4.6 mm, 5 µm). The mobile phases consisted of potassium dihydrogen phosphate (KH_2PO_4)/acetonitrile (90:10 v/v). The flow rate was maintained at 1.0 mL/min, and the run time was 8 min. Serotonin content in the amygdala was analyzed at + 320 mV. The injection volume (20 µl) of tissue homogenate was injected into the HPLC system. The retention time (RT) of each sample peak was recorded and compared with the RT of the external standard of serotonin (Sigma, USA; Cat No. H9523) prepared in 0.02 N perchloric acid. The area under the curve (AUC) of each chromatogram was quantified using Empower 3 software (Waters Instrument) and expressed as mean ± standard error of the mean (SEM) (Awathale et al. 2020).

2.8. Statistical analysis

The data are represented as mean ± SEM. The statistical analysis of the data was performed by one-way ANOVA, followed by post-hoc Bonferroni's multiple comparisons tests using GraphPad Prism software. The normal distribution of data was calculated by using the online calculator present at the Statistics Kingdom website (https://www.statskingdom.com/shapiro-wilk-test-calculator.html); all the data were found to be normally distributed. Applications of Grubbs' test revealed no outliers across the data. The data was considered significant if $p < 0.05$.

3. Results

3.1. Effect of etanercept on SAT, and its modulation by minocycline in BCG-inoculated mice

The effect of BCG inoculation on the aggressive behavior of test mice with unfamiliar mice was screened using SAT. Further, the effects of etanercept, alone and jointly with minocycline, were screened. Application of one-way ANOVA showed a significant change in the number of attacks within different groups of mice [$F (6, 35) = 54.04$, $p < 0.0001$, Fig. 2A]. Post-hoc Bonferroni's multiple comparisons test revealed a significant elevation in the number of attacks in BCG- inoculated mice as compared to the control ($p < 0.0001$). This suggests the induction of aggressive behavior by BCG. Etanercept at the dose of 1.5 mg/kg produced a non-significant decrease in the number of attacks ($p > 0.05$) and at the 3 mg/kg dose decreased the number of attacks as compared to the disease control ($p < 0.0001$), suggesting it reduces aggressive behavior.

Minocycline at a 25 mg/kg dose produced a non-significant reduction in the number of attacks compared to disease control ($p > 0.05$). Interestingly, its combination with a subeffective dose of etanercept (1.5 mg/kg) resulted in a significant decrease in the number of attacks as compared to disease control ($p < 0.0001$) and etanercept alone at 1.5 mg/kg ($p < 0.01$). Likewise, minocycline potentiated the effect of etanercept (3 mg/kg) and significantly decreased the number of attacks as compared to the BCG only ($p < 0.0001$) and etanercept alone ($p < 0.001$) (Fig. 2A).

3.2. Effect of etanercept on RBT, and its modulation by minocycline in BCG-inoculated mice

Application of one-way ANOVA showed a significant change in the number of bites within different groups of mice [$F (6, 35) = 148.9$, $p < 0.0001$, Fig. 2B]. Post-hoc Bonferroni's multiple comparisons test revealed that the number of Bites was increased in BCG-inoculated mice as compared to the control ($p < 0.0001$). Etanercept at the dose of 1.5 mg/kg produced a non-significant decrease in the number of bites ($p > 0.05$) and at the 3 mg/kg dose decreased the number of Bites as compared to the disease control ($p < 0.0001$).

Minocycline at 25 mg/kg dose produced a non-significant reduction in the number of Bites compared to disease control ($p > 0.05$). Interestingly, its combination with a subeffective dose of etanercept (1.5 mg/kg) resulted in a significant decrease in the number of Bites as compared to disease control ($p < 0.0001$) and per se treatment of etanercept (1.5 mg/kg) ($p < 0.0001$). Likewise, minocycline significantly potentiated the effect of etanercept (3 mg/kg) and decreases the number of Bites as compared disease control ($p < 0.0001$) and etanercept alone (3 mg/kg) ($p < 0.01$) (Fig. 2B). Although per se treatment with minocycline (25 mg/kg) or etanercept (1.5 mg/kg) failed to show a noticeable effect on Aggressive Biting behavior, a non-significantly decreased number of bites were observed ($p > 0.05$) as compared to disease control. This suggests that the combination of minocycline and etanercept reduces aggressive behavior through IDO1 and TNF-α inhibition. These data reflect that there is functional synergism between etanercept and minocycline with respect to aggressive behavior.

3.3. Effect of etanercept on locomotor activity, and its modulation by minocycline in BCG-inoculated mice

The effect of etanercept and minocycline on locomotor activity using OFT in different groups of mice. The data were analyzed by one-way ANOVA revealed no significant changes in locomotor count among all experimental groups [$F (6, 35) = 99.44$, $p = 0.4443$, Fig. 2C]. The post-hoc analysis using Bonferroni's multiple comparisons test demonstrated no significant alterations in locomotor activity among all groups of animals ($p > 0.05$). These results indicate that neither etanercept nor its combination with minocycline affects locomotor activity, thereby ruling out any cofounding influence of locomotion on the aggressive behavior with unfamiliar mice and with rod activity.

3.4. Effect of etanercept on TNF-α concentration in the plasma and amygdala of mice with aggressive-like behavior, and their modulation by minocycline

The concentrations of pro-inflammatory marker, TNF-α, in BCG-treated mice with etanercept (1.5 and 3 mg/kg), alone and in combination with minocycline (25 mg/kg), were determined in plasma using ELISA. Application of one-way ANOVA showed a significant variation in the plasma concentration of TNF-α among the different groups of mice [$F (6, 35) = 97.14$, $p < 0.0001$, Fig. 3A] and in amygdala [$F (6, 35) = 14.88$, $p < 0.0001$, Fig. 3B]. Post-hoc Bonferroni's multiple comparisons test revealed a significant increase in the concentrations of plasma and amygdala TNF-α in the BCG-treated mice as compared to the control (both $p < 0.001$), indicating that BCG increases pro-inflammatory cytokine concentration in periphery as well as in brain (amygdala). Subeffective dose of etanercept (1.5 mg/kg) shows a non-significant decrease in the level of TNF-α in plasma (Fig. 3A) and amygdala (Fig. 3B) as compared to disease control mice ($p > 0.05$). Moreover, effective dose of etanercept (3 mg/kg) decreases the level of TNF-α in plasma and amygdala as compared to disease control mice (both $p < 0.05$).

Administration of minocycline at a 25 mg/kg dose showed a non-significant change in the level of TNF-α ($p > 0.05$). Co-administration of etanercept (1.5 mg/kg) with minocycline (25 mg/kg) further reduced the TNF-α content in plasma ($p < 0.05$) and amygdala ($p < 0.01$). However, minocycline potentiated the effect of etanercept (3 mg/kg) ($p < 0.001$) and significantly decreased plasma and amygdala TNF-α level as

compared to etanercept alone both ($p < 0.001$) (Fig. 3A and B). This suggests that minocycline potentiated the effect of etanercept and significantly reduced plasma and amygdala TNF-α level, which was accompanied by alleviating the aggression-like phenotype in mice.

3.5. Effect of BCG, etanercept and their combination with minocycline on levels of serotonin in the mice brain

The effect of etanercept and combination with minocycline on the brain serotonin levels was determined using HPLC systems. Application of one-way ANOVA showed significant alteration in the levels of serotonin [chromatograms in Fig. 4A–E; AUC, $F(3, 20) = 1471$, $p < 0.0001$, Fig. 4E]. Post-hoc Bonferroni's multiple comparisons test revealed that the AUC of the 5-HT were significantly decreased in the BCG-injected mice as compared to control ($p < 0.001$, Fig. 4E).

However, etanercept at the dose of 3 mg/kg significantly increased the AUC of serotonin as compared to the disease control mice ($p < 0.001$, Fig. 4E). Furthermore, etanercept (3 mg/kg) in combination with minocycline (25 mg/kg) significantly increased the AUC of the serotonin as compared to the disease control mice and etanercept alone ($p < 0.0001$, Fig. 4E). These data indicate that BCG lowers the levels of serotonin which may be contributed to the induction of Aggressive behavior in mice. Moreover, the injection of etanercept alone and in combination with minocycline improves the levels of 5-HT, suggesting its alleviating aggressive behavior that might be associated with the modulation of the TNF-α receptor and IDO1 enzyme.

4. Discussion

Aggression can be induced by a number of risk factors, including biological and psychological, for example, genetics and mental illnesses, and socioeconomic (Fritz et al. 2023). The current study investigated the mechanisms through which BCG-induced systemic inflammation and influences aggression-like behavior in mice and examined the therapeutic potential of etanercept, a TNF-α inhibitor, and minocycline, an IDO1 inhibitor, alone and in combination. One of the key outcomes of this research is the development of BCG-induced aggression as a reproducible and stable model to investigate the effects of chronic neuroinflammation on behavior. BCG immunization robustly enhanced aggressive-like behaviors in the SIT and RBT. These behavioral modifications were accompanied by biochemical changes in proinflammatory cytokines and serotonin content, highlighting the neuroimmune connection in aggression.

Pathological aggression levels in intermittent IED or psychosis seem to be associated with increased proinflammatory cytokines (Bhatt et al. 2008; Das et al. 2016). Our results support the perspective that immune system activation is important in the modulation of behavioral states. The BCG vaccine, although conventionally employed as a protective measure against tuberculosis, is recognized to cause systemic immune activation. In our research, it robustly increased plasma TNF-α levels, an important cytokine that affects CNS function and is frequently involved in mood and behavioral disorders.

In dendritic cells TNF-α activate the JAK/STAT pathway & these stimuli result in IDO1 activation (Manches et al. 2012). Blood-brain barrier endothelial cells utilize IDO1 for the degradation of tryptophan into kynurenine for the maintenance of their function (Adam et al. 2005). L-tryptophan is not only a building block of proteins but also a precursor to serotonin synthesis (Comai et al. 2020). However, the major metabolic pathway of L-tryptophan is the catabolic pathway catalyzed by IDO1 enzyme that converts L-tryptophan to kynurenine and its metabolites, especially in inflammatory states (Badawy 2017; Pallotta et al. 2022). Under conditions of inflammation, over 95% of free L-tryptophan can be catabolized via the kynurenine pathway.

In the present investigation, mice subjected to restraint stress showed elevated TNF-α concentration in the amygdala, associated with defensive rage (Pesce et al. 2011). The role of the central amygdala in the pathology of aggression and violence has been well documented (Haller 2018). Maternal separation-induced aggression triggers a higher activation of the amygdala (Ohta et al. 2023). Therefore, in the present investigation, the involvement of TNF-α of the amygdala in the regulation of aggressive phenotype was tested. While restraint stress equipped with a biting rod elevates TNF-α in the amygdala, etanercept alone and in combination with minocycline significantly reduces its level. These results demonstrated that antagonism of the TNF-α-IDO1 interaction in the amygdala might have a regulatory effect on aggressive biting behavior in BCG-inoculated mice.

Whereas IDO1 activation in neuroinflammation induces serotonin production deficiency that can in-turn trigger psychiatric diseases (Jeon and Kim 2017). Dysregulation of the serotonin system has been implicated in the hyperexpression of aggression (Miczek et al. 2007). In line with this, our HPLC results indicated a marked reduction in brain serotonin levels in BCG-inoculated mice as compared to controls, which supports the mechanistic pathway of systemic inflammation leading to modified serotonergic signaling.

The behavioral effects showed that etanercept, especially at a higher dose of 5 mg/kg, highly decreased aggressive behaviors in both tests. This indicates that inhibition of TNF-α signaling alone is enough to weaken some of the effects of BCG for inducing aggression. Interestingly, although minocycline alone had a non-significant effect, a combination of it with a sub-effective and effective dose of etanercept was highly effective in reducing aggression. This suggests that there is a synergistic interaction between TNF-α and IDO1 pathways in controlling aggression. Combined treatment perhaps has dual inhibition preventing the release of pro-inflammatory cytokines and inhibiting tryptophan catabolism, thus maintaining serotonin levels and mood and behavioral stabilization.

This observation fits with the wider context of neuroimmune communication, in which cytokines and immune-activated enzymes communicate with neurotransmitter systems to have an effect on behavior. Stress-induced increased peripheral inflammation modulates BBB permeability, facilitating infiltration of freely circulating inflammatory factors into mood-associated brain regions' parenchyma, leading to damage and neural circuit modification (Miller and Raison 2016; Menard et al. 2017; Beurel et al. 2020). Inflammatory stimuli were found to affect brain areas associated with emotional processing, such as the

amygdala (Anisman et al. 2008; Mehta et al. 2022). The amygdala is the core of a neural system that deals with affective stimulus, particularly negatively valenced stimulus and aggression (Singh and Gobrogge 2024; Chaudhari et al. 2025b). Violent criminals have lower levels of serotonin than do nonviolent criminals, and criminals convicted of impulsive violent crimes have lower serotonin levels than criminals convicted of premeditated crimes (Virkkunen et al. 1987). So, the decreased serotonin in our BCG-injected mice probably caused this imbalance, impairing behavioral inhibition and increasing reactivity to provocation.

In addition, our research provides a translational perspective through the employment of clinical agents, pharmacologically available etanercept and minocycline. Etanercept, a biologic inhibitor of TNF have since been clinically effective in lowering inflammation related to a number of autoimmune disorders, including rheumatoid arthritis, psoriasis and Crohn's disease (Croft et al. 2024). It exhibits a significant effect on the inhibition of inflammation (Li et al. 2022). Minocycline is a tetracycline antibiotic with extensive anti-inflammatory effects and excellent central nervous system penetration. Thus, it has anti-inflammatory and neuroprotective effects and has been investigated for mood disorders as well as in other psychiatric diseases like bipolar disease and schizophrenia (Nettis 2021). The efficacy of these agents in suppressing aggression in our model suggests their potential for repurposing towards the treatment of neuroinflammation-related behavioral disorders.

5. Conclusion

The cytokine model of depression suggests that raised levels of proinflammatory cytokines including IL-6 and TNF-α, may be involved in the etiology of depressive symptoms and influence mood regulation and play a role in the episodic nature of the disorder. Neuroinflammation may interfere with neurotransmitter systems involved in depression, such as serotonin, dopamine, and glutamate (Levin 2024). Moreover, high states of anger (acute episodes of anger) also induce proinflammatory cytokine release (Kiecolt-Glaser et al. 2005). A modulating function of cytokines in mammalian aggressive behavior is implicated by direct experimental manipulation in lower mammals (Zalcman and Siegel 2006; Bhatt et al. 2008; Coccaro et al. 2015), by inflammatory cytokines' effect on anger and aggression in patients undergoing cytokine immunotherapy (McHutchison et al. 1998; Kraus et al. 2003). It is also noteworthy that the above data support the cytokine theory of affective disorders, which posits that elevated levels of proinflammatory cytokines can influence mood, behavior, and emotional regulation, thereby contributing to increased anger and aggression.

Declarations

Acknowledgements

Kartik T. Nakhate acknowledges the Indian Council of Medical Research (ICMR), Government of India, New Delhi (Ad-hoc Project ID: 2021–11133) and SEED Grant SVKM’s Institute of Pharmacy, Dhule, MH, India for research funding (Grant No. SVKM/IOP/ 2023–24/1303H).

CRediT authorship contribution statement

Pooja Patil and Dhiraj Vispute: Conceptualization, Writing-original draft, Methodology, Formal analysis, Investigation, Writing-review & editing, Visualization. **Sameer N. Goyal:** Writing-review & editing, Validation, Resources, Formal analysis, Funding acquisition. **Sanjay Awathale and Kartik T. Nakhate:** Conceptualization, Methodology, Supervision, Writing-review & editing, Visualization, Validation, Investigation, Formal analysis, Project administration. All authors reviewed the results and approved the final manuscript version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Declaration

This study was supported by the Indian Council of Medical Research (ICMR), Government of India, New Delhi (Ad-hoc Project ID: 2021–11133) and SEED Grant SVKM's Institute of Pharmacy, Dhule, MH, India for research funding (Grant No. SVKM/IOP/ 2023–24/1303H).

Data availability statement

Data will be made available on request.

References

1. Adam R, Rüssing D, Adams O, et al (2005) Role of human brain microvascular endothelial cells during central nervous system infection. Significance of indoleamine 2,3-dioxygenase in antimicrobial defence and immunoregulation. Thromb Haemost 94:341–346. https://doi.org/10.1160/TH05-01-0053
2. Anisman H, Merali Z, Hayley S (2008) Neurotransmitter, peptide and cytokine processes in relation to depressive disorder: Comorbidity between depression and neurodegenerative disorders. Progress in Neurobiology 85:1–74. https://doi.org/10.1016/j.pneurobio.2008.01.004
3. Awathale SN, Dudhbhate BB, Rahangdale RR, et al (2020) Denial of food to the hungry rat: a novel paradigm for induction and evaluation of anger-like emotion. Journal of Neuroscience Methods 341:108791
4. Awathale SN, Shirasath KR, More BR, et al (2025) Naringenin prevents ethanol-induced reward behavior in adolescent rats through inhibition of TRPM3-dependent inflammation in the pVTA. J Nutr Biochem 110078. https://doi.org/10.1016/j.jnutbio.2025.110078
5. Badawy AA-B (2017) Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects. Int J Tryptophan Res 10:1178646917691938. https://doi.org/10.1177/1178646917691938

6. Beurel E, Toups M, Nemeroff CB (2020) The Bidirectional Relationship of Depression and Inflammation: Double Trouble. Neuron 107:234–256. https://doi.org/10.1016/j.neuron.2020.06.002
7. Bhatt S, Bhatt R, Zalcman SS, Siegel A (2008) Role of IL-1 beta and 5-HT2 receptors in midbrain periaqueductal gray (PAG) in potentiating defensive rage behavior in cat. Brain Behav Immun 22:224–233. https://doi.org/10.1016/j.bbi.2007.07.011
8. Blair RJR (2016) The Neurobiology of Impulsive Aggression. J Child Adolesc Psychopharmacol 26:4–9. https://doi.org/10.1089/cap.2015.0088
9. Chaudhari AK, Shirasath KR, Goyal SN, et al (2025a) A novel biting rod model for assessing aggressive behavior in restraint-stressed rats: Role of 5-HT3 receptor and NF-κB-kynurenine pathways. Physiol Behav 115091. https://doi.org/10.1016/j.physbeh.2025.115091
10. Coccaro EF, Lee R, Coussons-Read M (2014) Elevated Plasma Inflammatory Markers in Individuals With Intermittent Explosive Disorder and Correlation With Aggression in Humans. JAMA Psychiatry 71:158–165. https://doi.org/10.1001/jamapsychiatry.2013.3297
11. Coccaro EF, Lee R, Coussons-Read M (2015) Cerebrospinal Fluid Inflammatory Cytokines and Aggression in Personality Disordered Subjects. Int J Neuropsychopharmacol 18:pyv001. https://doi.org/10.1093/ijnp/pyv001
12. Comai S, Bertazzo A, Brughera M, Crotti S (2020) Chapter Five - Tryptophan in health and disease. In: Makowski GS (ed) Advances in Clinical Chemistry. Elsevier, pp 165–218
13. Croft M, Salek-Ardakani S, Ware CF (2024) Targeting the TNF and TNFR superfamilies in autoimmune disease and cancer. Nat Rev Drug Discov 23:939–961. https://doi.org/10.1038/s41573-024-01053-9
14. Das S, Deuri SK, Sarmah A, et al (2016) Aggression as an independent entity even in psychosis- the role of inflammatory cytokines. Journal of Neuroimmunology 292:45–51. https://doi.org/10.1016/j.jneuroim.2016.01.012
15. Deore R, Ansari R, Awathale SN, et al (2024a) Lycopene alleviates BCG-induced depressive phenotypes in mice by disrupting 5-HT3 receptor - IDO1 interplay in the brain. Eur J Pharmacol 977:176707. https://doi.org/10.1016/j.ejphar.2024.176707
16. Duke AA, Bègue L, Bell R, Eisenlohr-Moul T (2013) Revisiting the serotonin-aggression relation in humans: a meta-analysis. Psychol Bull 139:1148–1172. https://doi.org/10.1037/a0031544
17. Fritz M, Soravia S-M, Dudeck M, et al (2023) Neurobiology of Aggression—Review of Recent Findings and Relationship with Alcohol and Trauma. Biology (Basel) 12:469. https://doi.org/10.3390/biology12030469
18. Haller J (2018) The role of central and medial amygdala in normal and abnormal aggression: A review of classical approaches. Neuroscience & Biobehavioral Reviews 85:34–43. https://doi.org/10.1016/j.neubiorev.2017.09.017
19. Halvachizadeh S, Mariani D, Pfeifer R (2025) Impact of trauma on society. Eur J Trauma Emerg Surg 51:155. https://doi.org/10.1007/s00068-025-02824-8

20. Jeon SW, Kim Y-K (2017) Inflammation-induced depression: Its pathophysiology and therapeutic implications. J Neuroimmunol 313:92–98. https://doi.org/10.1016/j.jneuroim.2017.10.016
21. Kiecolt-Glaser JK, Loving TJ, Stowell JR, et al (2005) Hostile marital interactions, proinflammatory cytokine production, and wound healing. Arch Gen Psychiatry 62:1377–1384. https://doi.org/10.1001/archpsyc.62.12.1377
22. Kim H-D, Call T, Carotenuto S, et al (2017) Testing Depression in Mice: a Chronic Social Defeat Stress Model. Bio Protoc 7:e2203. https://doi.org/10.21769/BioProtoc.2203
23. Kraus MR, Schäfer A, Faller H, et al (2003) Psychiatric symptoms in patients with chronic hepatitis C receiving interferon alfa-2b therapy. J Clin Psychiatry 64:708–714. https://doi.org/10.4088/jcp.v64n0614
24. Kuchiiwa S, Kuchiiwa T (2014) A novel semi-automated apparatus for measurement of aggressive biting behavior in mice. J Neurosci Methods 228:27–34. https://doi.org/10.1016/j.jneumeth.2014.02.017
25. Levin M (2024) The Role of Neuroinflammation in Psychiatric Disorders. Neuroscience and Psychiatry: Open Access 7:265–267. https://doi.org/10.47532/npoa.2024.7(5).265-267
26. Li Y, Fan H, Ni M, et al (2022) Etanercept Reduces Neuron Injury and Neuroinflammation via Inactivating c-Jun N-terminal Kinase and Nuclear Factor-κB Pathways in Alzheimer's Disease: An In Vitro and In Vivo Investigation. Neuroscience 484:140–150. https://doi.org/10.1016/j.neuroscience.2021.11.001
27. Lischinsky JE, Lin D (2020) Neural mechanisms of aggression across species. Nat Neurosci 23:1317–1328. https://doi.org/10.1038/s41593-020-00715-2
28. Liu F, Yin X (2024) Psychological and pharmacological treatments of intermittent explosive disorder: a meta-analysis protocol. BMJ Open 14:e083896. https://doi.org/10.1136/bmjopen-2024-083896
29. Manches O, Fernandez MV, Plumas J, et al (2012) Activation of the noncanonical NF-κB pathway by HIV controls a dendritic cell immunoregulatory phenotype. Proc Natl Acad Sci U S A 109:14122–14127. https://doi.org/10.1073/pnas.1204032109
30. Manuck SB, Flory JD, Ferrell RE, et al (1999) Aggression and anger-related traits associated with a polymorphism of the tryptophan hydroxylase gene. Biol Psychiatry 45:603–614. https://doi.org/10.1016/s0006-3223(98)00375-8
31. McHutchison JG, Gordon SC, Schiff ER, et al (1998) Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 339:1485–1492. https://doi.org/10.1056/NEJM199811193392101
32. Mehta ND, Stevens JS, Li Z, et al (2022) Inflammation, Amygdala-Ventromedial Prefrontal Functional Connectivity and Symptoms of Anxiety and PTSD in African American Women Recruited from an Inner-City Hospital: Preliminary Results. Brain Behav Immun 105:122–130. https://doi.org/10.1016/j.bbi.2022.06.013
33. Menard C, Pfau ML, Hodes GE, et al (2017) Social stress induces neurovascular pathology promoting depression. Nat Neurosci 20:1752–1760. https://doi.org/10.1038/s41593-017-0010-3

34. Meyer JH, Wilson AA, Rusjan P, et al (2008) Serotonin2A receptor binding potential in people with aggressive and violent behaviour. J Psychiatry Neurosci 33:499–508
35. Miczek KA, de Almeida RMM, Kravitz EA, et al (2007) Neurobiology of escalated aggression and violence. J Neurosci 27:11803–11806. https://doi.org/10.1523/JNEUROSCI.3500-07.2007
36. Miller AH, Raison CL (2016) The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol 16:22–34. https://doi.org/10.1038/nri.2015.5
37. Mommersteeg PMC, Vermetten E, Kavelaars A, et al (2008) Hostility is related to clusters of T-cell cytokines and chemokines in healthy men. Psychoneuroendocrinology 33:1041–1050. https://doi.org/10.1016/j.psyneuen.2008.05.007
38. Nettis MA (2021) Minocycline in Major Depressive Disorder: And overview with considerations on treatment-resistance and comparisons with other psychiatric disorders. Brain Behav Immun Health 17:100335. https://doi.org/10.1016/j.bbih.2021.100335
39. O'Connor JC, André C, Wang Y, et al (2009) Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guerin. J Neurosci 29:4200–4209. https://doi.org/10.1523/JNEUROSCI.5032-08.2009
40. Ohta K, Araki C, Ujihara H, et al (2023) Maternal separation early in life induces excessive activity of the central amygdala related to abnormal aggression. Journal of Neurochemistry 167:778–794. https://doi.org/10.1111/jnc.16020
41. Pallotta MT, Rossini S, Suvieri C, et al (2022) Indoleamine 2,3-dioxygenase 1 (IDO1): an up-to-date overview of an eclectic immunoregulatory enzyme. FEBS J 289:6099–6118. https://doi.org/10.1111/febs.16086
42. Pan W, Kastin AJ (2007) Tumor necrosis factor and stroke: role of the blood-brain barrier. Prog Neurobiol 83:363–374. https://doi.org/10.1016/j.pneurobio.2007.07.008
43. Pardeshi GN, Ali N, Shirasath KR, et al (2025) Inhibition of TRPM3 channels in the medial prefrontal cortex mitigates OCD symptoms following traumatic brain injury. Inflammopharmacol. https://doi.org/10.1007/s10787-025-01763-5
44. Passamonti L, Crockett MJ, Apergis-Schoute AM, et al (2012) Effects of acute tryptophan depletion on prefrontal-amygdala connectivity while viewing facial signals of aggression. Biol Psychiatry 71:36–43. https://doi.org/10.1016/j.biopsych.2011.07.033
45. Patel A, Siegel A, Zalcman SS (2010) Lack of aggression and anxiolytic-like behavior in TNF receptor (TNF-R1 and TNF-R2) deficient mice. Brain Behav Immun 24:1276–1280. https://doi.org/10.1016/j.bbi.2010.05.005
46. Pesce M, Speranza L, Franceschelli S, et al (2011) Biological role of interleukin-1beta in defensive-aggressive behaviour. J Biol Regul Homeost Agents 25:323–329
47. Ren J, Isakova A, Friedmann D, et al (2019) Single-cell transcriptomes and whole-brain projections of serotonin neurons in the mouse dorsal and median raphe nuclei. eLife 8:e49424. https://doi.org/10.7554/eLife.49424

48. Rosell DR, Siever LJ (2015) The neurobiology of aggression and violence. CNS Spectr 20:254–279. https://doi.org/10.1017/S109285291500019X
49. Sarangi P (2024) Role of indoleamine 2, 3-dioxygenase 1 in immunosuppression of breast cancer. Cancer Pathogenesis and Therapy 2:246–255. https://doi.org/10.1016/j.cpt.2023.11.001
50. Shirasath KR, Chaudhari AK, Nakhate KT, et al (2025) Plumbagin Alleviates Mild Traumatic Brain Injury-Induced Obsessive-Compulsive Disorder in Mice by Inhibiting nNOS and Augmenting Cortico-Striatal Serotonin Levels. Phytother Res. https://doi.org/10.1002/ptr.70105
51. Siever LJ (2008) Neurobiology of Aggression and Violence. Am J Psychiatry 165:429–442. https://doi.org/10.1176/appi.ajp.2008.07111774
52. Singh R, Gobrogge K (2024) Aggression Unleashed: Neural Circuits from Scent to Brain. Brain Sci 14:794. https://doi.org/10.3390/brainsci14080794
53. Suarez EC, Lewis JG, Krishnan RR, Young KH (2004) Enhanced expression of cytokines and chemokines by blood monocytes to in vitro lipopolysaccharide stimulation are associated with hostility and severity of depressive symptoms in healthy women. Psychoneuroendocrinology 29:1119–1128. https://doi.org/10.1016/j.psyneuen.2004.01.002
54. Takahashi A (2024) Associations of the immune system in aggression traits and the role of microglia as mediators. Neuropharmacology 256:110021. https://doi.org/10.1016/j.neuropharm.2024.110021
55. Vijaya Kumar K, Rudra A, Sreedhara MV, et al (2014) Bacillus Calmette-Guérin vaccine induces a selective serotonin reuptake inhibitor (SSRI)-resistant depression like phenotype in mice. Brain Behav Immun 42:204–211. https://doi.org/10.1016/j.bbi.2014.06.205
56. Virkkunen M, Nuutila A, Goodwin FK, Linnoila M (1987) Cerebrospinal fluid monoamine metabolite levels in male arsonists. Arch Gen Psychiatry 44:241–247. https://doi.org/10.1001/archpsyc.1987.01800150053007
57. Zalcman SS, Siegel A (2006) The neurobiology of aggression and rage: role of cytokines. Brain Behav Immun 20:507–514. https://doi.org/10.1016/j.bbi.2006.05.002

Figures

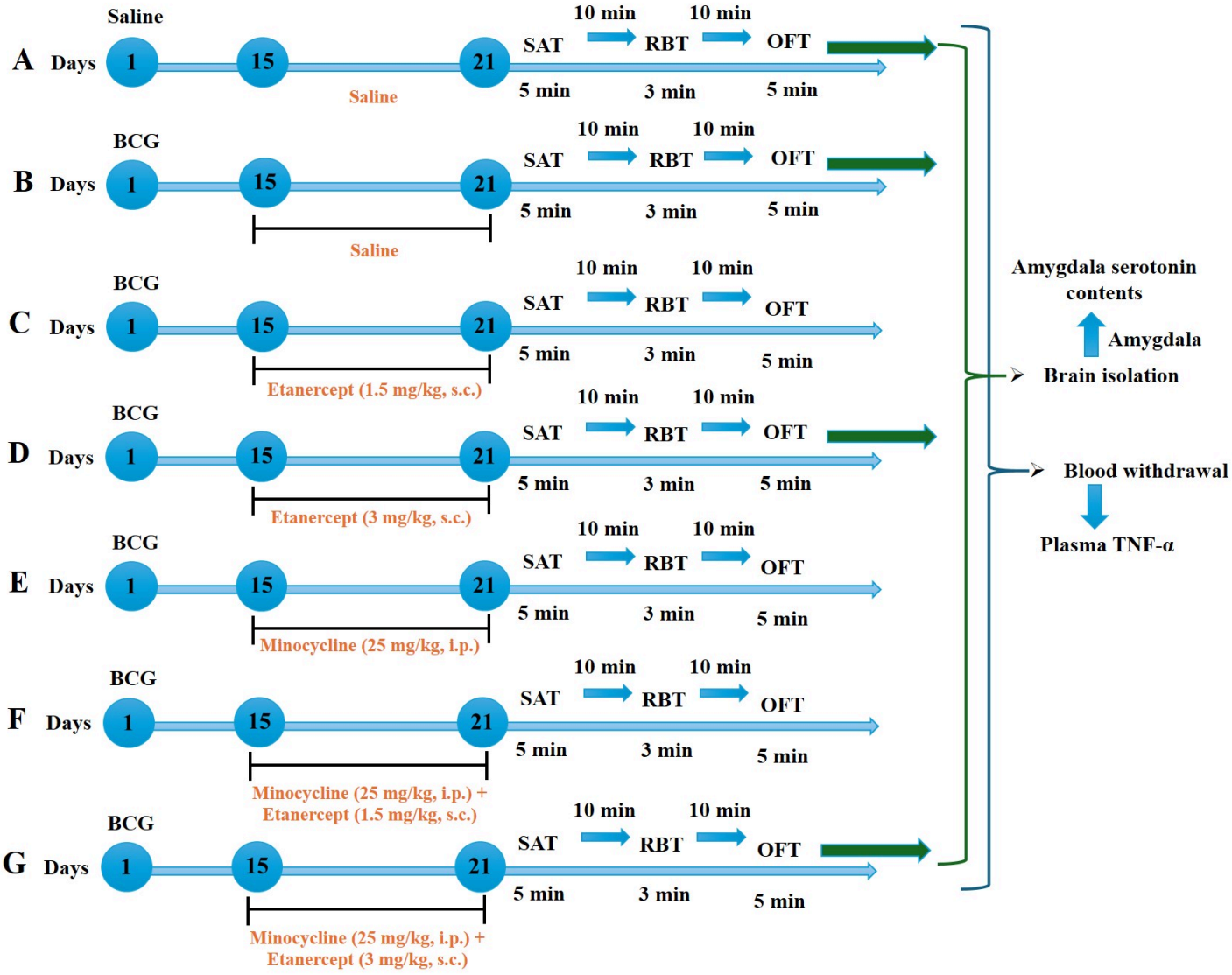


Figure 1

Legend not included with this version

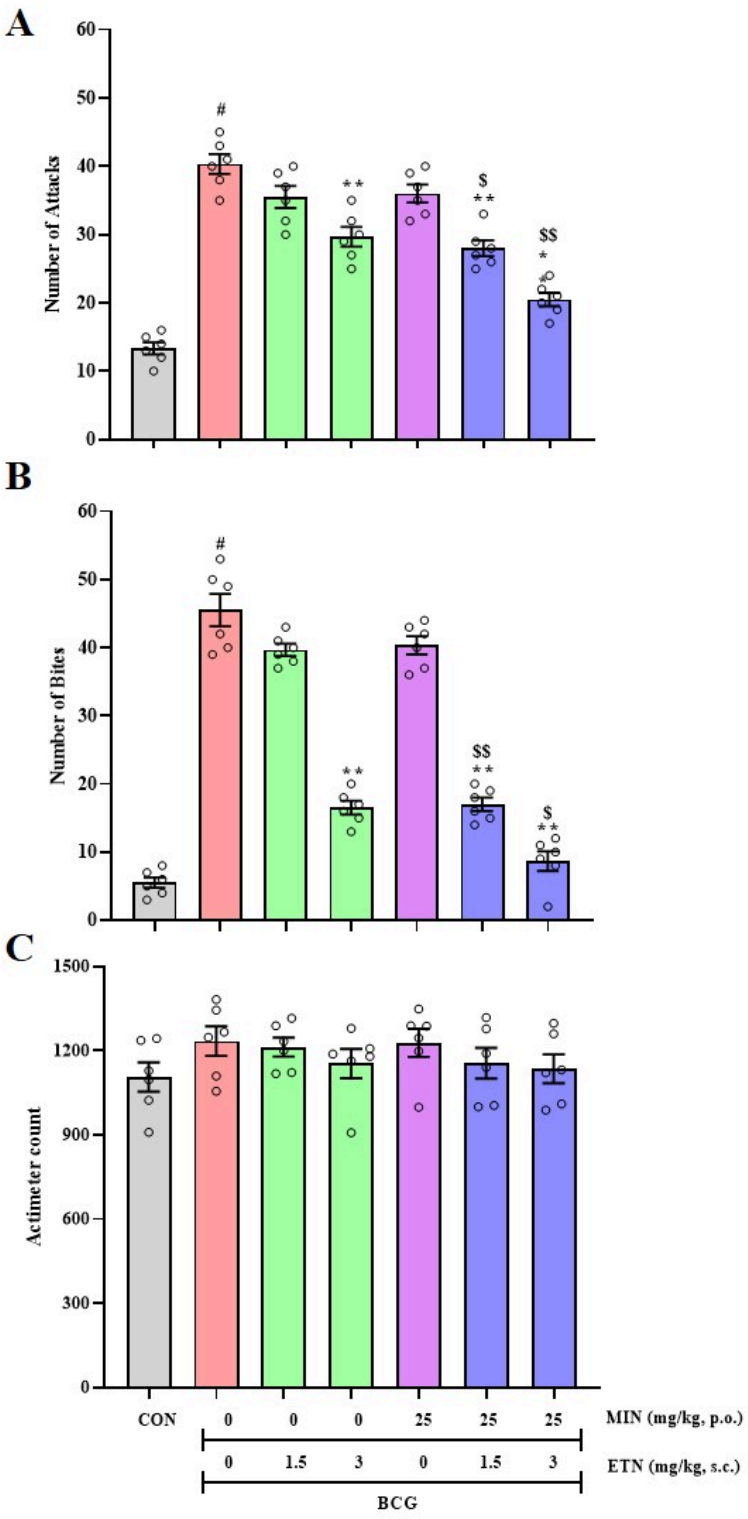


Figure 2

Legend not included with this version

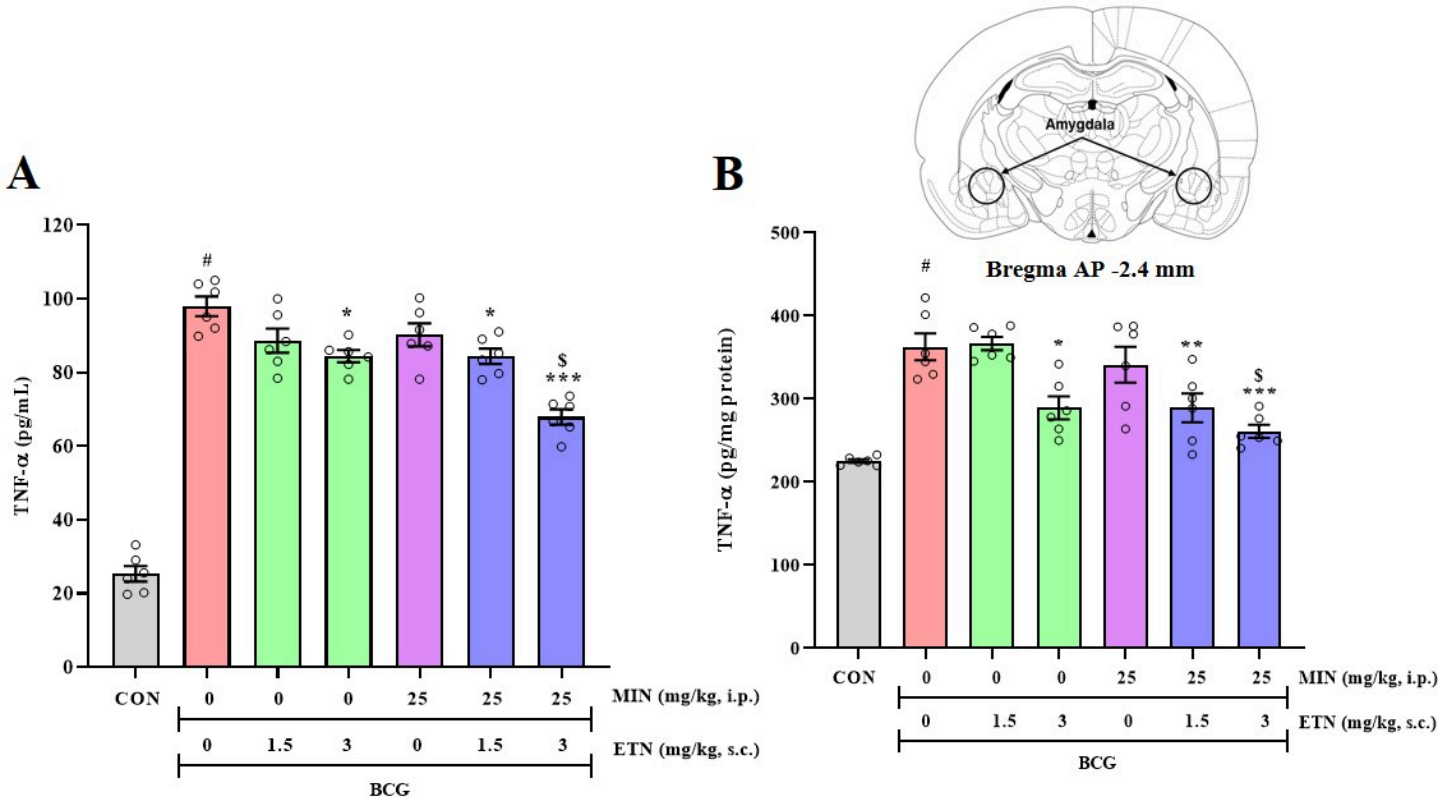


Figure 3

Legend not included with this version

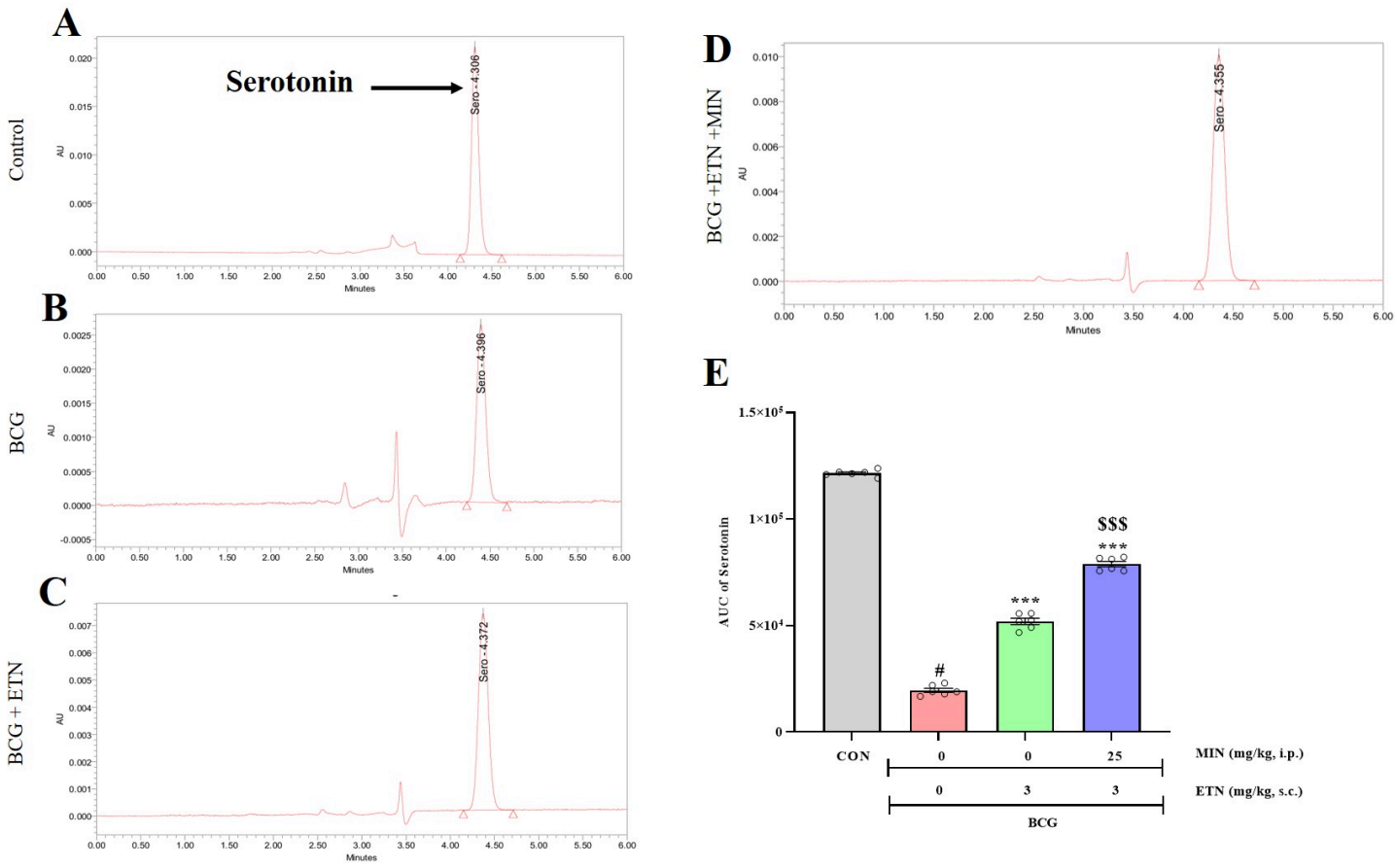


Figure 4

Legend not included with this version