Published in final edited form as:

Curr Opin Behav Sci. 2017 April ; 14: 123–132. doi:10.1016/j.cobeha.2017.01.005.

Immune signaling mechanisms of PTSD risk and symptom development: insights from animal models

Jessica Deslauriers^{1,2}, Susan Powell^{1,2}, and Victoria B Risbrough^{1,2}

¹Department of Psychiatry, University of California San Diego, La Jolla, CA, USA

²Center of Excellence for Stress and Mental Health, Veterans Affairs Hospital, La Jolla, CA, USA

Abstract

Post-traumatic stress disorder (PTSD) is characterized by persistent re-experiencing of a traumatic event, avoidance, and increased arousal. The approved pharmacological treatments for PTSD have limited efficacy (~60% treatment response), supporting the need for identification of biomarkers and novel pharmacological therapies. Mounting evidence suggests increased inflammatory markers and altered immune gene expression correlate with the severity of symptoms in PTSD patients. However a causal role of immune signaling in development and maintenance of PTSD symptoms is not clear, as inflammation may also be an epiphenomenon related to metabolic and behavioral effects of stress. Animal studies have been critical in understanding the potential causal role of immune signaling in PTSD. In this review we will present the most recent evidence, primarily focusing on the last 3 years, for inflammatory dysfunction both preceding and following PTSD, and how animal models of PTSD have contributed to our understanding of immune mechanisms involved in enduring anxiety after trauma. We will particularly focus on the role of peripheral vs. central immune signaling, the differences between single vs. chronic stress models of PTSD and recent utilization of these models to investigate novel anti-inflammatory treatments. We also highlight some current gaps in the literature including models of TBI/PTSD comorbidity, lack of translational peripheral markers of inflammation and the relatively incomplete understanding of the inflammatory trajectory after severe stress.

Keywords

inflammation; stress; PTSD; behavior; anxiety

Corresponding author: Victoria Risbrough, Ph.D., University of California San Diego, 9500 Gilman Dr. MC0804, La Jolla CA 92093-0804, Tel: 16195433582; Fax: 16195432475: vrisbrough@ucsd.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflicts of interest

The authors have no conflict of interest to declare.

Introduction

Post-traumatic stress disorder (PTSD) is a complex psychiatric disorder that can develop after experiencing a severe trauma. In the US, the overall prevalence of PTSD is 8%, with the rate increasing up to 22% in veterans that have been deployed to Iraq or Afghanistan [1]. PTSD is mainly characterized by persistent re-experiencing of the traumatic event through spontaneous memories or environmental cues, avoidance of trauma-related cues, and hyperarousal. To date, the only FDA-approved pharmacological treatments, selective serotonin reuptake inhibitors (SSRIs), have limited efficacy, with only ~60% of patients responding to treatment and 20–30% achieving full remission [2], supporting the urgency in identifying prognostic biomarkers and novel pharmacological therapies.

Clinical evidence for link between inflammation and PTSD symptoms: In the last decade, mounting evidence supports a role for inflammation as a potential pathophysiological mechanism underlying PTSD. Elevated serum levels of pro-inflammatory cytokines interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), and the complement protein C-reactive protein (CRP) are consistently observed in PTSD individuals compared to healthy controls and combat-exposed controls [3–5]. The levels of pro-inflammatory cytokines and CRP are positively correlated with severity of symptoms [5]. These data suggest that inflammation is present during high symptom state, but do not address if inflammation plays a role in PTSD risk. A prospective study (Marine Resiliency Study) of PTSD development in service members deployed to combat zones reported that high levels of plasma CRP collected before deployment were associated with increased risk for developing PTSD after combat [6]. Similarly, those that developed PTSD after combat exhibited altered gene expression patterns in peripheral immune cells collected before combat compared to those that did not go on to develop PTSD after combat [7]. Putatively functional mutations in immune genes (CRP) are also associated with increased risk for PTSD [8]. Finally, higher glucocorticoid-dependent cytokine production and T-cell proliferation before deployment is associated with increased PTSD symptoms after combat [9]. These findings suggest that immune factors might not only be markers for symptom state, but also contribute to pre-existing risk for PTSD upon trauma exposure. Overall, these findings support the emerging theory that alterations in the immune system may promote the development of PTSD symptoms, but the causal link between symptoms and inflammation remains to be understood. It is unclear if immune abnormalities increase risk for behavioral symptoms of PTSD (e.g. represent a risk trait) or are simply an epiphenomenon of other risk mechanisms (e.g. abnormal glucocorticoid signaling [10], high trauma burden or poor health choices) or if trauma itself causes a disruption of the immune system leading to PTSD symptoms (e.g. stress-induced inflammation). It is also unclear how much and if peripheral immune signaling, the predominant marker of immune abnormalities in human studies, plays a role in PTSD and how well it corresponds to inflammation in the central nervous system. Animal studies have been critical for addressing both the causal relationship between inflammation and PTSD symptoms, as well as identifying the relationship between peripheral and central immune signaling to alter anxiety behaviors. Here, we present a review of the studies investigating the role of the immune system in stress-induced

behavioral abnormalities in animal models that have predictive and construct validity for PTSD [11,12].

Animal models examining the effects of single vs. chronic stress on inflammatory signaling

The repeated social defeat (RSD) stress model and predator stress model, in which the rodent is exposed to a cat or a cat scent, are two animal models with both predictive and construct validity for PTSD, based on sensitivity to clinically effective pharmacological interventions and similar biological and behavioral characteristics to human PTSD [12]. In the chronic RSD model in which mice are repeatedly exposed to an aggressor for 6 consecutive nights, increased levels of circulating monocytes are found in blood, associated with higher macrophage recruitment to the brain (microglial activation) and enhanced anxiety-like behavior 8 days after the last stress exposure [13]. Up to 24 days after RSD, social avoidance and high mRNA levels of pro-inflammatory cytokines, such as IL-1 β , TNF- α and IL-6 were observed in microglial cells [13]. Importantly, splenectomy prior to stress attenuated macrophage recruitment to the brain and prevented the development of anxiety-like behavior in rodents [13], supporting the important role of the peripheral monocyte trafficking from the spleen to the brain in the development of anxiety-like behavior. It has to be noted that anxiety-like behavior in the previous study was assessed using the open field test. Thus further work investigating anxiety-like phenotype in other approach (e.g. elevated plus maze (EPM), light-dark box) and non-approach-based tasks (e.g., startle reactivity, cued avoidance, defensive burial), would strengthen the generalizability of these findings. Interestingly, persistent myocarditis and cardiac fibrosis were also correlated with increased inflammatory response following RSD in mice [14,15], in line with similar co-morbidities, such as diabetes and heart disease, observed in veterans diagnosed with PTSD [1,16]. Interestingly physical injury in addition to the psychosocial component appears to be required for the peripheral, but not central, inflammatory cascade driven by RSD [17], suggesting that the RSD model may best model PTSD involving physical trauma. Overall these data support the idea that peripheral immune signaling can directly affect central inflammation and relevant anxiety-like behaviors.

Using a repeated predator stress regimen involving repeated protected predator stress exposures and chronic mild social stress, an enduring anxiety-like phenotype (measured by elevated plus maze) was accompanied by increased levels of IL-1 β and NACHT/LYY/PYD domain containing protein 3 (NALP3) inflammasome in several brain regions including prefrontal cortex (PFC), hippocampus, and amygdala 3 weeks after the last stress exposure [2,18]. These studies suggest that neuroinflammatory mechanisms following chronic/repeated psychosocial stress promote anxiety-like behaviors. However, much less is known regarding the neuroinflammatory pathways associated with behavioral abnormalities reported in single stress models of PTSD, including predator stress or single prolonged stress (SPS) models [3–5,19–21]. SPS is a model in which rodents are exposed to multiple stressors over the course of one day (physical and emotional), putatively mimicking the effects of one extreme stress or traumatic event. Predator stress involves exposure to a predator, either protected, or under supervision with direct contact. Two studies associated

SPS-induced anxiety-like behaviors and neuroinflammation, specifically increasing hippocampal IL-6, IL-1 β and TNF- α expression 2 weeks after exposure [5,22,23]. Less is known about the long-term effects of a single predator stress on inflammation. Exposure to predator odor acutely increases expression of cytokines and microglia activation in brain [6,24], however further work is needed to investigate the relationship between peripheral and central markers in these single stress models and the longevity of inflammation after single stress exposure.

As suggested in the introduction, an important aspect of PTSD is the individual risk in developing and enduring response to trauma, and evidence suggests that stress hormones and immune function may regulate PTSD vulnerability. Modeling risk via identification of vulnerable and resilient animals enables identification of mechanism underlying PTSD risk [25]. In the RSD model, IL-6 circulating levels are significantly higher after the first defeat in mice that go on to develop a susceptible phenotype (social avoidance) [26]. Similar to increased immune responsivity pre-trauma associations with increased risk to develop PTSD after combat [9], mice that exhibit higher circulating levels of monocytes and elevated release of IL-6 in LPS-stimulated leukocytes predicts the susceptibility phenotype [26]. Furthermore, transplant of hematopoietic progenitor cells from stress-susceptible mice to naïve mice leads to increased social avoidance, whereas cell transplant from IL-6 knockout (IL-6^{KO}) mice or systemic treatment with an IL-6 monoclonal antibody in naïve mice promoted resilience to stress [26]. These findings support the role of IL-6 in stress susceptibility and that immune mediators before stress regulate the response to stress, but also strongly suggest that a peripheral immune mechanism mediates the individual differences in response to stress.

Role of peripheral vs. central immune signaling in enduring stress responses

Several studies have sought to delineate the role of specific cytokine pathways in stress-induced anxiety using mice deficient in central signaling involving pro-inflammatory cytokines. IL-1 β is a pro-inflammatory cytokine involved in stress-induced behavioral changes that has both peripheral and central signaling capabilities. Both intracerebroventricular (ICV) and systemic administration of IL-1 β increase anxiety-like behavior and impair contextual fear conditioning [7,27]. ICV IL-1 β disrupted contextual but not auditory fear conditioning [8,27] suggesting that IL-1 β signaling can modulate hippocampal specific circuits [10,28]. Conversely, mice deficient for the IL-1 receptor type-1 (IL-1R^{KO} mice) exhibited reduced anxiety-like behavior in the EPM and light-dark box, and improved contextual and auditory fear conditioning [29,30]. These findings support the notion that IL-1 signaling may increase “general” anxiety-like behaviors and disrupt fear learning processes [13,28]. Similarly, ICV infusion of IL-1 receptor antagonist (IL-1Ra) normalizes contextual and auditory fear extinction in chronically stressed rats (socially isolated rats [13,27] or rats exposed to repeated footshocks [13,31]). In IL-1R^{KO} mice, elevation of IL-1 β in the brain, microglial activation, and development of anxiety-like symptoms did not occur following RSD [14,15,32]. Although these findings indicate that IL-1 β plays a significant role in RSD-induced neuroinflammation and anxiety, the

mechanisms underlying the neuroimmune alterations and behavioral effects following stress remained unclear. A following study by Wohleb et al. showed that the RSD-induced decrease of peripheral monocytes in blood in IL-1R^{KO} mice correlates with decreased macrophage trafficking to the brain and reduced microglia activation after RSD [33]. Endothelial-specific knockdown of IL-1R1 in mice (eIL-1R1kd) results in attenuated circulating monocytes, recruitment of macrophages to the brain, and microglia activation following RSD [33]. Also, the same mice exhibited reduced stress-induced anxiety-like behavior and expression of IL-1 β , TNF- α and IL-6 mRNA levels in microglial cells [33], suggesting that IL-1 signaling in endothelial cells promotes stress-induced anxiety and neuroinflammation. The same group demonstrated that mice deficient for either chemokine receptor-2 (CCR2) or fractalkine receptor (CX3CR1), two receptors involved in peripheral monocytes trafficking to the brain [34], did not develop anxiety-like behaviors and failed to recruit macrophages to the brain following RSD, as shown by decreased monocytes cells in blood and microglial activation in the brain [35]. These studies altogether indicate that IL-1 β primes stress-induced neuroinflammation and anxiety-like behavior by facilitating monocyte trafficking/recruitment of macrophages from the spleen to the brain.

TNF- α is another cytokine regulating brain-endocrine-immune interactions during the acute response to stress through its receptors TNF receptors type 1 (TNFR1) and type 2 (TNFR2). TNF- α is also known to modulate several behavioral constructs, including anxiety, as ICV injections of TNF- α induces anxiety-like behavior in rats [36]. Endogenous levels of TNF- α , however, may play a role in reducing anxiety, as mice with reduced TNF- α signaling exhibit increased anxiety-like behavior. Specifically, TNFR1/TNFR2 double knockout mice, but not mice with single knockout of either TNFR1 or TNFR2, show increased anxiety-like behaviors [37,38]. One potential mechanism through which TNF- α inhibits anxiety is via activation of CD4⁺ T cells. Double knockdown of TNFR1 and TNFR2 resulted in a greater anxiety-like behavior following RSD, correlating with a higher elevation of corticosterone serum levels and decreased number of T cells in the spleen [39]. Nude mice, known to exhibit reduced formation of CD4⁺ helper and CD8⁺ cytotoxic T cells, display increased avoidance and startle responding after predator scent stress [40]. However, the type of T cells activated by stress appears to be a critical factor in mediating stress-induced changes in avoidance behavior. Mice specifically deficient for the suppressive T_{reg} CD4⁺/CD25⁺ cells (T_{MBP}/Rag^{KO}) do not exhibit enduring anxiety-like behavior after predator stress and specific depletion of T_{reg} cells in nude mice attenuates anxiety-like behaviors [40]. Taken together these data suggest that the overall proportion of different T-cell types could play a role in the adaptive behavioral responses after a stressful event, with either increased or resilient responses depending on the proportion of certain T-cell types [41]. Although the involvement of TNF- α in peripheral inflammation and consequent behavior is well demonstrated [42], the role of this cytokine in peripheral signaling to brain is less clear and remains to be studied *in vivo*.

The previous studies provide insights on the role of specific cytokines in the development of PTSD-related behaviors *in vivo*. However, an overall increase of inflammatory state is found in PTSD patients [5,6], leading researchers to investigate direct effects of activation of peripheral and central immune signaling on PTSD-related phenotypes. The most common method for inducing immune response in these studies is through systemic administration of

lipopolysaccharide (LPS), which activates the immune system by binding the Toll-like receptor 3 (TLR3). LPS treatment disrupts auditory cued and contextual fear extinction and increases anxiety-like behaviors in rodents [43–46] mimicking phenotypes reported in PTSD patients. Subcutaneous treatment with IL-1 receptor antagonist (IL-1Ra) prevents LPS-induced impairments in contextual fear conditioning [44], supporting again the role of IL-1 β in hippocampal-dependent fear learning. It is not clear if these LPS effects are also via IL-1-induced macrophage trafficking or other peripheral-central signaling mechanisms (e.g. vagal nerve signaling). Overall these data suggest that LPS-induced inflammation can mimic some of the phenotypes described in PTSD. However, the effects of immune activation in terms of increased sensitivity (i.e. PTSD risk) to enduring effects of trauma has not yet been investigated in PTSD models. This is an exciting new area given the recent evidence in humans that inflammation is associated with increased PTSD risk.

Interestingly, sub-chronic treatment with candesartan, an angiotensin II receptor type 1 (AT₁) antagonist, prevents the development of the LPS-induced anxiety-like behavior and impairment of fear extinction [45,46]. The prophylactic effects of candesartan correlates with an attenuated release of pro-inflammatory cytokines TNF- α , IL-6, and IL-1 β in plasma and brain [46]. Angiotensin II (Ang II) is a peptide that can promote neuroinflammation by stimulating of AT₁ receptors, and AT₁ receptor antagonists are commonly used to regulate blood pressure [47]. In line with these findings, decreased PTSD symptoms are associated with the use of blood pressure medication selectively targeting the renin-angiotensin pathway – angiotensin converting enzyme inhibitors or AT₁ receptor antagonists – but not beta-blockers, calcium channel blockers, or diuretics [48]. These findings altogether suggest not only that inflammatory mechanisms might be good targets for improving the prognosis of PTSD patients, but also that drugs targeting the renin-angiotensin pathway might possess therapeutic potential. Nevertheless, the mechanisms underlying the renin-angiotensin pathway and inflammation remain to be investigated.

Clinically available drugs targeting immune mechanisms in animal models of PTSD

The findings described above support the involvement of inflammation in the development of PTSD-related behaviors and suggest the therapeutic potential of anti-inflammatory drugs. Minocycline, a tetracycline antibiotic with anti-inflammatory properties [49], administered a few hours after stress exposure, attenuates predator stress-induced anxiety-like behaviors in rats [50]. The reduction in anxiety-like behavior correlates with normalized levels of pro-inflammatory cytokines IL-1 α , IL-6 and TNF- α in several brain regions, including the hippocampus, hypothalamus and PFC, three regions known to be altered in PTSD patients [50]. However, the mechanisms by which minocycline reduces inflammation and anxiety-like behaviors remain to be studied. Because recruitment of macrophages to the brain and subsequent microglial activation might be mechanisms underlying the development of anxiety-like symptoms in vivo [32,33,35], minocycline might have neuroprotective effects through direct microglial inhibition [49]. Additionally, the nonsteroidal anti-inflammatory drug (NSAID) ibuprofen, chronically administered for 2 weeks following stress, normalizes anxiety-like behavior and TNF- α and IL-1 β hippocampal expression in the SPS model [23].

These studies indicate that anti-inflammatory drugs might be potential preventive treatments in PTSD, and that a pharmacological intervention within the first hours after a traumatic event might be sufficient to prevent the onset of the full spectrum of PTSD symptoms.

Aga-Mizarachi et al. investigated the effects of methylphenidate, the most common treatment for attention deficit hyperactivity disorder (ADHD), on three core PTSD-like symptoms, including re-experiencing, avoidance, and hyperarousal [51]. In a stress paradigm consisting of repeated footshock exposures, chronic treatment with methylphenidate reverses the stress-related impairment in fear extinction (re-experiencing), decrease in locomotor activity (avoidance), and increase in startle response (hyperarousal). The effects of methylphenidate are enhanced when combined with desipramine, a noradrenergic reuptake inhibitor, and correlate with an attenuation of the stress-induced elevation of IL-1 β and IL-6 serum levels [51], linking immune alterations and PTSD-like phenotype in vivo. In line with this study, a recent randomized placebo-controlled trial showed that methylphenidate improves PTSD symptoms in mixed head injury/PTSD patients [52], supporting the potential of this drug for treatment of PTSD. Still, further work will be necessary to understand the underlying mechanisms of the anti-inflammatory effects of methylphenidate, especially combined with desipramine or a SSRI approved for the treatment of PTSD symptoms.

Studies linking PTSD-associated traumatic brain injury (TBI) and inflammation

Co-occurring traumatic brain injury (TBI), a risk factor for PTSD [53], and psychological trauma is common in U.S. veterans, leading to higher risk of PTSD in this population [54]. These findings highlight an urgent need to investigate the role of immune mediators in the onset of PTSD following psychological trauma combined with TBI. Despite several studies highlighting the overlapping clinical symptoms between PTSD and TBI, many individuals with PTSD that have endorsed TBI before have no brain abnormalities as seen with imaging techniques [55]. Although the consequences and mechanisms following TBI combined with psychological trauma remain unclear, specific signaling pathways might be involved, such as neuroinflammation and oxidative stress [54,56]. Some studies reported increased pro-inflammatory status in the brain (IL-1 α , IL-6 and microglia activation) correlating with anxiety-like behaviors and disrupted fear extinction in a rat model of blast-induced TBI [57–59], but causal mechanisms for neuroimmune alterations in TBI models have yet to be tested. Two studies have investigated the effects of chronic stress combined with TBI on PTSD-like phenotypes and neuroinflammation to model comorbid TBI/PTSD. Blast-induced TBI (severe TBI) exerts additive/synergistic effects on chronic stress-induced anxiety-like behaviors and memory impairments, correlating with higher expression of interferon- γ (IFN- γ) and astrogliosis in PFC and hippocampus [60]. Conversely, milder blunt trauma (no skull fracture or subdural hemorrhage) had relatively little effect or attenuated stress effects on fear learning, social preference and anxious-like behaviors, and on IL-1 β and TNF- α plasma levels and microglia activation [61]. TBI models, however, present a challenge since both the anesthesia required when inflicting the injury and the loud sounds associated with pressure blasts (used in blast models of TBI) can induce release of central proinflammatory

markers and increase anxiety-like behavior [62]. Therefore, the neuroimmune mechanisms underlying behavioral abnormalities in a case of co-occurring psychological trauma and TBI may be difficult to disentangle from model-driven confounders.

Conclusion

There are no FDA-approved pharmacological interventions available to prevent the onset of PTSD symptoms in traumatized individuals. Identifying prognostic biomarkers and potential pre-symptomatic therapeutic targets for PTSD became a new priority in the study of this anxiety disorder. Although animal models have their limitations, they allow assessing physiological parameters prior to PTSD-like phenotype, which is a major component missing in most human research. In the last decade, the involvement of inflammation as a potential pathophysiological mechanism of PTSD emerged, with several studies showing altered immune system function in PTSD patients [10,63]. Overall, the findings in chronic stress models and using modulation of the immune function support the hypothesis that chronic inflammation is part of the pathophysiology of PTSD and its co-morbidities and that previous inflammatory dysfunctions lead to a number of PTSD-like phenotypes including avoidance, increased fear expression, increased acoustic startle and social avoidance (Table 1). To date, a major limitation of these studies is that sex-dependent effects have not been investigated, although sex hormones are known to modulate inflammatory response [64].

Among all the inflammatory markers correlating with behavioral impairments relevant to PTSD in vivo, the pro-inflammatory cytokine IL-1 β plays a major role in the onset of these behavioral abnormalities, by mediating the mandatory trafficking of peripheral monocytes from the spleen to the brain and microglial activation. Also, autoreactive T cells and suppressive T_{reg} cells mediate the adaptive response to stress [40]. Since IL-1 β and its receptors are known to regulate proliferation and survival of T cells following a stimulus [65], T cells regulation might be another mechanism by which IL-1 β mediates the onset of PTSD. Altogether, these findings suggest that IL-1 β might be a new target for treatment of PTSD. The time course studies in the RSD chronic stress model [13,32,35] support the association between trauma-induced inflammatory responses and anxiety-like behaviors relevant to PTSD (Figure 1). However, further time-course experiments by performing repeated sampling will be necessary to assess the trajectory of inflammatory signaling after severe stress/trauma to inform how these signals coincide with development, maintenance and recovery of PTSD-like behaviors. Genetic and pharmacological manipulations that mimic specific cytokine response patterns associated with PTSD risk (e.g. exogenously induced elevations in CRP) should also be investigated to identify if chronic dysregulation of specific cytokines and signaling factors play a causal role in PTSD risk. Studies showing that LPS, an activator of the peripheral and central immune system, lead to increased PTSD-related behavioral abnormalities [44–46] supports the hypothesis that altered inflammatory state, including peripheral inflammation, might be a risk factor for the development of PTSD, however further work however is needed using both single and chronic immune challenge models to identify specific risk mechanisms. Finally, comorbid TBI/PTSD animal models need to be developed to understand the neuroimmune mechanisms underlying the onset of PTSD-like behaviors following psychological trauma combined with TBI.

Acknowledgments

Jessica Deslauriers, Ph.D. is recipient of a CIHR (Canadian Institutes of Health Research) postdoctoral fellowship. Support came from a VA Merit Award to VBR and ES025585 to SBP. VR is also funded by the Veterans Affairs Center of Excellence for Stress and Mental Health and SBP is funded by the Veterans Affairs Mental Illness Research and Clinical Core.

References

1. Pace TWW, Heim CM. A short review on the psychoneuroimmunology of posttraumatic stress disorder: from risk factors to medical comorbidities. *Brain Behav Immun*. 2011; 25:6–13. [PubMed: 20934505]
2. Asnis GM, Kohn SR, Henderson M, Brown NL. SSRIs versus non-SSRIs in posttraumatic stress disorder: an update with recommendations. *Drugs*. 2004; 64:383–404. [PubMed: 14969574]
3. Guo M, Liu T, Guo J-C, Jiang X-L, Chen F, Gao Y-S. Study on serum cytokine levels in posttraumatic stress disorder patients. *Asian Pac J Trop Med*. 2012; 5:323–325. [PubMed: 22449527]
4. Lindqvist D, Wolkowitz OM, Mellon S, Yehuda R, Flory JD, Henn-Haase C, Bierer LM, Abu-Amara D, Coy M, Neylan TC, et al. Proinflammatory milieu in combat-related PTSD is independent of depression and early life stress. *Brain Behav Immun*. 2014; 42:81–88. [PubMed: 24929195]
5. Michopoulos V, Norrholm SD, Jovanovic T. Diagnostic Biomarkers for Posttraumatic Stress Disorder: Promising Horizons from Translational Neuroscience Research. *Biol Psychiatry*. 2015; doi: 10.1016/j.biopsych.2015.01.005
- 6**. Eraly SA, Nievergelt CM, Maihofer AX, Barkauskas DA, Biswas N, Agorastos A, O'Connor DT, Baker DG. Assessment of Plasma C-Reactive Protein as a Biomarker of Posttraumatic Stress Disorder Risk. *JAMA Psychiatry*. 2014; 71:423. This was the first prospective, longitudinal study showing that a marker of peripheral inflammation, C-reactive protein, is higher pre-trauma in those that go on to develop PTSD after trauma exposure. [PubMed: 24576974]
7. Breen MS, Maihofer AX, Glatt SJ, Tylee DS, Chandler SD, Tsuang MT, Risbrough VB, Baker DG, O'Connor DT, Nievergelt CM, et al. Gene networks specific for innate immunity define post-traumatic stress disorder. *Mol Psychiatry*. 2015; doi: 10.1038/mp.2015.9
- 8**. Michopoulos V, Rothbaum AO, Jovanovic T, Almlie LM, Bradley B, Rothbaum BO, Gillespie CF, Ressler KJ. Association of CRP Genetic Variation and CRP Level With Elevated PTSD Symptoms and Physiological Responses in a Civilian Population With High Levels of Trauma. *AJP*. 2015; 172:353–362. Following quickly after the Eraly et al. report of circulating CRP is associated with PTSD risk, the Smith and colleagues showed that a putative functional polymorphism in the CRP gene is associated both with increased CRP levels as well as increased prevalence for PTSD.
9. van Zuiden M, Heijnen CJ, Maas M, Amarouchi K, Vermetten E, Geuze E, Kavelaars A. Glucocorticoid sensitivity of leukocytes predicts PTSD, depressive and fatigue symptoms after military deployment: A prospective study. *Psychoneuroendocrinology*. 2012; 37:1822–1836. [PubMed: 22503138]
10. Rohleder N, Joksimovic L, Wolf JM, Kirschbaum C. Hypocortisolism and increased glucocorticoid sensitivity of pro-Inflammatory cytokine production in Bosnian war refugees with posttraumatic stress disorder. *Biol Psychiatry*. 2004; 55:745–751. [PubMed: 15039004]
11. Daskalakis NP, Yehuda R, Diamond DM. Animal models in translational studies of PTSD. *Psychoneuroendocrinology*. 2013; 38:1895–1911. [PubMed: 23845512]
12. Matar MA, Zohar J, Cohen H. Translationally relevant modeling of PTSD in rodents. *Cell Tissue Res*. 2013; 354:127–139. [PubMed: 23912242]
- 13**. Wohleb ES, McKim DB, Shea DT, Powell ND, Tarr AJ, Sheridan JF, Godbout JP. Re-establishment of Anxiety in Stress-Sensitized Mice Is Caused by Monocyte Trafficking from the Spleen to the Brain. *Biol Psychiatry*. 2014; 75:970–981. This study showed that splenectomy prior to stress attenuated macrophage recruitment to the brain and prevented the development of chronic stress-induced anxiety-like behavior in rodents, supporting the important role of the

- peripheral monocyte trafficking from the spleen to the brain in stress-induced increases in anxiety-like behavior. [PubMed: 24439304]
14. Cho J-H, Lee I, Hammamieh R, Wang K, Baxter D, Scherler K, Etheridge A, Kulchenko A, Gautam A, Muhie S, et al. Molecular evidence of stress-induced acute heart injury in a mouse model simulating posttraumatic stress disorder. *Proceedings of the National Academy of Sciences*. 2014; 111:3188–3193.
 15. Gautam A, D'Arpa P, Donohue DE, Muhie S, Chakraborty N, Luke BT, Grapov D, Carroll EE, Meyerhoff JL, Hammamieh R, et al. Acute and chronic plasma metabolomic and liver transcriptomic stress effects in a mouse model with features of post-traumatic stress disorder. *PLoS ONE*. 2015; 10:e0117092. [PubMed: 25629821]
 16. Levine AB, Levine LM, Levine TB. Posttraumatic stress disorder and cardiometabolic disease. *Cardiology*. 2014; 127:1–19. [PubMed: 24157651]
 17. Reader BF, Jarrett BL, McKim DB, Wohleb ES, Godbout JP, Sheridan JF. Peripheral and central effects of repeated social defeat stress: monocyte trafficking, microglial activation, and anxiety. *Neuroscience*. 2015; 289:429–442. [PubMed: 25596319]
 18. Wilson CB, McLaughlin LD, Nair A, Ebenezer PJ, Dange R, Francis J. Inflammation and oxidative stress are elevated in the brain, blood, and adrenal glands during the progression of post-traumatic stress disorder in a predator exposure animal model. *PLoS ONE*. 2013; 8:e76146. [PubMed: 24130763]
 19. Liberzon I, López JF, Flagel SB, Vazquez DM, Young EA. Differential regulation of hippocampal glucocorticoid receptors mRNA and fast feedback: relevance to posttraumatic stress disorder. *J Neuroendocrinol*. 1999; 11:11–17. [PubMed: 9918224]
 20. Adamec R, Walling S, Burton P. Long-lasting, selective, anxiogenic effects of feline predator stress in mice. *Physiology & behavior*. 2004; 83:401–410. [PubMed: 15581662]
 21. Toth M, Flandreau EI, Deslauriers J, Geyer MA, Mansuy I, Merlo-Pich E, Risbrough V. Overexpression of Forebrain CRH During Early Life Increases Trauma Susceptibility in Adulthood. *Neuropsychopharmacology*. 2015; doi: 10.1038/npp.2015.338
 22. Peng Z, Wang H, Zhang R, Chen Y, Xue F, Nie H, Chen Y, Wu D, Wang Y, Wang H, et al. Gastrodin ameliorates anxiety-like behaviors and inhibits IL-1 β level and p38 MAPK phosphorylation of hippocampus in the rat model of posttraumatic stress disorder. *Physiol Res*. 2013; 62:537–545. [PubMed: 24020812]
 23. Lee B, Sur B, Yeom M, Shim I, Lee H, Hahm D-H. Effects of systemic administration of ibuprofen on stress response in a rat model of post-traumatic stress disorder. *Korean J Physiol Pharmacol*. 2016; 20:357–366. [PubMed: 27382352]
 24. Barnum CJ, Pace TW, Hu F, Neigh GN, Tansey MG. Psychological stress in adolescent and adult mice increases neuroinflammation and attenuates the response to LPS challenge. *J Neuroinflammation*. 2012; 9:9. [PubMed: 22248083]
 25. Daskalakis NP, Cohen H, Nievergelt CM, Baker DG, Buxbaum JD, Russo SJ, Yehuda R. New translational perspectives for blood-based biomarkers of PTSD: From glucocorticoid to immune mediators of stress susceptibility. *Experimental Neurology*. 2016; 284:133–140. [PubMed: 27481726]
 26. Hodes GE, Pfau ML, Leboeuf M, Golden SA, Christoffel DJ, Bregman D, Rebusi N, Heshmati M, Aleyasin H, Warren BL, et al. Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. *Proceedings of the National Academy of Sciences*. 2014; 111:16136–16141.
 27. Pugh CR, Nguyen KT, Gonyea JL, Fleshner M, Wakins LR, Maier SF, Rudy JW. Role of interleukin-1 β in impairment of contextual fear conditioning caused by social isolation. *Behav Brain Res*. 1999; 106:109–118. [PubMed: 10595426]
 28. Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci*. 1992; 106:274–285. [PubMed: 1590953]
 29. Bourin M, Petit-Demouliere B, Dhonnchadha BN, Hascöet M. Animal models of anxiety in mice. *Fundam Clin Pharmacol*. 2007; 21:567–574. [PubMed: 18034657]
 30. Koo JW, Duman RS. Interleukin-1 receptor null mutant mice show decreased anxiety-like behavior and enhanced fear memory. *Neurosci Lett*. 2009; 456:39–43. [PubMed: 19429130]

- 31**. Jones ME, Lebonville CL, Barrus D, Lysle DT. The role of brain interleukin-1 in stress-enhanced fear learning. *Neuropsychopharmacology*. 2015; 40:1289–1296. This report showed that IL-1 signaling in brain “primes” enhanced fear conditioning, providing a potential link between high IL-1 levels and the high fear conditioning phenotype consistently reported in PTSD patients. [PubMed: 25430780]
32. Wohleb ES, Hanke ML, Corona AW, Powell ND, Stiner LM, Bailey MT, Nelson RJ, Godbout JP, Sheridan JF. β -Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. *Journal of Neuroscience*. 2011; 31:6277–6288. [PubMed: 21525267]
- 33**. Wohleb ES, Patterson JM, Sharma V, Quan N, Godbout JP, Sheridan JF. Knockdown of Interleukin-1 Receptor Type-1 on Endothelial Cells Attenuated Stress-Induced Neuroinflammation and Prevented Anxiety-Like Behavior. *Journal of Neuroscience*. 2014; 34:2583–2591. This work by Wohleb et al. showed that endothelial-specific knockdown of IL-1R1 in mice results in attenuated circulating monocytes, recruitment of macrophages to the brain, microglia activation, and anxiety-like behavior following chronic stress, suggesting that IL-1 signaling in endothelial cells potentiates stress-induced anxiety and neuroinflammation. [PubMed: 24523548]
34. Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, Sarnacki S, Cumano A, Lauvau G, Geissmann F. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science*. 2007; 317:666–670. [PubMed: 17673663]
35. Wohleb ES, Powell ND, Godbout JP, Sheridan JF. Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior. *Journal of Neuroscience*. 2013; 33:13820–13833. [PubMed: 23966702]
36. Connor TJ, Song C, Leonard BE, Merali Z, Anisman H. An assessment of the effects of central interleukin-1 β , -2, -6, and tumor necrosis factor- α administration on some behavioural, neurochemical, endocrine and immune parameters in the rat. *Neuroscience*. 1998; 84:923–933. [PubMed: 9579794]
37. Simen BB, Duman CH, Simen AA, Duman RS. TNF α signaling in depression and anxiety: behavioral consequences of individual receptor targeting. *Biol Psychiatry*. 2006; 59:775–785. [PubMed: 16458261]
38. Patel A, Siegel A, Zalcman SS. Lack of aggression and anxiolytic-like behavior in TNF receptor (TNF-R1 and TNF-R2) deficient mice. *Brain Behav Immun*. 2010; 24:1276–1280. [PubMed: 20685290]
39. Gimsa U, Kanitz E, Otten W, Tuchscherer M, Tuchscherer A, Ibrahim SM. Tumour necrosis factor receptor deficiency alters anxiety-like behavioural and neuroendocrine stress responses of mice. *Cytokine*. 2012; 59:72–78. [PubMed: 22561136]
40. Cohen H, Ziv Y, Cardon M, Kaplan Z, Matar MA, Gidron Y, Schwartz M, Kipnis J. Maladaptation to mental stress mitigated by the adaptive immune system via depletion of naturally occurring regulatory CD4⁺CD25⁺ cells. *J Neurobiol*. 2006; 66:552–563. [PubMed: 16555237]
41. Yoles E, Hauben E, Palgi O, Agranov E, Gothilf A, Cohen A, Kuchroo V, Cohen IR, Weiner H, Schwartz M. Protective autoimmunity is a physiological response to CNS trauma. *Journal of Neuroscience*. 2001; 21:3740–3748. [PubMed: 11356861]
42. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol*. 2003; 3:745–756. [PubMed: 12949498]
43. Swiergiel AH, Dunn AJ. Effects of interleukin-1 β and lipopolysaccharide on behavior of mice in the elevated plus-maze and open field tests. *Pharmacol Biochem Behav*. 2007; 86:651–659. [PubMed: 17360031]
44. Pugh CR, Kumagawa K, Fleshner M, Watkins LR, Maier SF, Rudy JW. Selective effects of peripheral lipopolysaccharide administration on contextual and auditory-cue fear conditioning. *Brain Behav Immun*. 1998; 12:212–229. [PubMed: 9769157]
45. Quinones MAMQ, Maldonado L, Velazquez B, Porter JT. Candesartan ameliorates impaired fear extinction induced by innate immune activation. *Brain Behav Immun*. 2016; 52:169–177. [PubMed: 26520214]

46. Benicky J, Sánchez-Lemus E, Honda M, Pang T, Orecna M, Wang J, Leng Y, Chuang D-M, Saavedra JM. Angiotensin II AT1 Receptor Blockade Ameliorates Brain Inflammation. *Neuropsychopharmacology*. 2010; 36:857–870. [PubMed: 21150913]
47. Savoia C, Schiffrin EL. Reduction of C-reactive protein and the use of antihypertensives. *Vasc Health Risk Manag*. 2007; 3:975–983. [PubMed: 18200816]
48. Khoury NM, Marvar PJ, Gillespie CF, Wingo A, Schwartz A, Bradley B, Kramer M, Ressler KJ. The renin-angiotensin pathway in posttraumatic stress disorder: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are associated with fewer traumatic stress symptoms. *J Clin Psychiatry*. 2012; 73:849–855. [PubMed: 22687631]
49. Garrido-Mesa N, Zarzuelo A, Gálvez J. What is behind the non-antibiotic properties of minocycline? *Pharmacol Res*. 2013; 67:18–30. [PubMed: 23085382]
- 50*. Levkovitz Y, Fenchel D, Kaplan Z, Zohar J, Cohen H. Early post-stressor intervention with minocycline, a second-generation tetracycline, attenuates post-traumatic stress response in an animal model of PTSD. *Eur Neuropsychopharmacol*. 2015; 25:124–132. This study showed that anti-inflammatory treatment given prophylactically attenuates development of PTSD-like behavior in rodents. This study supports the concept that anti-inflammatory interventions immediately after trauma may reduce PTSD risk. [PubMed: 25487770]
- 51**. Aga-Mizrachi S, Cymerblit-Sabba A, Gurman O, Balan A, Shwam G, Deshe R, Miller L, Gorodetsky N, Heinrich N, Tzezana O, et al. Methylphenidate and desipramine combined treatment improves PTSD symptomatology in a rat model. *Transl Psychiatry*. 2014; 4:e447–9. This was a novel study showing that methylphenidate treatment attenuated PTSD-like behavior and associated brain markers of inflammation. This study was later found to be predictive of methylphenidate efficacy in humans (see McAllister et al. below), and also provided a novel mechanism of catecholamine modulation of inflammation for these effects. [PubMed: 25247592]
- 52**. McAllister TW, Zafonte R, Jain S, Flashman LA, George MS, Grant GA, He F, Lohr JB, Andaluz N, Summerall L, et al. Randomized Placebo-Controlled Trial of Methylphenidate or Galantamine for Persistent Emotional and Cognitive Symptoms Associated with PTSD and/or Traumatic Brain Injury. *Neuropsychopharmacology*. 2015; In line with a previous in vivo study in animals (see Aga-Mizrachi et al. above), this randomized placebo-controlled trial showed that methylphenidate improves PTSD symptoms in mixed head injury/PTSD patients, supporting the potential therapeutic effect of methylphenidate. Further work will be necessary to assess the anti-inflammatory properties of this drug. doi: 10.1038/npp.2015.282
- 53*. Yurgil KA, Barkauskas DA, Vasterling JJ, Nievergelt CM, Larson GE, Schork NJ, Litz BT, Nash WP, Baker DG. Association Between Traumatic Brain Injury and Risk of Posttraumatic Stress Disorder in Active-Duty Marines. *JAMA Psychiatry*. 2014; 71:149. This was the first large, prospective, longitudinal study to show that traumatic brain injury uniquely and robustly predicts increased risk for development of PTSD. [PubMed: 24337530]
54. McAllister TW, Stein MB. Effects of psychological and biomechanical trauma on brain and behavior. *Ann N Y Acad Sci*. 2010; 1208:46–57. [PubMed: 20955325]
55. Levin HS, Wilde E, Troyanskaya M, Petersen NJ, Scheibel R, Newsome M, Radaideh M, Wu T, Yallampalli R, Chu Z, et al. Diffusion tensor imaging of mild to moderate blast-related traumatic brain injury and its sequelae. *Journal of Neurotrauma*. 2010; 27:683–694. [PubMed: 20088647]
56. Cernak I. The importance of systemic response in the pathobiology of blast-induced neurotrauma. *Front Neurol*. 2010; 1:151. [PubMed: 21206523]
57. Elder GA, Dorr NP, De Gasperi R, Gama Sosa MA, Shaughness MC, Maudlin-Jeronimo E, Hall AA, McCarron RM, Ahlers ST. Blast exposure induces post-traumatic stress disorder-related traits in a rat model of mild traumatic brain injury. *Journal of Neurotrauma*. 2012; 29:2564–2575. [PubMed: 22780833]
58. Kochanek PM, Dixon CE, Shellington DK, Shin SS, Bayir H, Jackson EK, Kagan VE, Yan HQ, Swauger PV, Parks SA, et al. Screening of Biochemical and Molecular Mechanisms of Secondary Injury and Repair in the Brain after Experimental Blast-Induced Traumatic Brain Injury in Rats. *Journal of Neurotrauma*. 2013; 30:920–937. [PubMed: 23496248]
59. Acosta SA, Diamond DM, Wolfe S, Tajiri N, Shinozuka K, Ishikawa H, Hernandez DG, Sanberg PR, Kaneko Y, Borlongan CV. Influence of post-traumatic stress disorder on neuroinflammation

- and cell proliferation in a rat model of traumatic brain injury. PLoS ONE. 2013; 8:e81585. [PubMed: 24349091]
60. Kwon S-KC, Kovesdi E, Gyorgy AB, Wingo D, Kamnaksh A, Walker J, Long JB, Agoston DV. Stress and traumatic brain injury: a behavioral, proteomics, and histological study. Front Neurol. 2011; 2:12. [PubMed: 21441982]
61. Ojo JO, Greenberg MB, Leary P, Mouzon B, Bachmeier C, Mullan M, Diamond DM, Crawford F. Neurobehavioral, neuropathological and biochemical profiles in a novel mouse model of comorbid post-traumatic stress disorder and mild traumatic brain injury [Internet]. Front Behav Neurosci. 2014; 8:1–23. [PubMed: 24478648]
62. Kamnaksh A, Kovesdi E, Kwon S-K, Wingo D, Ahmed F, Grunberg NE, Long J, Agoston DV. Factors Affecting Blast Traumatic Brain Injury. Journal of Neurotrauma. 2011; 28:2145–2153. [PubMed: 21861635]
63. Baker DG, Nievergelt CM, O'Connor DT. Biomarkers of PTSD: neuropeptides and immune signaling. Neuropharmacology. 2012; 62:663–673. [PubMed: 21392516]
64. Corcoran MP, Meydani M, Lichtenstein AH, Schaefer EJ, Dillard A, Lamon-Fava S. Sex hormone modulation of proinflammatory cytokine and C-reactive protein expression in macrophages from older men and postmenopausal women. The Journal of endocrinology. 2010; 206:217–224. [PubMed: 20484148]
65. Sims JE, Smith DE. The IL-1 family: regulators of immunity. Nat Rev Immunol. 2010; 10:89–102. [PubMed: 20081871]
66. Weber MD, Godbout JP, Sheridan JF. Repeated Social Defeat, Neuroinflammation, and Behavior: Monocytes Carry the Signal. Neuropsychopharmacology. 2016; doi: 10.1038/npp.2016.102

Highlights

- Animal models support role for peripheral and central immune signaling in anxiety/fear behavior
- Futures studies must address how specific immune mechanisms contribute to PTSD risk.
- Future studies must address the effects of single vs multiple traumas on immune function and anxiety

Figure 1. Inflammatory responses to a severe stress associated with PTSD-like phenotype in rodents

A severe stressor primes central and peripheral inflammatory response. The activation of central fear circuits induces microglial activation, which facilitates the release of pro-inflammatory cytokines, putatively leading to the onset of PTSD-like symptoms. Central inflammation can also promote peripheral inflammation by facilitating bone marrow-induced production of monocytes and their release by the spleen, with the pro-inflammatory cytokine IL-1 β facilitating the trafficking of peripheral monocytes to the brain, leading to microglial activation. Also, a severe stressor associated with physical injury can also directly promote the peripheral immune mechanisms (see Weber et al., 2016 [66] for review). Thus, microglia-derived IL-1 β affects the neuronal functionality, triggering the development of PTSD-like symptoms. CCL2, chemokine (C-C-motif) ligand 2; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α . Figure created in the Mind the Graph platform www.mindthegraph.com.

Studies investigating the peripheral and central immune mechanisms associated with the development of PTSD-like behaviors in animal models

Curr Opin Behav Sci. Author manuscript; available in PMC 2018 April 01.

Animal models examining the effects of chronic and single stress on inflammatory signaling						
Stressor	Species	Behaviors	Inflammatory markers	Response to stress in genetic models	Pharmacological treatments	References
Subchronic/chronic stressors (>1 day)						
Repeated social defeat stress (2 h on 6 consecutive nights)	Mice	↑ avoidance (OF) (12 h and 8 days); ↑ social avoidance (12h, 8 and 24 days)	Plasma: ↑ IL-6 (12 h); ↑ monocytes (12h and 8 days) Brain: ↑ macrophages (12 h and 8 days); ↑ microglial cells in PFC (12 h, 8 and 24 days), amygdala and HP (12 h and 8 days); ↑ mRNA for IL-1β and IL-6 in microglial cells (12h, 8 and 24 days)	No changes in IL-1R1KO, eIL-1R1kd, CCR2KO and CX3CR1KO mice; ↑ avoidance behaviors (EPM) and ↓ T cells in the spleen in TNFR1/TNFR2KO mice	N/A	[13,32,33, 35,39]
Repeated social defeat stress (10 min on 10 consecutive days)	Mice	↑ social avoidance (11 days)	Plasma: ↑ monocytes (before 1 st defeat); ↑IL-6 (20 min after 1 st defeat) Brain: N/A	Transplant of HPCs from susceptible mice ↓ social interaction in BM chimera; transplant of HPCs from IL-6KO mice ↑ social interaction in BM chimera	Monoclonal antibody IL-6 (4 ug/day IP) ↑ social interaction	[26]
Repeated predator stress exposures (2 × 1 h and cages changes for 10 days)	Rat	↑ avoidance (EPM) (3 wks)	Plasma: N/A Brain: ↑ mRNA and protein for IL-1β and NALP3 in PFC, HP and amygdala (3 wks)	N/A	N/A	[18]
Repeated footshock exposures (0.8 mA 2 s; 2 ×/day, 4 days/wk during 2 wks)	Rat	↑ context fear expression (5 wks); ↑ acoustic startle response and ↓ PPI (5 wks)	Plasma: ↑ IL-1β and IL-6 (5 wks) Brain: N/A	N/A	Methylphenidate 2.6 mg/kg IP (5 wks) ↓ behavioral abnormalities and inflammatory response	[51]
Single Stressors (limited to 1 day)						

Animal models examining the effects of chronic and single stress on inflammatory signaling						
Stressor	Species	Behaviors	Inflammatory markers	Response to stress in genetic models	Pharmacological treatments	References
Repeated (15) footshock exposures (2 mA 1 s)	Rat	↑ cued fear expression (1–7 days)	Plasma: N/A Brain: ↑ IL-1β in DG(6–72 h) and mRNA for IL-1β in DH (48 h)	N/A	ICV infusions (2) of IL-1Ra (10 ug) ↓ cued fear expression (1–7 days)	[31]
Soiled cat litter (10 min)	Rat	↑ avoidance (EPM) (7 days); ↑ acoustic startle response (7 days); ↑ contextual fear expression (8 days)	Plasma: N/A Brain: ↑ protein for IL-1α in HP and hypothalamus (9 days); ↑ protein for IL-6 in PFC, HP and hypothalamus (9 days); ↑ protein for TNF-α in HP (9 days)	N/A	Minocycline (35 mg/kg IP 1 h after stress) ↓ behavioral abnormalities and inflammatory response to stress	[50]
SPS (restraint stress 2 h, forced swimming 20 min, diethyl ether exposure, footshock 1 mA 1 s)	Rat	↑ avoidance (OF/EPM) (2 wks)	Plasma: N/A Brain: ↑ protein for IL-6 and IL-1β in HP (2 wks)	N/A	Ibuprofen (40 mg/kg IP 14 days) ↓ avoidance and inflammatory response	[22,23]

Studies investigating the effects of immune activation or administration of cytokines						
Treatment/Immune activation	Species	Behaviors	Inflammatory markers	Response to stress in genetic models	Pharmacological treatments	References
ICV Infusions of IL-1β (20–30 ng)	Rat	↑ avoidance (EPM) (1–2 h)	N/A	N/A	N/A	[27,30,36]
IL-1β (100, 300 or 1000 ng) IP	Mice	↑ avoidance (OF) (60 min)	N/A	N/A	N/A	[43]
LPS 50, 100 or 250 ug/kg or 1 mg/kg IP	Rat	↑ fear expression during extinction recall; ↓ context fear expression (48 h); ↑ avoidance (EPM)	Plasma: ↑ TNF-α, IL-6, IL-1β and IL-10 Brain: ↑ TNF-α, IL-6 and IL-1β in PFC, HP and amygdala	N/A	Candesartan (1 mg/kg/day IP or SC 4 days) ↓ behavioral and inflammatory response; IL-1Ra 100 mg/kg SC ↓ context fear expression	[44–46]
LPS (1 or 5 ug) IP	Mice	↑ avoidance (OF) (2 h)	N/A	N/A	N/A	[43]
ICV infusions of TNF-α (40 ng)	Rat	↑ avoidance (EPM) (45 min)	N/A	N/A	N/A	[36]

Animal models of comorbid TBI/PTSD						
TBI Model	Stressor	Species	Behaviors	Inflammatory markers	Response to stress in genetic models	Pharmacological treatments
Mild TBI (impact with blunt metal tip under anesthesia, no days- regimen)	Repeated predator scent exposures (11 × 30 min within a 21 days- regimen)	Mice	Stress ↑ context and cued fear expression (1 wk); TBI ↓ avoidance (EPM) and social	Plasma: Stress ↑ TNF-α (2 wks); TBI ↑ IL-17A (2 wks) Brain: TBI ↑ astrocytes in CC, parietal cortex, HP and BLA; and microglia in CC (2 wks)	N/A	N/A
						[61]

Animal models of comorbid TBI/PTSD							
TBI Model	Stressor	Species	Behaviors	Inflammatory markers	Response to stress in genetic models	Pharmacological treatments	References
skull fracture of subdural hemorrhage)			avoidance in stressed mice (1 wk)				
Severe TBI (whole body blast overpressure 20.6 ± 3 psi under anesthesia)	Repeated unpredictable stressors (predator scent, loud noises, cage movements)	Rat	↑↑ avoidance (OF/EPM) (24–48 h); ↓↓ spatial learning and memory (Barnes maze) (5–9 wks)	Plasma: ↑↑ GFAP Brain: Stress ↑ protein for IL-6 in HP (regardless of TBI); ↑↑ protein for IFN-γ and GFAP in HP and PFC; ↑↑ apoptotic cells in HP and PFC	N/A	N/A	[60]