

TNFR1 signaling is required for the consolidation of fear extinction memory during the resolution phase of neuroinflammation

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Research Article

Keywords: Neuroinflammation, Fear extinction, TNFR1, Memory consolidation, BDNF

Posted Date: October 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11157237/v1>

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Additional Declarations: No competing interests reported.

Abstract

Acute neuroinflammation induces cognitive deficits, but the mechanisms persisting during its resolution phase remain poorly understood. This study investigated the role of constitutive TNF receptor 1 (TNFR1) signaling in aversive memory dynamics and fear extinction during this resolution phase. Wild-type (WT) and knockout (TNFR1^{-/-}) male mice received systemic lipopolysaccharide (LPS; 1 mg/kg, 3 days), and behavioral assessments began on day 4 post-injection, following recovery from transient sickness behavior. Cytokine expression (ELISA) and glial and neurotrophic markers (Western blotting) were quantified in the prefrontal cortex, amygdala, and hippocampus. TNFR1 deletion selectively impaired contextual fear memory, without altering anxiety-like behavior or object recognition. LPS pre-exposure impaired intrasession fear extinction acquisition across all groups; however, during the 24-h retention test, WT mice successfully consolidated extinction memory, whereas the TNFR1^{-/-} LPS group exhibited retention deficits. This impairment coincided with a failure to upregulate hippocampal brain-derived neurotrophic factor (BDNF), which increased in WT but not TNFR1^{-/-} LPS animals, despite higher hippocampal tropomyosin receptor kinase B (TrkB) levels in the knockout. The resolution phase of neuroinflammation lacked generalized astrogliosis, instead presenting sub-basal levels of IL-1 β and IL-10. In conclusion, TNFR1 signaling is essential for consolidating fear extinction memory following an immune challenge, likely by supporting a hippocampal BDNF-mediated compensatory response. The cognitive inflexibility observed without this receptor reflects a locally altered neuroimmune and neurotrophic microenvironment rather than active chronic inflammation.

Introduction

Neuroinflammation is a core pathophysiological component underlying several neurodegenerative diseases and psychiatric disorders, such as major depressive disorder (Guzman-Martinez et al. 2019; Wu and Zhang 2023). To investigate these neuroimmune interactions, the systemic administration of lipopolysaccharide (LPS) remains a widely established model (Skrzypczak-Wiercioch and Sałat 2022). Historically, experimental designs have focused primarily on the acute phase of neuroinflammation, mediated by the activation of toll-like receptor 4 (TLR4) and the subsequent nuclear factor kappa B (NF- κ B) transcription factor signaling, that leads to a cytokine storm during this initial stage, culminating in a robust release of proinflammatory cytokines, including tumor necrosis factor alpha (TNF- α), interleukin (IL)-1 β , and IL-6 (Beutler 2000; Glezer et al. 2003; Skelly et al. 2013).

The acute TLR4 signaling induces sickness behavior, characterized by hypolocomotion, reduced social interaction, and transient body weight loss due to decreased food intake (Henry et al. 2008; Hines et al. 2013). Importantly, this acute neuroinflammatory state also induces significant cognitive impairments, which can be reversed by TLR4 inhibitors (Zhao et al. 2019). As a substantial portion of the literature restricts behavioral evaluations to this acute time window, experimental interventions have predominantly targeted the immediate suppression of proinflammatory cytokines, oxidative stress, and other early inflammatory mediators. This time window, however, is also marked by a high prevalence of physical malaise (Belarbi et al. 2012; Choi et al. 2024).

Among these early inflammatory mediators, TNF- α acts as a master cytokine orchestrating this inflammatory cascade. Its proinflammatory signaling occurs primarily through tumor necrosis factor receptor 1 (TNFR1), whose activation induces NF- κ B translocation to the nucleus, thereby stimulating the production of additional cytokines and further upregulating TNF- α itself (Van Loo and Bertrand 2023). In models of neurodegeneration, neuroinflammation and glutamate-induced cytotoxicity are attenuated by TNF- α blockade using specific inhibitors, demonstrating its critical role in proinflammatory regulation, particularly through TNFR1 signaling (Tweedie et al. 2012; Li et al. 2022; Ortí-Casañ et al. 2023; Udaiyappan et al. 2026). Beyond this canonical role in the immune response, TNF also regulates homeostatic synaptic plasticity under basal physiological conditions, dynamically modulating the surface expression of glutamate and GABA receptors and thereby directly modifying neuronal excitability thresholds (Leonoudakis et al. 2008; Pribiag and Stellwagen 2013; Heir and Stellwagen 2020). During acute neuroinflammation, however, this regulatory function is disrupted, contributing to the cognitive deficits observed in rodents (Rizzo et al. 2018).

Among the molecular pathways governing activity-dependent synaptic plasticity, brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin receptor kinase B (TrkB) are particularly important for the acquisition and consolidation of memory, since BDNF-TrkB signaling supports the structural and functional changes underlying long-term potentiation (Numakawa and Odaka 2022). Neuroinflammation directly interferes with this system, as LPS challenge reduces BDNF and phosphorylated TrkB expression across the hippocampus and prefrontal cortex, an effect that parallels the extent of the accompanying cognitive impairment (Zhang et al. 2022). This overlap extends to structural mechanisms as well, since constitutive TNF deficiency reduces dendritic spine density in hippocampal granule cells, with the remaining spines undergoing compensatory enlargement, an effect that is partially replicated by the isolated loss of TNFR1 (Smilovic et al. 2022; Smilovic et al. 2023). TNF also acts presynaptically, as it is required for astrocyte-mediated glutamate release during gliotransmission in the dentate gyrus (Santello et al. 2011). Given that TNF and BDNF act on shared synaptic plasticity mechanisms, the neurotrophic status of these circuits may represent a relevant, yet underexplored, readout of how TNFR1 signaling shapes memory processes beyond the acute inflammatory window.

While the mechanisms driving cognitive impairment during the acute phase of neuroinflammation are well documented, the transition period into the resolution phase remains poorly understood. Emerging evidence indicates that cognitive deficits persist for days after systemic LPS challenge, a phenomenon linked to transient microglial activation and exacerbated pruning of both excitatory and inhibitory synapses (Jung et al. 2023; Liu et al. 2025). Nonetheless, the specific pathways by which an immune insult continues to disrupt cognitive performance long after the initial acute response has subsided remain unclear.

To address this gap, the present study investigated the role of TNFR1 signaling during the resolution phase of LPS-induced neuroinflammation, using a complete TNFR1 knockout mouse strain to determine

whether the absence of this immunomodulatory receptor affects aversive memory dynamics and extinction learning.

Methods

Animals and treatment

Founders for the wild-type (WT) and $Tnfr1^{tm1Mak}$ knockout ($TNFR1^{-/-}$) colonies were provided by the Animal Facility of the School of Medicine (University of São Paulo). The experimental animals were bred and maintained in microisolator cages at the Department of Pharmacology Animal Care Unit (Institute of Biomedical Sciences, USP). Following genotyping confirmation, litters were randomly allocated into groups of two to five per cage. The housing environment was strictly controlled and featured a 12-h light/dark cycle, stable temperature ($22 \pm 1^\circ\text{C}$), and appropriate humidity, with standard rodent chow and water supplied ad libitum. All handling and experimental protocols strictly adhered to the ARRIVE guidelines and Brazilian National Council for the Control of Animal Experimentation (CONCEA) guidelines. The procedures were formally approved by the Institutional Ethics Committee on Animal Use of the Biomedical Sciences Institute (CEUA-ICB/USP, protocol no. 1345200524).

Eighty-seven male mice (8–12 weeks old) were used and randomly assigned to four experimental groups: (A) WT SAL, (B) WT LPS, (C) $TNFR1^{-/-}$ SAL, and (D) $TNFR1^{-/-}$ LPS. The experimental sample sizes (n) varied according to the specific assay and are detailed in the respective figure legends. Lipopolysaccharide (LPS, *E. coli* O111:B4; Sigma–Aldrich) was dissolved in sterile saline and freshly prepared on the day of administration. Intraperitoneal injections (1 mg/kg) were administered for three consecutive days 24 hours apart (Jung et al. 2023). The animals were weighed daily until day post-injection (dpi) 4, at which point the behavioral testing was also initiated.

Behavioral experiments

Elevated plus maze

Anxiety-like behavior was evaluated via an elevated plus maze following the methods of (Walf and Frye 2007). The apparatus, positioned 60 cm above the floor, comprised a central square and four perpendicular arms (25×5 cm each): two enclosed arms and two open arms (1 cm height). The animal started in the central zone (dpi 4), facing an open arm directed away from the experimenter, followed by 5 minutes of exploration. Locomotor and spatial parameters were automatically tracked and calculated via ANY-maze software (Stoelting, Kiel, WI, USA).

Novel object recognition

The protocol was based on our group's previous work (Lima et al. 2024), with minor modifications. The test was conducted over two days (dpi 4–5). On the first day, the training phase consisted of introducing

the animal into a rectangular open field apparatus (40 × 34 × 15 cm) containing two identical and equidistant objects. The animals were allowed to freely explore the objects for 10 minutes. After a 2-hour interval, the animal was reintroduced to the apparatus to explore identical objects for another 10 minutes. Twenty-four hours after the final training session, the animals were placed back into the apparatus, where one of the objects had been replaced by a novel one. The animals were allowed to explore freely for 5 minutes. The novel object recognition index was calculated via the following formula: (novel object exploration/[novel object + familiar object exploration]) × 100. Exploration was defined as directing the snout toward the object within a 2-cm radius.

Fear conditioning

In accordance with the protocol of (Kawamoto et al. 2014), the animals were placed in a sound-attenuated conditioning chamber equipped with a metal grid floor capable of delivering electric footshocks. On the first day (dpi 6), each animal was placed in the chamber and allowed to explore freely for 2 minutes. A sequence of three auditory tones (90 dB, 30 s duration) was subsequently presented, each coterminating with a 2-second electric footshock stimulus (0.5 mA), separated by an intertrial interval of 30 seconds. Twenty-four hours later, the animals were re-exposed to the same conditioning chamber (original context) and allowed free exploration for 5 minutes. An additional 24 hours later, the animals were introduced into a completely modified context (altered flooring, modified walls, and a new olfactory cue). Following a 3-minute free exploration period, a sequence of five auditory tones (30 s duration) was presented in the absence of the electric shock, separated by 30-second intervals. All the sessions were recorded. Freezing behavior, defined as the complete absence of movement except for respiration, was manually scored.

Fear extinction

The conditioned fear extinction test was adapted from Chang et al. (2009). On day 1 (dpi 6), the auditory tone was paired with an electric shock in the same chamber and following the same procedure used for standard fear conditioning. On day 2, extinction training began, with animals placed in a fully modified chamber context and allowed 3 min of free exploration, followed by a sequence of 25 auditory tones (90 dB, 30 s duration) separated by 5 s intertrial intervals (ITIs). On day 3, extinction memory retention was assessed by presenting a shorter sequence of 5 tones (5 s ITI). Freezing behavior during tone presentations was scored manually and grouped into blocks of 5 tones each (b1-b5), with the average freezing time per block expressed as a percentage.

Tissue collection and protein extraction

The animals were anesthetized with isoflurane and euthanized by decapitation. The prefrontal cortex (PFC), amygdala, and hippocampus were bilaterally dissected and storage until homogenate preparation. Dissections were performed on the basis of the following anteroposterior (AP) coordinates relative to

the bregma: approximately + 1.98 mm to + 1.34 mm for the PFC and bregma – 0.82 mm to -1.70 mm for the amygdala (Paxinos and Franklin 2019). For total protein extraction, samples were homogenized in supplemented radioimmunoprecipitation assay (RIPA) buffer (150 mM NaCl; 50 mM Tris-HCl; 5 mM EGTA; 0.5% sodium deoxycholate; 0.1% SDS, pH 7.5) with the addition of 0.5 mM phenylmethylsulfonyl fluoride (PMSF), protease and phosphatase inhibitors (2.5 µL/mL antipain; 2.5 µL/mL leupeptin; 5 mM B-GP; 30 mM NaF; 20 mM sodium pyrophosphate) and centrifuged exactly as previously described by Dos Santos et al. (2024). The protein concentration of the resulting supernatants was subsequently quantified via the standard Bradford colorimetric assay (Bio-Rad Laboratories, Hercules, CA, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

Tissue homogenates were utilized to quantify the concentrations of IL-1 β , IL-6, IL-10 and BDNF via ELISA (DuoSet IL-1 β : #DY400; DuoSet IL-6: #DY406, DuoSet IL-10: #DY417 and DuoSet BDNF: #DY248). All the assays were performed in accordance with the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). The obtained results were normalized to the total protein content of each sample, and the final data are expressed as pg/mg.

Western blotting

Protein samples (20 µg/lane) were prepared in Laemmli buffer (Laemmli 1970), boiled for 5 min at 95°C, and resolved on 10% SDS–PAGE gels. Proteins were then transferred to nitrocellulose membranes (400 mA, 2 h). Following blockade with 5% BSA, the membranes were incubated overnight at 4°C with specific primary antibodies (glial fibrillary acidic protein (GFAP), 1:1000, Cell Signaling, Danvers, MA, USA; TrkB, 1:1000, Cell Signaling; β -Actin, 1:10000, Cell Signaling). After standard Tris Buffered Saline Tween-20 (TBS-T) (10 mM Tris, pH 8.0; 150 mM NaCl; 0.5% Tween 20) washes, the membranes were incubated with appropriate secondary antibodies for 2 h. Immunoreactive bands were detected via enhanced chemiluminescence (SignalFire Elite, Cell Signaling) and quantified by optical density using ImageJ software (NIH, NY, USA). Protein expression was normalized to that of β -actin as an internal loading control. When necessary, the membranes were subjected to mild stripping buffer (1.5% glycine, 0.1% SDS, and 1% Tween 20; pH 2.2) for reprobing.

Statistical analysis

The data were analyzed via two-way analysis of variance (ANOVA) (genotype vs. treatment) or two-way repeated-measures ANOVA (time vs. group), followed by Bonferroni post hoc correction. Statistical significance was accepted at $p < 0.05$. The assumption of data normality was evaluated via the Shapiro–Wilk test; in instances where this assumption was not met, the data were naturally log-transformed prior to parametric analysis. Outliers were removed using the interquartile range (IQR) method (excluded if their values fell below $Q1 - 1.5 \text{ IQR}$ or above $Q3 + 1.5 \text{ IQR}$). All graphical representations present values as

the mean $\pm$ standard error of the mean (SEM). Statistical analyses and graphical plotting were performed via GraphPad Prism software, version 9 (GraphPad Software, San Diego, CA, USA).

Results

Constitutive TNFR1 deletion selectively impairs contextual fear memory and extinction consolidation following an immune challenge

To confirm the sickness behavior induced by systemic LPS administration, we analysed the body weight across time. We found that the LPS administration induced a significant reduction in body weight (Fig. 1A) across the LPS-treated groups compared with the saline controls from the first injection until dpi 3 [Interaction: $F(18, 288) = 60.12$, $p < 0.0001$]. By dpi 4, body weight had largely normalized, with a significant difference remaining only between the WT SAL and WT LPS groups ($p < 0.05$). This transient body weight loss confirmed the successful induction of LPS-driven sickness behavior, which effectively resolved by this time point.

Constitutive deletion of TNFR1 significantly impaired contextual fear memory (Fig. 1B). Compared with WT control mice, TNFR1^{-/-} mice presented significantly lower freezing levels, independent of the immune challenge [genotype: $F(1, 48) = 18.54$, $p < 0.0001$]. In contrast, neither cued fear memory (Fig. 1C) nor object recognition memory (Fig. 1D) were affected by genotype or LPS administration.

To determine whether the contextual fear memory deficit was confounded by generalized alterations in emotional reactivity, we also evaluated the elevated plus maze test in the same animals (Fig. 1E, F). No significant differences were observed in the time spent in the open arms or the number of closed arm entries. Together, these results indicate a selective impairment in contextual fear memory driven by the absence of TNFR1 signaling, without concurrent modifications in other memory domains or anxiety-like behavior during the late resolution phase of LPS administration.

We used an independent cohort of animals to investigate how the resolution phase of neuroinflammation impacts conditioned fear extinction learning. During mass extinction training (Fig. 2A), a significant interaction between training blocks and groups was observed [interaction: $F(12, 124) = 3.700$, $p < 0.0001$]. A significant reduction in freezing behavior from block 1 (b1) to block 5 (b5) was detected exclusively in the saline-treated groups ($p < 0.01$), indicating successful intrasession extinction learning only in control animals. Conversely, the freezing levels of the TNFR1^{-/-} LPS group were significantly greater than those of the corresponding saline control groups during blocks 3 and 4 ($p < 0.05$). By the final training block (b5), both LPS-treated groups maintained significantly greater freezing expression than the saline groups did ($p < 0.05$).

To quantify the overall freezing expression throughout the training session, we analyzed the area under the curve (AUC; Fig. 2B). This analysis revealed a significant main effect of the immune challenge

[treatment: $F(1, 31) = 29.88$, $p < 0.0001$], with LPS-treated animals displaying significantly greater total freezing than saline controls did ($p < 0.05$). These data suggest that the LPS challenge, despite occurring days earlier, still impaired the acute acquisition of fear extinction across all genotypes.

Twenty-four hours later, the animals were re-exposed to the context to evaluate extinction memory retention (Fig. 2C). In this test, a significant interaction was detected [interaction: $F(1, 31) = 5.378$, $p < 0.05$]. Post hoc comparisons revealed that, compared with both the WT LPS and the $TNFR1^{-/-}$ SAL groups, the $TNFR1^{-/-}$ LPS group presented significant increases in freezing behavior ($p < 0.01$). Together, these results demonstrate that although LPS challenge impairs extinction acquisition regardless of genotype during the resolution phase, $TNFR1$ signaling is specifically required to support the long-term consolidation of extinction memory following an immune insult.

Region-specific neuroimmune dynamics during the resolution phase of neuroinflammation

In the prefrontal cortex, biochemical analysis revealed a significant reduction in IL-1 β levels (Fig. 3A) driven by both the genotype [genotype: $F(1, 11) = 18.71$, $p < 0.01$] and the immune challenge [treatment: $F(1, 11) = 26.81$, $p < 0.001$]. Post hoc comparisons confirmed that the level of IL-1 β was significantly lower in the $TNFR1^{-/-}$ mice than in the WT controls ($p < 0.05$) and in the LPS-treated groups than in the saline controls ($p < 0.01$). Furthermore, prefrontal IL-10 expression was significantly altered (Fig. 3D) by genotype [genotype: $F(1, 11) = 12.54$, $p < 0.05$], with the $TNFR1^{-/-}$ SAL group presenting lower baseline levels than the WT SAL group did ($p < 0.05$).

Within the amygdala, a distinct neuroimmune dynamic was observed. A significant interaction was detected for IL-1 β (Fig. 3B) [interaction: $F(1, 11) = 7.381$, $p < 0.05$], and compared with the WT SAL group, the $TNFR1^{-/-}$ SAL group presented elevated baseline expression ($p < 0.05$). Conversely, the late effects of LPS administration significantly suppressed IL-1 β levels in both WT ($p < 0.05$) and $TNFR1^{-/-}$ ($p < 0.0001$) mice. For IL-10, a significant interaction was also identified (Fig. 3E) [interaction: $F(1, 11) = 11.06$, $p < 0.01$]. Specifically, the IL-10 levels were markedly lower in the $TNFR1^{-/-}$ LPS group than in both the $TNFR1^{-/-}$ SAL and WT LPS groups ($p < 0.01$).

In contrast to the alterations observed in the prefrontal cortex and amygdala, no significant differences were observed in the expression of IL-1 β or IL-10 within the hippocampus (Fig. 3C, F). Additionally, IL-6 levels remained completely unaffected across all three analyzed brain structures, regardless of genotype or treatment (Fig. 3G-I). However, we observed a significant treatment effect on BDNF expression [treatment: $F(1, 14) = 5.014$, $p < 0.05$], with WT LPS animals showing a significant increase compared with WT SAL ($p < 0.05$), whereas no significant differences were found in the other regions (Fig. 3J-L).

Collectively, these results suggest a structure-specific shift in the neuroimmune profile during the resolution phase of this model, one that appears to depend on intact $TNFR1$ signaling. This is illustrated by the genotype-specific rise in hippocampal BDNF, observed only in WT LPS animals, and by the

requirement of TNFR1 signaling for the proper restoration of basal IL-10 levels following systemic immune challenge.

To further characterize the neuroimmune landscape and the expression of TrkB during the resolution phase, we assessed the protein expression of the astrocytic marker GFAP and TrkB in the prefrontal cortex (Fig. 4A, B), amygdala (Fig. 4C, D) and hippocampus (Fig. 4E, F) via Western blotting.

We assessed the astrocyte reactivity through GFAP expression and found that it remained completely stable across all experimental groups and treatments in the prefrontal cortex, amygdala, and hippocampus. This widespread absence of reactive astrogliosis confirms that the late resolution phase of this LPS model is not characterized by astrocyte sustained activation.

No significant differences in TrkB expression were detected in the prefrontal cortex or amygdala. In the hippocampus, TrkB expression showed a significant genotype effect [genotype: $F(1, 14) = 14.00$, $p < 0.01$], with TNFR1^{-/-} LPS animals presenting significantly higher levels than their WT LPS counterparts ($p < 0.01$).

Discussion

Our findings demonstrate that constitutive TNFR1 signaling is required for the consolidation of extinction memory during the resolution phase of neuroinflammation, suggesting a role for this receptor in the stabilization of fear-related plasticity beyond the acute inflammatory window.

The transient body weight reduction observed in our study reflects the classic sickness behavior driven by reduced food and water intake, confirming the induction of a robust neuroinflammatory state (Lasselin et al. 2020). By tracking the temporal progression of body weight and initiating behavioral assessments only after complete normalization (Jung et al. 2023), we established a critical temporal window for cognitive evaluation. To further confirm the validity of our behavioral tests, we verified that LPS-challenged animals displayed neither increased anxiety-like behavior nor alterations in baseline exploratory activity within the testing apparatus, confirming the absence of confounding motor or emotional impairments. Consequently, it is unlikely that the selective memory impairments observed in subsequent trials were confounded by generalized fear, altered emotional reactivity, or the persistent hypolocomotion characteristic of acute sickness behavior (Bassi et al. 2012).

In addition to the acute sickness response, our behavioral profiling revealed a specific cognitive signature associated with the constitutive loss of TNFR1. While recognition and cued fear memory remained completely intact, the isolated deletion of TNFR1 was sufficient to significantly impair contextual fear memory, a deficit that occurred independently of the LPS challenge. This genotypic difference suggests that TNFR1 signaling does not exert a global cognitive influence, instead governing specific cognitive domains through the localized modulation of neural plasticity in hippocampus (Singh et al. 2022). Our findings align with previous reports utilizing this same knockout strain, which similarly highlighted baseline deficits in fear memory processing (Simen et al. 2006). This specific vulnerability in

contextual memory points directly to the well-documented role of TNF- α in homeostatic synaptic scaling (Singh et al. 2022). Particularly within the hippocampus, TNF- α is fundamentally required for the dynamic trafficking and surface anchoring of GABA-A and AMPA receptors (Stellwagen et al. 2005; Pribiag and Stellwagen 2013). Given that contextual, but not cued, fear memory relies heavily on hippocampal processing, the absence of TNFR1 signaling likely disrupts this receptor-mediated synaptic scaling specifically within the hippocampus, consistent with evidence that microglia-derived TNF- α in this region modulates aversive memory encoding and conditioned fear extinction (Yu et al. 2017). Our data, showing that the absence of TNFR1 signaling impairs contextual fear memory, align with evidence that excessive TNF signaling is equally disruptive, as the exogenous infusion of TNF- α into the dorsal hippocampus impairs the retrieval and reconsolidation of fear memory (Takahashi et al. 2021), together supporting the notion that a precise physiological window of TNF signaling is required for proper memory dynamics.

Building on the baseline contribution of TNFR1 to contextual fear memory, our data revealed an impairment in extinction learning dynamics during the resolution phase of neuroinflammation. During the extinction test, LPS challenge impaired the acquisition of extinction across both genotypes, preventing a reduction in freezing even under a massed training protocol. Retention testing revealed a genotypic difference, as WT mice, despite having also failed to exhibit adequate intrasession learning, successfully consolidated their extinction memory, suggesting that their underlying plasticity mechanisms remained sufficiently intact to process safety signals over time. In contrast, the constitutive loss of TNFR1 abolished this capacity, culminating in significant retention deficits. This difference between acquisition and retention indicates that TNFR1 signaling mediates the consolidation processes required to stabilize extinction memory following an immune challenge.

The altered neuroimmune landscape during the resolution of inflammation may help explain this consolidation impairment. As neuroinflammation shifts microglia toward a reactive, phagocytic phenotype, the exacerbated pruning of both excitatory and inhibitory synapses reduces the pool of mature synaptic connections, a structural degradation directly linked to the cognitive impairments observed days after LPS administration (Morimoto et al. 2023; Wu et al. 2023). Given that extinction acquisition was similarly impaired in both genotypes, whereas retention failed specifically in TNFR1^{-/-} animals, a synergistic effect between neuroinflammation-induced synaptic pruning and TNFR1 deletion likely compromises extinction learning. Specifically, the degradation of mature synapses may weaken the networks essential for encoding the new extinction trace, while the absence of TNFR1 further impairs memory consolidation by preventing homeostatic stabilization of the remaining synapses. Although this hypothesis requires direct molecular validation in future studies, it provides a plausible basis for the cognitive deficit observed in the TNFR1^{-/-} LPS group.

To contextualize these behavioral findings within the biochemical microenvironment, we evaluated the cytokine profile in regions critical for the acquisition and expression of extinction learning: the prefrontal cortex, the amygdala, and the hippocampus (Myers and Davis 2007). Unexpectedly, the resolution phase of neuroinflammation in our model was characterized by a distinct subbasal expression profile of IL-1 β

and IL-10, specifically in the prefrontal cortex and amygdala, whereas hippocampal levels remained unchanged. Typically, central cytokine expression peaks hours after acute LPS administration and decays to baseline within approximately 48 hours, with spatiotemporal variations depending on the brain region (Yue et al. 2026). While high-dose endotoxin models often report persistently elevated cytokine levels for days, a state generally associated with cell death and chronic inflammation (Qin et al. 2007), our data highlight fundamentally different biological trajectories during the resolution phase. Further supporting the absence of a classic, active inflammatory state during this period, our evaluation of glial marker revealed no widespread gliosis, as indicated by the stable expression of the astrocytic marker GFAP across the prefrontal cortex, amygdala, and hippocampus.

The literature presents heterogeneous inflammatory profiles following an immune challenge. While long-lasting morphological alterations in activated microglia and astrocytes are frequently described (Ayyubova et al. 2023), a reduction in cytokine expression below basal levels is a much less common finding. Nevertheless, this specific profile has been previously reported and linked to the polarization of microglia toward an anti-inflammatory M2 phenotype (Shimada et al. 2022). Conversely, other models have reported delayed increases in proinflammatory mRNAs (IL-1 β and TNF- α) up to 7 days post-LPS in rats (Pienaar et al. 2025). Ultimately, our cytokine data emphasize the regional specificity of the neuroinflammatory response. Distinct brain regions process the LPS challenge and its subsequent resolution through different patterns (Yue et al. 2026), demonstrating that the failure of cognitive tasks, such as extinction consolidation, can occur within a localized, immunosuppressed milieu rather than requiring a state of chronic, active inflammation.

The role of hippocampal BDNF in the consolidation of conditioned fear extinction is well established in the literature, particularly through the ventral hippocampus-infralimbic cortex (IL) circuit. Peters et al. (2010) demonstrated that animals failing to extinguish fear exhibit reduced BDNF levels specifically within hippocampal afferents projecting to the IL, and that pharmacologically increasing BDNF in this circuit is sufficient to prevent extinction failure. Convergent, extinction training itself induces increased neuronal BDNF expression in the ventral hippocampus, and that direct BDNF infusion into this region is sufficient to reduce conditioned freezing, reproducing the behavioral effect of extinction (Rosas-Vidal et al. 2014). The significant increase in hippocampal BDNF observed in LPS-challenged WT animals, accompanied by normal consolidation of extinction memory 24 h later, is consistent with this previously described role of hippocampal BDNF. Although TNFR1 signaling is classically associated with driving the acute proinflammatory response, TNF- α itself has also been shown to upregulate BDNF expression in glial cells, supporting a neuroprotective function for this pathway (Saha et al. 2006). Our findings suggest that, during the resolution phase, TNFR1 signaling may similarly be required for mounting this compensatory trophic response to the preceding inflammatory insult. The absence of the BDNF increase in TNFR1^{-/-} LPS animals, together with their failure to consolidate extinction over the same interval, indicates that this compensatory mechanism becomes unavailable in the absence of TNFR1 signaling, leaving the system unable to provide sufficient trophic support once the acute response has subsided.

The absence of a significant BDNF difference in the prefrontal cortex is consistent with the hippocampal origin of the BDNF pool implicated in extinction, since the relevant increase occurs in hippocampal afferents projecting to the IL rather than being locally synthesized in the cortex (Peters et al. 2010; Brockway et al. 2023), and may be further explained by subregional specificity within the medial PFC itself, as BDNF in the prelimbic cortex supports original fear memory but not extinction, unlike its infralimbic counterpart (Choi et al. 2010). In the hippocampus, TrkB expression was significantly higher in TNFR1^{-/-} LPS animals than in their WT LPS counterparts, whereas no such difference was detected in the prefrontal cortex, indicating that receptor availability, whose surface expression is itself subject to activity-dependent regulation (Andreska et al. 2020), is not a limiting factor for BDNF signaling in TNFR1^{-/-} LPS animals. Rather, these animals have more TrkB available in the hippocampus, suggesting that their failure to consolidate extinction memory is more likely explained by insufficient BDNF signaling, secondary to the abolished rise in its ligand, than by a limitation in receptor availability.

Conclusion

The present study demonstrates that TNFR1 signaling is required for the successful consolidation of fear extinction memory following a systemic immune challenge. The requirement extends beyond the classical proinflammatory role of TNFR1, as its absence also prevented the hippocampal rise in BDNF observed in WT animals during the resolution phase, without a corresponding limitation in TrkB availability. This pattern indicates that TNFR1^{-/-} animals fail to consolidate extinction memory not because of persistent inflammation, but because they cannot mount the compensatory trophic response needed to support memory-related plasticity once the acute inflammatory insult has subsided. Together with the locally altered, rather than chronically active, neuroimmune profile observed across brain regions, these findings position TNFR1 as a receptor whose relevance shifts across the course of neuroinflammation, from driving the acute proinflammatory response to supporting neurotrophic recovery during its resolution. This dual role highlights the resolution phase as a distinct and therapeutically relevant window, in which cognitive vulnerability may result less from ongoing inflammation than from a failure to restore the trophic support required for adaptive memory remodeling.

Declarations

Ethics declarations

Ethics approval

All experimental protocols involving animals were conducted in accordance with the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and were approved by the Institutional Ethics Committee on Animal Use of the Biomedical Sciences Institute, University of São Paulo (CEUA-ICB/USP, protocol no. 1345200524).

Competing interests:

The authors declare no conflicts of interests.

Generative AI use

Generative AI tools were used exclusively for language-related assistance, such as correcting grammar and rephrasing sentences for clarity. All scientific content, including the experimental design, data analysis, interpretation, and conclusions, was developed entirely by the authors.

Funding:

We thank FAPESP (grant number 2024/10817-9; 2021/06009-6, 2023/04906-6), CNPq and CAPES for their financial supports.

Author Contribution

T. A. S. LIMA: Methodology, Data curation, Formal analysis, Investigation, Visualization, Writing - original draft. M. R. RIBEIRO: Investigation, Validation, Visualization, Writing - review & editing. A. SIENA: Investigation, Visualization, Writing – review & editing. E. M. KAWAMOTO: Formal analysis, Funding acquisition, Project administration, Conceptualization, Methodology, Visualization, Writing - review & editing, Resources.

Acknowledgement

We thank FAPESP (grant number 2024/10817-9; 2021/06009-6, 2023/04906-6), CNPq and CAPES for their financial supports.

Data Availability

All data supporting the findings of this study are available within the paper.

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Figures

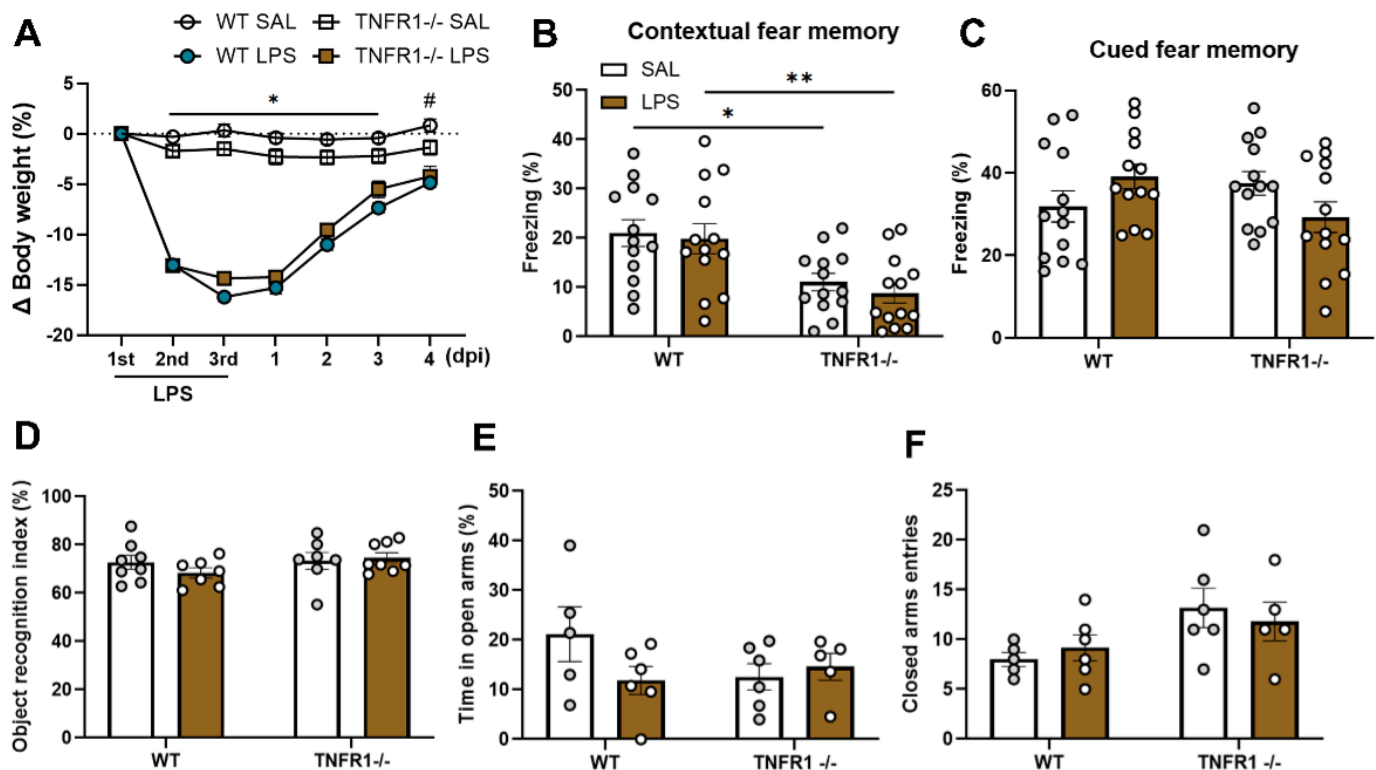


Figure 1

Body weight, fear and recognition memory, and anxiety-like behavior. (A) Repeated LPS injections (1 mg/kg) induced body weight loss, with recovery observed after 4 days. Animals were subjected to fear conditioning to evaluate (B) contextual and (C) cued fear memory. Additionally, evaluations included (D) recognition memory, (E) anxiety-like behavior, and (F) exploratory activity. (A) Repeated measures two-way ANOVA, (B-F) two-way ANOVA. Values were expressed as mean $\pm$ SEM. (WT SAL: n = 5–13; WT LPS:

n = 6–13; TNFR1^{-/-} SAL: n = 6–13; TNFR1^{-/-} LPS: n = 5–13). (A) #p < 0.05 WT SAL vs WT LPS; *p < 0.05 SAL vs LPS (B-F) *p < 0.05; **p < 0.01

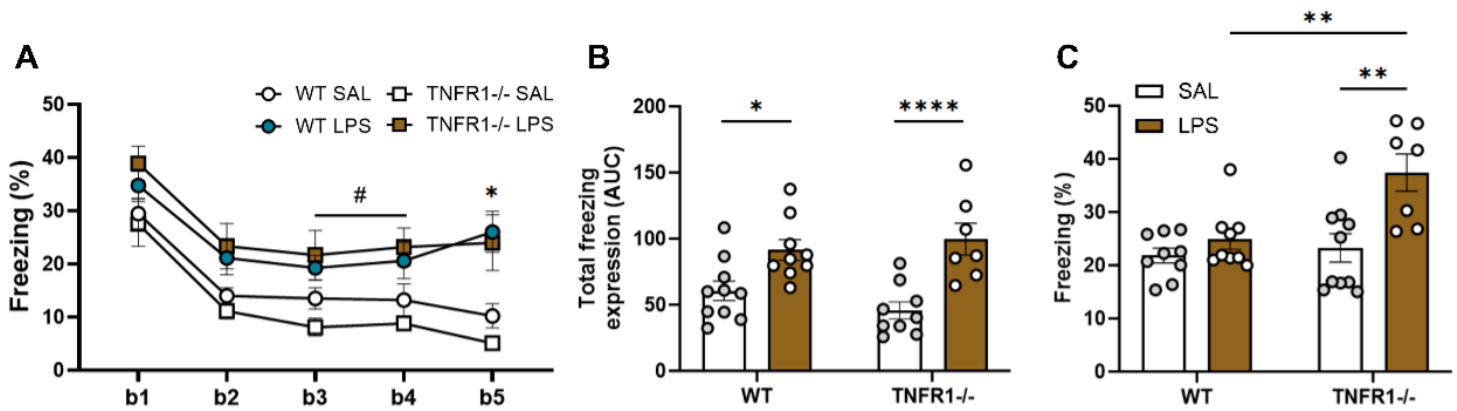


Figure 2

Cognitive impairment is induced during the resolution phase of neuroinflammation. (A) Twenty-five auditory tones were presented during conditioned fear extinction training. (B) Total fear expression during extinction training was quantified by calculating the area under the curve (AUC). (C) Retention of extinction learning was assessed after a 24-h interval. (A) Log-transformed repeated measures two-way ANOVA, (B) log-transformed two-way ANOVA and (C) two-way ANOVA. Values were expressed as mean $\pm$ SEM. (WT SAL: n = 10; WT LPS: n = 9; TNFR1^{-/-} SAL: n = 9; TNFR1^{-/-} LPS: n = 7). (A) #p < 0.05 TNFR1^{-/-} SAL vs TNFR1^{-/-} LPS; *p < 0.05 SAL vs LPS (B-C) *p < 0.05; **p < 0.01; ****p < 0.0001

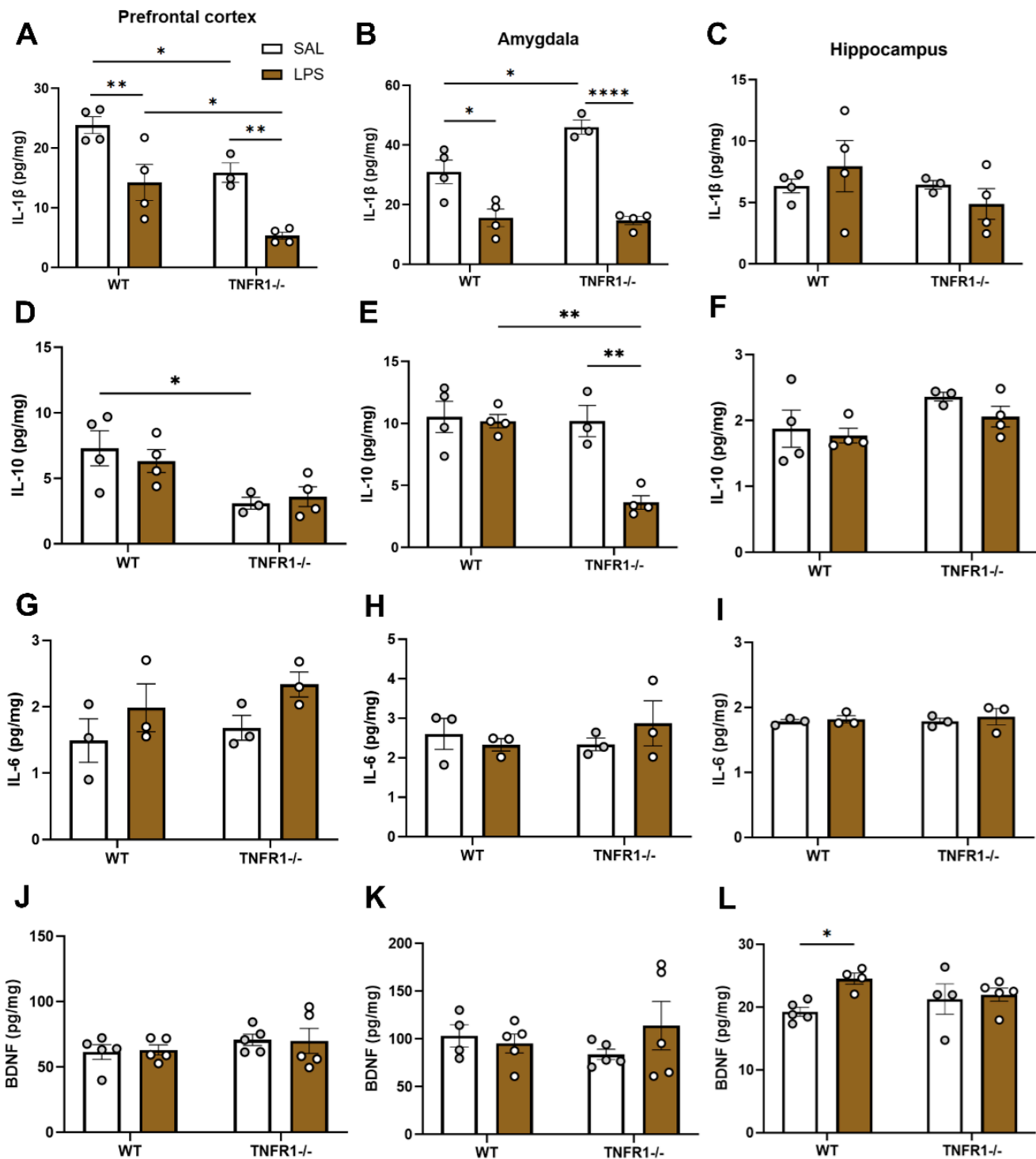


Figure 3

Cytokines and BDNF profiles in the prefrontal cortex, amygdala, and hippocampus. The levels of (A–C) IL-1 β , (D–F) IL-10, (G–I) IL-6 and (J–L) BDNF were quantified across the three regions of interest. (A–L) Two-way ANOVA. Values were expressed as mean $\pm$ SEM. (WT SAL: n = 3-5; WT LPS: n = 3-5; TNFR1^{-/-} SAL: n = 3-5; TNFR1^{-/-} LPS: n = 3-5). *p < 0.05; **p < 0.01; ****p < 0.0001

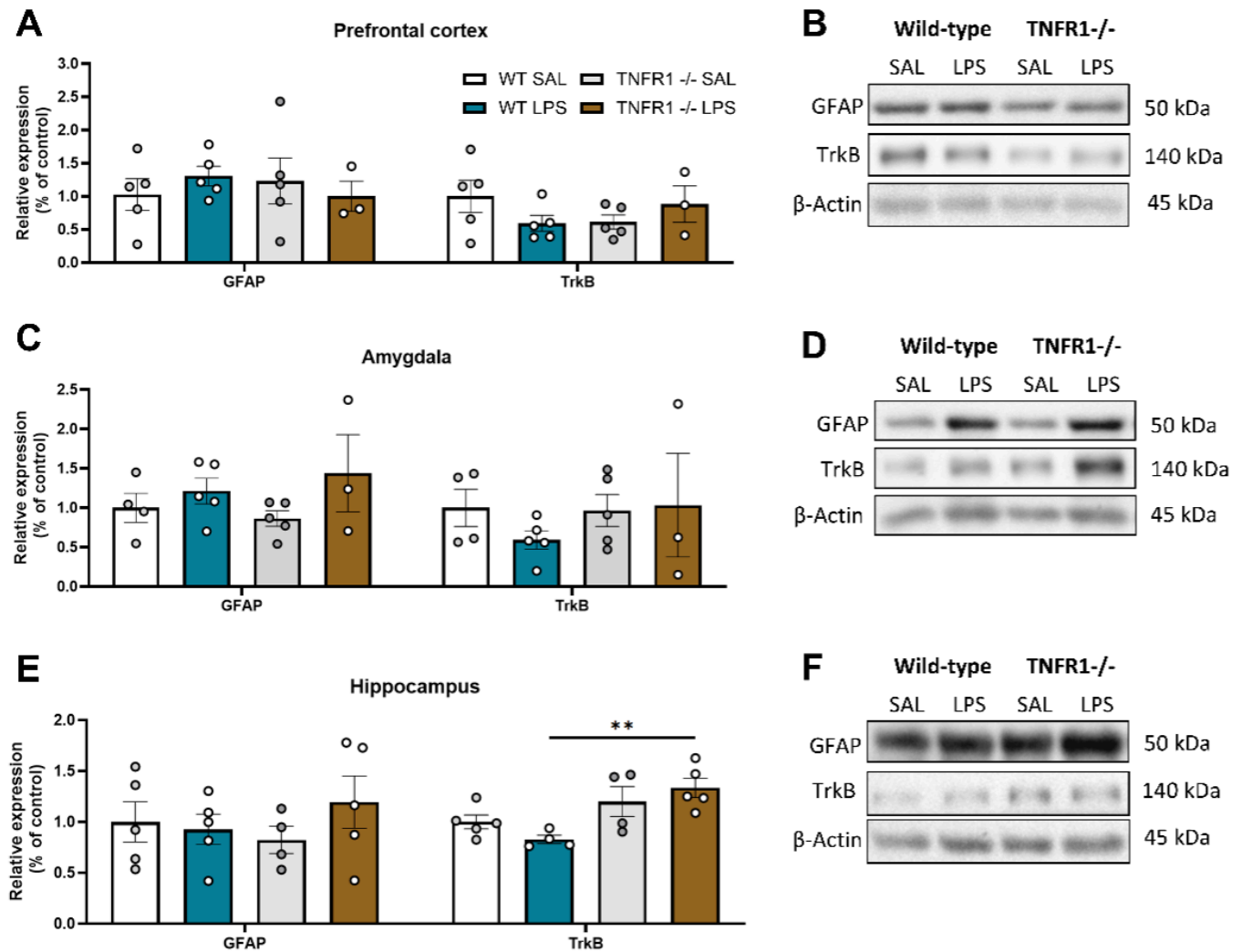


Figure 4

Astrocyte reactivity and TrkB expression. GFAP and TrkB markers were used to evaluate the astrocyte reactivity and TrkB expression in the (A–B) prefrontal cortex, (C–D) amygdala, and (E–F) hippocampus. (A, C, E) Two-way ANOVA. Values were expressed as mean $\pm$ SEM. (WT SAL: n = 4-5; WT LPS: n = 5; TNFR1^{-/-} SAL: n = 4-5; TNFR1^{-/-} LPS: n = 3-5). **p < 0.01