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The neuroimmunology of social-stress-induced sensitization

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Abstract

Myriad clinical findings provide links between chronic stressors, inflammation, and mood disorders. Furthermore, traumatic or chronic exposure to psychological stressors may promote stress sensitization, in which individuals have long-term complications, including increased vulnerability to subsequent stressors. Post-traumatic stress disorder (PTSD) is a clinically relevant example of stress sensitization. PTSD alters neuronal circuitry and mood; however, the mechanisms underlying long-term stress sensitization within this disorder are unclear. Rodent models of chronic social defeat recapitulate several key physiological, immunological, and behavioral responses associated with psychological stress in humans. Repeated social defeat (RSD) uniquely promotes the convergence of neuronal, central inflammatory (microglial), and peripheral immune (monocyte) pathways, leading to prolonged anxiety, social withdrawal, and cognitive impairment. Moreover, RSD promotes stress sensitization, in which mice are highly sensitive to subthreshold stress exposure and recurrence of anxiety weeks after the cessation of stress. Therefore, the purpose of this Review is to discuss the influence of social-defeat stress on the immune system that may underlie stress sensitization within three key cellular compartments: neurons, microglia, and monocytes. Delineating the mechanisms of stress sensitization is critical in understanding and treating conditions such as PTSD.

Alterations in bidirectional communication between the brain and immune system contribute to the etiology of many psychiatric disorders related to stress[1–6]. Although acute exposure to stress can be beneficial, repeated or chronic stress has myriad negative psychological

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and physiological effects. For instance, chronic social stress can increase inflammation, which is correlated with worsened mental health[7,8]. Increased inflammation, both peripheral and centrally (brain and spinal cord) mediated, is relevant to many stress-related disorders, including anxiety, depression, and PTSD[7,9–12]. Thus, identification of key pathways within the immune system may elucidate the underlying pathophysiology of chronic-stress-related complications.

Clinical significance

There is clinical evidence of peripheral immune changes following chronic stress in both men and women. For instance, the chronic stress of caregiving or lower socioeconomic status promotes anxiety that is associated with increased pro-inflammatory monocytes in circulation[13–16]. Moreover, increases in circulating monocytes ($CD16^{-}CD14^{+}$) have been reported in individuals with PTSD, anxiety, and depression[13,16–18]. As such, the severity of PTSD and depressive symptoms is correlated with inflammatory NF-κB signaling and glucocorticoid resistance in monocytes[19–21]. The chronic stress of low socioeconomic status is associated with an inflammatory leukocyte transcriptional fingerprint. There are transcriptional indications of immune activation with increased pro-inflammatory gene transcription (for example, tumor necrosis factor (*TNF*), matrix metalloproteinase-9 (*MMP9*), superoxide dismutase (*SOD2*), *CD163*) and decreased anti-inflammatory-related pathways[13,15,16,22]. A recent study has identified five alleles on the human leukocyte antigen (*HLA*) locus that are associated with an increased risk of developing PTSD[23]. Collectively, there are clinical links between chronic stress, inflammatory monocytes, and neuropsychiatric complications.

Chronic stress also affects microglia, the myeloid cells of the central nervous system. Increased inflammatory profiles of microglia have been reported in depression, anxiety, and PTSD[7,24,25]. For instance, structural alterations in microglia have been detected in regions associated with cognitive function, memory formation and memory processing (for example, the hippocampus and prefrontal cortex) in individuals with depression, anxiety, or PTSD[7,24–27]. In addition, there were increased microglia with a ‘reactive’ morphology (for example, de-ramified branches, enlarged soma) and increased gene expression of ionized-calcium-binding adaptor molecule-1 (IBA-1) in individuals with depression who had committed suicide[7]. Moreover, positron emission tomography (PET) using carbon-11 ($[^{11}C]$PBR28) detected decreased levels of 18-kDa translocator protein (TSPO), a microglia biomarker, in prelimbic regions associated with increased severity of PTSD symptoms[28]. Another study using PET ($[^{18}F]$FEPPA) reported enhanced microglia activation in the hippocampus and frontal cortex of responders to the 2001 World Trade Center attack in the United States. The increased TSPO was associated with higher severity of PTSD symptoms[24]. Similar results were detected in individuals with major depression[25]. These findings indicate that microglia contribute to inflammation and symptom severity in individuals with psychiatric disorders.

Social defeat stress

As noted above, clinical findings link chronic stressors, inflammation, and mood disorders. Experimental models using chronic or repeated social defeat (RSD) in mice parallel some of these clinical connections among stress, inflammation, and anxiety. For instance, social defeat in mice promotes the convergence of neuronal, central inflammatory (microglia), and peripheral immune (monocytes) pathways that enhance inflammatory pathways and augment anxiety. Anxiety in rodents is associated with thigmotaxis and avoidance of the center in open field tests, and neophobic behaviors in the light/dark preference test and elevated plus maze paradigms[29]. Additionally, social defeat causes social avoidance (of the aggressor mouse), reduced social interaction (with a juvenile conspecific), and cognitive impairment[30–36]. Social avoidance (SA) is determined by using a two-trial social test[37]. Avoidant mice explore the empty cage area but avoid the caged aggressor in the second trial. These anxiety behaviors promoted by social defeat in mice parallel aspects of mood disorders.

Key to the augmentation of anxiety following social stress is the recruitment of monocytes to the brain by microglia[33]. In addition, social stress promotes longer term influences on behavior, physiology, and immunity, termed ‘stress sensitization.’ For instance, socially defeated mice developed social-avoidance behavior that maintained for 4 weeks after the initial exposure to social stress[35]. Stress sensitization is associated with amplified responses to acute stress exposure weeks after the initial stressor. For example, there was an exaggerated inflammatory response to the subthreshold stressor, mediated by microglia and monocytes, that caused the recurrence of anxiety[38]. Notably, this acute stress is termed ‘subthreshold’ because it does not elicit these exaggerated inflammatory responses in control mice[38]. Thus, it is important to discuss the pathways that are induced by the initial stress exposure and to delineate the pathways that may result in long-term sensitization.

There are several protocols to elicit social stress in mice. In general, an intruder mouse (older, larger, and more aggressive) is introduced into a cage with a mouse or a group of mice for a specific duration of time, such as acute (for example, 10–15 minutes for 1 day), repeated (for example, 2 hours for 6 consecutive days), or chronic (for example, up to 35 days of consecutive repeated social defeat)[39]. In a classic version of this stressor, chronic social defeat or paired fighting, an experimental mouse is placed into the home cage of an aggressor mouse for 10 minutes. Following the 10 minutes, a perforated clear divider is placed in the cage to separate the mice for 24 hours[40]. This stress cycle is repeated for 10 consecutive days. This model is relevant for investigating the neurobiological differences between stress-susceptible and stress-resilient mice[35,37,40–44]. For example, resilient mice do not have social avoidant behavior to the aggressor following the 10 days of defeat[35,42]. Social avoidance was positively correlated with increased volume in regions associated with fear, memory, and the hypothalamic–pituitary–adrenal (HPA) axis, including hippocampus, periaqueductal gray, and hypothalamus[45]. In a related version of social defeat, RSD, an aggressor mouse is introduced into the home cage of three to five resident C57BL/6 mice for 2 hours per day for 6 consecutive days. During the 2-hour interaction period, the aggressor asserts dominance and disrupts the previously established social hierarchy of the residents. Although these models are similar in their construction and outcomes, RSD is a more

intense social stressor in which resilience to behavioral complications from the stress are rare.

The primary response to these social stressors is the perception of this threat within the central nervous system with corresponding activation of neurons, glia, and endothelia. Social defeat in mice causes a region-dependent increase in neuronal activation (cFos and ΔFosB expression) in fear- and threat-appraisal regions[30], including the prefrontal cortex, amygdala, hippocampus, lateral septum, bed nucleus of the stria terminalis, hypothalamus[30,33,46], and nucleus accumbens[37,47]. Additionally, there is both behavioral and biochemical evidence of a fear response persisting after the cessation of RSD[48]. This interpretation of fear and threat appraisal was regionally specific within the brains of mice after social stress[33,38,46,49,50]. Neuronal activation with RSD also spatially coincides with the activation of microglia and endothelia[33,46]. For example, there is significant morphological restructuring and increased cytokine and chemokine expression in the microglia after RSD. Additionally, RSD-induced brain endothelia express adhesion molecules and interleukin-1 receptor-1 (IL-1R1). Consistent with the idea that threat interpretation is the start of this cascade, inhibiting the fear and threat representation after RSD with anxiolytics blocks all downstream responses[30,51,52]. For instance, propranolol, a non-selective beta blocker, prevents neuronal activation and the corresponding activation in both the endocrine and immune systems upon RSD[30]. Overall, there is convergence of neuronal, endocrine, and immune signals with social defeat.

RSD induces significant activation of both the sympathetic nervous system (SNS) and HPA axis, which provide key signals for regulation of the immune system (Fig. 1). SNS activation results in the release of catecholamines that promote a peripheral ‘fight or flight’ response, with increased heart rate, muscle tone, and energy breakdown. HPA activation increases the release of glucocorticoids, which are steroid hormones involved in metabolism. Notably, both pathways have direct communication with the immune system. For example, SNS nerve fibers are hardwired into primary and secondary lymph tissues, including the bone marrow, lymph nodes, and spleen[53]. Thus, increased beta-adrenergic signaling with stress influences the profile, maturation, and release of immune cells into circulation[30,54]. In addition, glucocorticoids, like corticosterone, provide anti-inflammatory pathways and induce apoptosis in macrophages[55]. This glucocorticoid response can become dysregulated with chronic stress, and immune cells develop glucocorticoid resistance[56]. Collectively, the activation of the SNS and HPA axis and glucocorticoid resistance with chronic stress results in the increased production and release of immune cells into the circulation.

RSD increases the production of monocytes and neutrophils in the bone marrow and the subsequent release of inflammatory (Ly6C^{hi}) monocytes into circulation[30,46] (Fig. 2). These monocytes traffick to the brain and are enriched in fear- and threat-appraisal regions (for example, the hippocampus, prefrontal cortex, and amygdala). This trafficking involves a stress-induced increase in chemokines, cell adhesion molecules (for example, intracellular CAM and vesicular CAM), and IL-1R1 signaling on endothelia[49,57]. Moreover, these monocytes have a pro-inflammatory, or primed, profile, with surface expression of pathogen-associated molecular patterns (PAMPS), including toll-like receptor (TLR)-2 and TLR-4 (ref. [58]), CCR2, and Ly6C. They also have increased mRNA expression

of *Il1b*, *Myd88*, *Cd14*, *Mmp8*, and *Stat3* (refs. [33,59]). Additionally, these inflammatory monocytes are more resistant to the anti-inflammatory regulation by glucocorticoids. This resistance results in a higher production and release of pro-inflammatory cytokines when the cell is activated. Indeed, monocytic priming may be influenced by the presence of the pro-inflammatory cytokine IL-6 in circulation after RSD[34,59–61]. For example, monocytes from IL-6-deficient mice that accumulated in the brain after RSD did not have this pro-inflammatory mRNA profile, and the mice did not develop anxiety-like behavior[59]. Taken together, these findings indicate that monocytes released into circulation with RSD are inflammatory and are influenced by high levels of glucocorticoids and IL-6.

Coinciding with the peripheral immune responses to RSD, there is central immune activation within the brain[30] and spinal cord[62]. In these tissues, microglial activation after RSD increases the expression of cytokines and chemokines[33]. For example, microglia increased expression of CCL2 after RSD. CCL2 is a chemokine involved in the recruitment of $CCR2^{+}$ monocytes to the brain[46]. RSD also induces a reactive endothelium through the increased expression of cell adhesion molecules (selectins and integrins) in fear- and threat-appraisal regions of the brain[49]. The consequence of this endothelial and microglial activation is the accumulation of inflammatory monocytes in the brain[33]. When microglia are inhibited by minocycline, a tetracycline antibiotic that inhibits the NF-κB pathway, or are eliminated by the CSF1R antagonist PLX5622 prior to RSD, the recruitment of monocytes to the brain is blocked[33]. Furthermore, the accumulation of peripheral $Ly6C^{hi}$ monocytes following RSD promotes a leukocyte ‘transcriptional fingerprint’ or profile similar to those in stressed or anxious individuals. Specific similarities included increased gene expression of pro-inflammatory and immune-activation genes (for example *TNF*, *MMP9*, *SOD2*, *CD163*)[14–16,22,59]. The inflammatory monocytes released by RSD are actively recruited to the brain by microglia, a key process that results in prolonged anxiety-like behavior[46]. Specifically, increased IL-1 signaling by these peripheral monocytes promotes anxiety. Caspase-1 is required for the post-translational processing of IL-1β into a mature and active protein. Monocytes with caspase-1 deficiency still accumulate in the brain after RSD, but without monocyte production of active IL-1β, there is no promotion of anxiety[33]. Taken together, these findings indicate that monocytes promote a robust IL-1 inflammatory signal in the brain in response to RSD that augments neuroinflammation and prolongs anxiety.

IL-1β production by inflammatory monocytes is critical in blood-to-brain signaling during the response to RSD. Brain endothelia are one target of IL-1 signaling with RSD. For instance, knockdown of endothelial IL-1R1 resulted in attenuation of RSD-induced anxiety-like behavior and reduction of neuroinflammation[57]. Thus, IL-1R1 on endothelia may play a critical role in modulating stress and augmenting the signal between the immune system (monocytes) and brain endothelia. Another cell type that highly expresses IL-1R1 is neurons[63,64]. A recent study using a modified version of RSD showed that IL-1R1 on excitatory $VGLUT2^{+}$ neurons in the hippocampus is critical for both neuronal sensitization and neuronal-driven behavioral deficits after RSD[50]. More specifically, $IL\text{-}1R1^{KO}$ in glutamatergic neurons ($VGLUT2^{+}IL\text{-}1R1^{KO}$) abrogated the stress-induced deficits in social interaction (with a juvenile) and in hippocampal-dependent working memory (Y-Maze)[50]. $VGLUT2^{+}IL\text{-}1R1^{KO}$, however, did not affect monocyte release or accumulation of

inflammatory monocytes in the brain. Therefore, blocking IL-1β signaling selectively within VGLUT2^{+} neurons ameliorated RSD-induced cognitive and social-interaction deficits.

There are numerous models of stress, including chronic variable stress, single prolonged stress, restraint stress, predator stress, and tail shock stress. These models promote anxiety-like behavior in rodents[65–68]. In addition, chronic mild stressors lead to changes in microglial activation and differences in stress susceptibility. For instance, increased susceptibility in mice after 3 weeks of chronic mild stress was associated with microglia with low expression of arginase 1, an enzyme involved in maintaining neurogenesis[69]. One key difference in these models is that these stressors do not have the same robust output to the immune system as that which results from RSD. RSD leads to activation in both the central nervous system and peripheral immune system and is key in the development of stress sensitization.

Social defeat and sensitization

A unique component of RSD is the development of stress sensitization, which persists weeks after cessation of the initial exposure to social stress. In this dynamic, there is a recurrence of anxiety-like behavior with exposure to acute stress at 24 days after stress sensitization (Fig. 3). This acute stress was considered a subthreshold stressor because it had no significant effect on immunity or behavior in non-sensitized mice[38]. Subthreshold stress 24 days after RSD re-establish the release of inflammatory monocytes and monocyte recruitment to the brain, augment neuroinflammation (by release of IL-1β), and cause recurrence of anxiety[38,70]. Moreover, subthreshold stress increases social avoidance in RSD-sensitized mice compared with controls. One difference with the recurrence of anxiety with stress sensitization is that the inflammatory monocytes derive from the spleen, not the bone marrow[71]. Discerning mechanisms of stress sensitization with social stress is clinically relevant and critical in understanding conditions related to PTSD. For instance, several clinical studies indicate that humans become sensitized to stress over time, such that minor stressors trigger complications[72]. Chronic or traumatic stressors may cause individuals to become more susceptible to developing psychiatric illnesses[73,74]. Indeed, individuals who experienced more childhood adversities have more depressive reactions to low levels of stress than do those who experienced fewer adversities[75]. Thus, negative early life events and adverse environments may contribute to stress sensitization. Collectively, these studies indicate that stress sensitization in mice parallels components of PTSD.

One month after RSD, several changes in behavior and physiology returned to baseline levels. For instance, RSD-associated monocyte accumulation in the brain, bone marrow myelopoiesis, splenomegaly, and increased circulating cytokine levels resolved 24 days after RSD[38,70,76]. The RSD-induced anxiety-like behavior and mild cognitive impairment also returned to baseline[32]. Nonetheless, some differences persisted 24 days after RSD, and these likely provide insight into the mechanisms of stress sensitization. Three key cellular components (neurons, microglia, and splenic monocytes) provide evidence of the influence of RSD on the immune system that may underlie stress sensitization.

Stress sensitization of neurons

Neurons are predicted to have a central role in stress sensitization and corresponding long-term effects on behavior and cognition. As mentioned above, an initial response to RSD is neuronal activation within the fear- and threat-appraisal centers of the brain (for example, the prefrontal cortex, hippocampus, and amygdala)[33,46]. Parallel to this idea, individuals with PTSD have abnormal neuronal functioning associated with a hyper-reactive amygdala and a reduction in the size of the hippocampus[77,78]. Abnormal functioning within these two regions may enhance adrenergic signaling and cause memory impairment. In some studies, propranolol, a beta-2 adrenergic antagonist, improved memory recall and retention in individuals with PTSD[79]. Nonetheless, PTSD is complex, and not all reports show benefits of propranolol[80]. RSD in mice promotes altered neuronal function that points to the sensitization of neurons[70]. Therefore, an understanding of neuronal activation is necessary to understanding long-term changes associated with stress sensitization.

There are a few behaviors that persisted longer term after RSD. For instance, there was prolonged social avoidance of an aggressor mouse after RSD. This behavior developed after one cycle of RSD[38]. Similarly, there was increased neuronal activation in fear- and threat-appraisal regions after one cycle of social defeat[30]. Social avoidance is primarily neuronal-activity-dependent. Along with the rapid induction of social avoidance, this behavior was unaffected by interventions that either blocked monocyte accumulation in the brain or inhibited microglia activation[33]. Consistent with neuronal sensitization and social avoidance, the interpretation of fear was enhanced after RSD, and this response persisted[48]. For example, mice had enhanced contextual fear memory (that is, memories associated with aversive stimuli), compared with that of controls, for 7 days after RSD. Fear memory in the stress-sensitized mice was blocked by endocannabinoid intervention[48]. There is also more direct evidence of sensitization of neurons within the threat-appraisal centers after RSD sensitization. For instance, acute defeat 24 days after RSD caused robust phosphorylation of the transcription factor CREB (p-CREB) and increased activity in hippocampal neurons of stress-sensitized mice compared with the control acute-stress group. Thus, there is increased neuronal reactivity to threatening stimuli following stress sensitization[70]. Additionally, this reactivity is relevant because increased expression of p-CREB is implicated in learning-induced synaptic plasticity[81]. Taken together, these results indicate that stress-sensitized mice are socially avoidant, which is associated with increased neuronal reactivity.

Stress sensitization of microglia

Microglia are sensitive to the long-term effects of injury, age, and trauma or psychological stress[70,82,83]. One reason for this is that microglia have a relatively low turnover rate compared with other myeloid cell populations[84,85]. Priming is used to describe immune changes in microglia that equate to an increased readiness to respond to an innate immune challenge[33]. For example, primed profiles of microglia with stress, injury, or age conferred hyper-reactivity to peripheral immune challenges with liposaccharide (LPS)[83,86–89]. Functionally, these primed microglia are activated and produce higher levels of inflammatory cytokines for a longer duration[51,86,89]. There is evidence of priming or sensitization of microglia 24 days after RSD[70]. Taken together, microglia are likely a critical component to the development of stress sensitization.

Resident microglia are important in the initial central immune responses to RSD and other preclinical stress paradigms in mice. For example, in mice subjected to restraint stress, a purinergic receptor antagonist inhibited microglial activation and decreased expression of IL-1β[90]. In RSD, there is region-dependent activation of microglia (for example, Iba-1, morphological restructuring), production of chemokines, and recruitment of monocytes to the brain vasculature[30,46]. Moreover, the de-ramified Iba-1 morphology was region-specific after RSD and paralleled the regions with neuronal activation. With stress sensitization (24 days after RSD), some structural differences in microglia remained in the prefrontal cortex but returned to baseline in the hippocampus and amygdala[38]. The evidence of microglia priming 24 days after stress sensitization was more apparent with RNA profiling and functional responses. For instance, microglia that underwent fluorescence-activated cell sorting maintained a unique mRNA signature 24 days after RSD with 137 differentially expressed genes. Pathway analysis showed that upstream regulators increased in microglia 24 days after stress sensitization, including genes encoding IL-1β, MyD88, TLR-4, and IFN-γ. In addition, isolated microglia from stress-sensitized mice expressed genes associated with PAMPS (*Tlr2* and *Tlr4*) and innate immunity (*Cd14, Cd22, Cd68*)[38,70]. Functionally, RSD increased microglial priming (higher expression of *Cd14, Tlr4, Il6*) and these cells were hyper-reactive to LPS challenge both ex vivo and in vivo[70] (Fig. 4). Enriched microglia had higher levels of *Il1b*, *Il6*, and *Tnf* when cultured ex vivo 24 days after RSD[51]. When these isolated microglia from RSD mice were cultured ex vivo 24 days after stress sensitization, they were more reactive to LPS and had amplified expression of *Cd14*, *Il6* and *Il1b* compared with controls[70]. Following an intraperitoneal injection of LPS, there was robust microglial reactivity in stress-sensitized mice that was associated with prolonged sickness behavior[70]. These data support the idea of microglia priming or sensitization weeks after the initial exposure to RSD.

A critical question is whether microglia underlie maintenance of stress sensitization and the recurrence of anxiety with acute stress. One strategy to explore priming after RSD is to either eliminate[33] or force the turnover of microglia[70]. Elimination of microglia using the CSF1R antagonist PLX5622 prior to RSD prevented monocyte accumulation in the brain and blocked anxiety recurrence following acute defeat (24 days). Next, microglia were eliminated prior to RSD and then allowed to repopulate prior to acute defeat at 24 days. This repopulation, however, did not affect the acute stress response in stress-sensitized mice. There was still acute-defeat-induced monocyte release, monocyte accumulation in the brain, and anxiety-like behavior in stress-sensitized mice. Thus, the splenic release of monocytes in stress-sensitized mice exposed to acute stress was independent of microglial priming and corresponded with increased monocyte accumulation in the brain. Stress reactivity to acute defeat remained when microglia were eliminated and repopulated after RSD. Overall, stress sensitization to acute defeat depends on microglia being present at time of acute defeat, but does not depend on microglial priming.

To further investigate microglial sensitization after RSD, depleted and repopulated microglia were cultured ex vivo and treated with LPS to induce inflammatory gene expression. Similar to the data above, microglia were eliminated prior to RSD (PLX5622) and then allowed to repopulate prior to acute defeat at 24 days. This microglial elimination and repopulation prevented amplified immune reactivity ex vivo and in vivo in stress-sensitized mice. Immune

reactivity of microglia to LPS (ex vivo and in vivo) was prevented when microglia were eliminated and repopulated after RSD. Thus, there was mRNA and functional evidence that microglia remained 'primed' or 'sensitized' to innate immune challenge weeks after RSD[70]. Additionally, repopulated, non-sensitized microglia were all that was necessary to recall these immune and behavioral responses in stress-sensitized mice. Thus, stress sensitization is a complex process in which microglia play a role in the recurrence of anxiety with acute defeat and are essential for the increased reactivity to immune challenge.

Stress sensitization of splenic monocytes

Another important compartment for sensitization after RSD is the spleen. Following primary exposure to RSD, monocytes are released from the bone marrow into circulation and traffic to several peripheral tissues, including the spleen. In fact, the spleen doubles in size after RSD[91]. This stress-induced enlargement in the spleen is caused by extramedullary hematopoiesis and the production of red blood cells[76]. Reduced production of red blood cells in the bone marrow after RSD appears to be compensated by the spleen-dependent enhancement of red blood cell production. Moreover, RSD induced the mobilization of stem progenitor cells from the bone marrow (β-adrenergic dependent) that enter circulation, engraft into the spleen, and establish a persistent extramedullary hematopoietic depot[38,71]. This response results in a unique pool of monocytes in the spleen after RSD.

Splenic production of monocytes ($CD11b^{+}$) persisted for 24 days after the cessation of social stress. These splenic data are relevant because there is a link between extramedullary hematopoiesis in the generation of inflammatory monocytes in atherosclerosis, myocardial infarction, and infection[92–98]. Notably, these $Ly6C^{hi}$ monocytes generated within the spleen are inflammatory. For instance, splenocytes cultured 24 days after RSD produced higher levels of IL-6 (ref. [71]). These data support the idea of priming within the splenic immune compartment.

The key here is that acute stress caused the release of monocytes from the spleen. For example, increased availability of releasable $Ly6C^{hi}$ monocytes 24 days after RSD is related to generation of monocytes within the spleen[71]. Although the spleen is not involved in the initial anxiety response to RSD[71], it is critical in stress sensitization and the recurrence of anxiety with acute stress[38,71]. Acute stress increased trafficking of $Ly6C^{hi}$ monocytes from the spleen to the brain in stress-sensitized mice[38,71]. Furthermore, removal of the spleen (by splenectomy) blocked the acute-stress-induced recurrence of anxiety[33,99]. Splenectomy, however, had no effect on monocyte accumulation or anxiety when determined 14 hours after primary exposure to RSD[71]. Thus, the spleen is a unique reservoir for maintaining progenitor myeloid cells and inflammatory monocytes in sensitized mice that are readily releasable into circulation after acute stress exposure[71]. Sympathetic release of splenic monocytes promotes recurring anxiety following RSD and is an important component of stress sensitization.

Conclusions

The pathophysiology of psychiatric disorders is complex, with myriad mechanisms contributing to the intricacy of determining how an individual will adapt or not adapt to

stress. RSD promotes stress sensitization, in which mice are highly sensitive to subthreshold stress and recurrence of anxiety weeks after the cessation of stress. Nonetheless, several important questions remain. For instance, the bidirectional communication between microglia and neurons with stress sensitization is unclear. IL-1R1 specifically on glutamatergic neurons may play a critical role in stress sensitization. Changes to IL-1R1 may account for altered neuronal reactivity and activation and behavioral changes following stress sensitization. Understanding these interactions may lead to more targeted therapeutics for individuals with long-lasting anxiety disorders, like PTSD. Therefore, understanding this multifactorial nature of stress sensitization could lead to a more strategic approach to helping individuals with psychiatric disorders.

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References

1. Haroon E, Raison CL & Miller AH Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 37, 137–162 (2012). [PubMed: 21918508]
2. Koo JW & Duman RS IL-1β is an essential mediator of the antineurogenic and anhedonic effects of stress. Proc. Natl Acad. Sci. USA 105, 751–756 (2008). [PubMed: 18178625]
3. Raison CL, Capuron L & Miller AH Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 27, 24–31 (2006). [PubMed: 16316783]
4. Dantzer R, O'Connor JC, Freund GG, Johnson RW & Kelley KW From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9, 46–56 (2008). [PubMed: 18073775]
5. Pace TW & Heim CM A short review on the psychoneuroimmunology of posttraumatic stress disorder: from risk factors to medical comorbidities. Brain Behav. Immun. 25, 6–13 (2012).
6. Stoddard FJ et al. Young burned children: the course of acute stress and physiological and behavioral responses. Am. J. Psychiatry 163, 1084–1090 (2006). [PubMed: 16741210]
7. Torres-Platas SG, Cruceanu C, Chen GG, Turecki G & Mechawar N Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides. Brain Behav. Immun. 42, 50–59 (2014). [PubMed: 24858659]
8. Schnieder TP et al. Microglia of prefrontal white matter in suicide. J. Neuropathol. Exp. Neurol. 73, 880–890 (2014). [PubMed: 25101704]
9. Jonker I, Rosmalen JGM & Schoevers RA Childhood life events, immune activation and the development of mood and anxiety disorders: the TRAILS study. Transl. Psychiatry 7, e1112 (2017). [PubMed: 28463238]
10. Köhler CA et al. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. Acta Psychiatr. Scandinavica 135, 373–387 (2017).
11. Wang Z, Mandel H, Levingston CA & Young MRI An exploratory approach demonstrating immune skewing and a loss of coordination among cytokines in plasma and saliva of veterans with combat-related PTSD. Hum. Immunol. 77, 652–657 (2016). [PubMed: 27216157]
12. Passos IC et al. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. Lancet Psychiatry 2, 1002–1012 (2015). [PubMed: 26544749]
13. Miller GE et al. Greater inflammatory activity and blunted glucocorticoid signaling in monocytes of chronically stressed caregivers. Brain Behav. Immun. 41, 191–199 (2014). [PubMed: 25242587]
14. Powell ND et al. Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via beta-adrenergic induction of myelopoiesis. Proc. Natl Acad. Sci. USA 110, 16574–16579 (2013). [PubMed: 24062448]

15. Cole SW, Hawkley LC, Arevalo JM & Cacioppo JT Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes. Proc. Natl Acad. Sci. USA 108, 3080–3085 (2011). [PubMed: 21300872]
16. Miller GE et al. A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-κB signaling. Biol. Psychiatry 64, 266–272 (2008). [PubMed: 18440494]
17. Lindqvist D et al. Increased circulating blood cell counts in combat-related PTSD: associations with inflammation and PTSD severity. Psychiatry Res 258, 330–336 (2017). [PubMed: 28942957]
18. Beumer W et al. The immune theory of psychiatric diseases: a key role for activated microglia and circulating monocytes. J. Leukoc. Biol. 92, 959–975 (2012). [PubMed: 22875882]
19. Pace TW et al. Increased peripheral NF-κB pathway activity in women with childhood abuse-related posttraumatic stress disorder. Brain Behav. Immun. 26, 13–17 (2011). [PubMed: 21801830]
20. Gola H et al. Posttraumatic stress disorder is associated with an enhanced spontaneous production of pro-inflammatory cytokines by peripheral blood mononuclear cells. BMC Psychiatry 13, 40 (2013). [PubMed: 23360282]
21. Carvalho LA et al. Inflammatory activation is associated with a reduced glucocorticoid receptor alpha/beta expression ratio in monocytes of inpatients with melancholic major depressive disorder. Transl. Psychiatry 4, e344 (2014). [PubMed: 24424390]
22. Cole SW et al. Social regulation of gene expression in human leukocytes. Genome Biol. 8, R189 (2007). [PubMed: 17854483]
23. Katrinli S et al. Association of HLA locus alleles with posttraumatic stress disorder. Brain Behav. Immun. 81, 655–658 (2019). [PubMed: 31310798]
24. Deri Y et al. Neuroinflammation in World Trade Center responders at midlife: a pilot study using [^{18}F]-FEPPA PET imaging. Brain Behav. Immun. Health 16, 100287 (2021). [PubMed: 34589784]
25. Setiawan E et al. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. JAMA Psychiatry 72, 268–275 (2015). [PubMed: 25629589]
26. Mondelli V, Vernon AC, Turkheimer F, Dazzan P & Pariante CM Brain microglia in psychiatric disorders. Lancet Psychiatry 4, 563–572 (2017). [PubMed: 28454915]
27. Réus GZ et al. The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. Neuroscience 300, 141–154 (2015). [PubMed: 25981208]
28. Bhatt S et al. PTSD is associated with neuroimmune suppression: evidence from PET imaging and postmortem transcriptomic studies. Nat. Commun. 11, 2360 (2020). [PubMed: 32398677]
29. Hall C & Ballachey EL A study of the rat's behavior in a field. A contribution to method in comparative psychology. University of California Publications in Psychology 6, 1–12 (1932).
30. Wohleb ES et al. β-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. J. Neurosci. 31, 6277–6288 (2011). [PubMed: 21525267]
31. Sawicki CM et al. Ropivacaine and bupivacaine prevent increased pain sensitivity without altering neuroimmune activation following repeated social defeat stress. Brain Behav. Immun. 69, 113–123 (2018). [PubMed: 29126979]
32. McKim DB et al. Neuroinflammatory dynamics underlie memory impairments after repeated social defeat. J. Neurosci. 36, 2590–2604 (2016). [PubMed: 26937001]
33. McKim DB et al. Microglial recruitment of IL-1β-producing monocytes to brain endothelium causes stress-induced anxiety. Mol. Psychiatry 23, 1421–1431 (2018). [PubMed: 28373688]
34. Hodes GE et al. Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proc. Natl Acad. Sci. USA 111, 16136–16141 (2014). [PubMed: 25331895]
35. Krishnan V et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131, 391–404 (2007). [PubMed: 17956738]
36. Bergamini G et al. Chronic social stress induces peripheral and central immune activation, blunted mesolimbic dopamine function, and reduced reward-directed behaviour in mice. Neurobiol. Stress 8, 42–56 (2018). [PubMed: 29888303]

37. Berton O et al. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311, 864–868 (2006). [PubMed: 16469931]
38. Wohleb ES et al. Re-establishment of anxiety in stress-sensitized mice is caused by monocyte trafficking from the spleen to the brain. Biol. Psychiatry 75, 970–981 (2014). [PubMed: 24439304]
39. Shimamoto A Social defeat stress, sex, and addiction-like behaviors. Int. Rev. Neurobiol. 140, 271–313 (2018). [PubMed: 30193707]
40. Golden SA, Covington HE, Berton O & Russo SJ A standardized protocol for repeated social defeat stress in mice. Nat. Protoc. 6, 1183–1191 (2011). [PubMed: 21799487]
41. Russo SJ, Murrough JW, Han MH, Charney DS & Nestler EJ Neurobiology of resilience. Nat. Neurosci. 15, 1475–1484 (2012). [PubMed: 23064380]
42. Takahashi A et al. Establishment of a repeated social defeat stress model in female mice. Sci. Rep. 7, 12838 (2017). [PubMed: 28993631]
43. Menard C et al. Social stress induces neurovascular pathology promoting depression. Nat. Neurosci. 20, 1752–1760 (2017). [PubMed: 29184215]
44. Tsyglakova M, McDaniel D & Hodes GE Immune mechanisms of stress susceptibility and resilience: lessons from animal models. Front. Neuroendocrinol. 54, 100771 (2019). [PubMed: 31325456]
45. Anacker C et al. Neuroanatomic differences associated with stress susceptibility and resilience. Biol. Psychiatry 79, 840–849 (2016). [PubMed: 26422005]
46. Wohleb ES, Powell ND, Godbout JP & Sheridan JF Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior. J. Neurosci. 33, 13820–13833 (2013). [PubMed: 23966702]
47. Christoffel DJ et al. IκB kinase regulates social defeat stress-induced synaptic and behavioral plasticity. J. Neurosci. 31, 314–321 (2011). [PubMed: 21209217]
48. Lisboa SF et al. Repeated social defeat-induced neuroinflammation, anxiety-like behavior and resistance to fear extinction were attenuated by the cannabinoid receptor agonist WIN55,212–2. Neuropsychopharmacology 43, 1924–1933 (2018). [PubMed: 29786066]
49. Sawicki CM et al. Social defeat promotes a reactive endothelium in a brain region-dependent manner with increased expression of key adhesion molecules, selectins and chemokines associated with the recruitment of myeloid cells to the brain. Neuroscience 302, 151–164 (2015). [PubMed: 25445193]
50. DiSabato DJ et al. Interleukin-1 receptor on hippocampal neurons drives social withdrawal and cognitive deficits after chronic social stress. Mol. Psychiatry 26, 4770–4782 (2021). [PubMed: 32444870]
51. Ramirez K, Shea DT, McKim DB, Reader BF & Sheridan JF Imipramine attenuates neuroinflammatory signaling and reverses stress-induced social avoidance. Brain Behav. Immun. 46, 212–220 (2015). [PubMed: 25701613]
52. Ramirez K, Niraula A & Sheridan JF GABAergic modulation with classical benzodiazepines prevent stress-induced neuro-immune dysregulation and behavioral alterations. Brain Behav. Immun. 51, 154–168 (2016). [PubMed: 26342944]
53. Bellinger DL et al. Sympathetic modulation of immunity: relevance to disease. Cell Immunol. 252, 27–56 (2008). [PubMed: 18308299]
54. Hanke ML, Powell ND, Stiner LM, Bailey MT & Sheridan JF β-adrenergic blockade decreases the immunomodulatory effects of social disruption stress. Brain Behav. Immun. 26, 1150–1159 (2012). [PubMed: 22841997]
55. Bellavance MA & Rivest S The HPA–immune axis and the immunomodulatory actions of glucocorticoids in the brain. Front Immunol. 5, 136 (2014). [PubMed: 24744759]
56. Niraula A, Wang Y, Godbout JP & Sheridan JF Corticosterone production during repeated social defeat causes monocyte mobilization from the bone marrow, glucocorticoid resistance, and neurovascular adhesion molecule expression. J. Neurosci. 38, 2328–2340 (2018). [PubMed: 29382712]
57. Wohleb ES et al. Knockdown of interleukin-1 receptor type-1 on endothelial cells attenuated stress-induced neuroinflammation and prevented anxiety-like behavior. J. Neurosci. 34, 2583–2591 (2014). [PubMed: 24523548]

58. Bailey MT, Engler H, Powell ND, Padgett DA & Sheridan JF Repeated social defeat increases the bactericidal activity of splenic macrophages through a Toll-like receptor-dependent pathway. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, R1180–R1190 (2007). [PubMed: 17596326]
59. Niraula A, Witcher KG, Sheridan JF & Godbout JP Interleukin-6 induced by social stress promotes a unique transcriptional signature in the monocytes that facilitate anxiety. Biol. Psychiatry 85, 679–689 (2019). [PubMed: 30447911]
60. Voorhees JL et al. Prolonged restraint stress increases IL-6, reduces IL-10, and causes persistent depressive-like behavior that is reversed by recombinant IL-10. PLoS ONE 8, e58488 (2013). [PubMed: 23520517]
61. Zhou D, Kusnecov AW, Shurin MR, DePaoli M & Rabin BS Exposure to physical and psychological stressors elevates plasma interleukin 6: relationship to the activation of hypothalamic–pituitary–adrenal axis. Endocrinology 133, 2523–2530 (1993). [PubMed: 8243274]
62. Sawicki CM et al. Microglia promote increased pain behavior through enhanced inflammation in the spinal cord during repeated social defeat stress. J. Neurosci. 39, 1139–1149 (2019). [PubMed: 30559153]
63. Liu X et al. Cell-type-specific interleukin 1 receptor 1 signaling in the brain regulates distinct neuroimmune activities. Immunity 50, 317–333 (2019). [PubMed: 30683620]
64. Takahashi A et al. Neuromodulatory effect of interleukin 1β in the dorsal raphe nucleus on individual differences in aggression. Mol. Psychiatry 27, 2563–2579 (2021). [PubMed: 33931727]
65. Guo YY et al. Scutellarin ameliorates the stress-induced anxiety-like behaviors in mice by regulating neurotransmitters. Phytother. Res. 35, 3936–3944 (2021). [PubMed: 33856723]
66. Wu YP et al. Predator stress-induced depression is associated with inhibition of hippocampal neurogenesis in adult male mice. Neural Regen. Res. 14, 298–305 (2019). [PubMed: 30531013]
67. Poulos AM et al. Amnesia for early life stress does not preclude the adult development of posttraumatic stress disorder symptoms in rats. Biol. Psychiatry 76, 306–314 (2014). [PubMed: 24231200]
68. Nahvi RJ, Nwokafor C, Serova LI & Sabban EL Single prolonged stress as a prospective model for posttraumatic stress disorder in females. Front Behav. Neurosci. 13, 17 (2019). [PubMed: 30804766]
69. Zhang J et al. IL4-driven microglia modulate stress resilience through BDNF-dependent neurogenesis. Sci. Adv. 7, abb9888 (2021).
70. Weber MD et al. The influence of microglial elimination and repopulation on stress sensitization induced by repeated social defeat. Biol. Psychiatry 85, 667–678 (2019). [PubMed: 30527629]
71. McKim DB et al. Sympathetic release of splenic monocytes promotes recurring anxiety following repeated social defeat. Biol. Psychiatry 79, 803–813 (2016). [PubMed: 26281717]
72. Harkness KL, Hayden EP & Lopez-Duran NL Stress sensitivity and stress sensitization in psychopathology: an introduction to the special section. J. Abnorm. Psychol. 124, 1–3 (2015). [PubMed: 25688427]
73. Bandoli G et al. Childhood adversity, adult stress, and the risk of major depression or generalized anxiety disorder in US soldiers: a test of the stress sensitization hypothesis. Psychol. Med. 47, 2379–2392 (2017). [PubMed: 28443533]
74. Park YM, Shekhtman T & Kelsoe JR Effect of the type and number of adverse childhood experiences and the timing of adverse experiences on clinical outcomes in individuals with bipolar disorder. Brain Sci. 10, 254 (2020). [PubMed: 32349367]
75. Hammen C, Henry R & Daley SE Depression and sensitization to stressors among young women as a function of childhood adversity. J. Consult Clin. Psychol. 68, 782–787 (2000). [PubMed: 11068964]
76. McKim DB et al. Social stress mobilizes hematopoietic stem cells to establish persistent splenic myelopoiesis. Cell Rep. 25, 2552–2562 (2018). [PubMed: 30485819]
77. Logue MW et al. Smaller hippocampal volume in posttraumatic stress disorder: a multisite ENIGMA-PGC study: subcortical volumetry results from posttraumatic stress disorder consortia. Biol. Psychiatry 83, 244–253 (2018). [PubMed: 29217296]
78. Badura-Brack A et al. Veterans with PTSD demonstrate amygdala hyperactivity while viewing threatening faces: a MEG study. Biol. Psychol. 132, 228–232 (2018). [PubMed: 29309826]

79. Vaiva G et al. Immediate treatment with propranolol decreases posttraumatic stress disorder two months after trauma. Biol. Psychiatry 54, 947–949 (2003). [PubMed: 14573324]
80. Raut SB et al. Effects of propranolol on the modification of trauma memory reconsolidation in PTSD patients: a systematic review and meta-analysis. J. Psychiatr. Res 150, 246–256 (2022). [PubMed: 35405409]
81. Gandolfi D et al. Activation of the CREB/c-Fos pathway during long-term synaptic plasticity in the cerebellum granular layer. Front Cell Neurosci. 11, 184 (2017). [PubMed: 28701927]
82. Niraula A, Sheridan JF & Godbout JP Microglia priming with aging and stress. Neuropsychopharmacology 42, 318–333 (2017). [PubMed: 27604565]
83. Fenn AM et al. Immune activation promotes depression 1 month after diffuse brain injury: a role for primed microglia. Biol. Psychiatry 76, 575–584 (2014). [PubMed: 24289885]
84. Réu P et al. The lifespan and turnover of microglia in the human brain. Cell Rep. 20, 779–784 (2017). [PubMed: 28746864]
85. Ajami B, Bennett JL, Krieger C, Tetzlaff W & Rossi FM Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat. Neurosci. 10, 1538–1543 (2007). [PubMed: 18026097]
86. Godbout JP et al. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. FASEB J. 19, 1329–1331 (2005). [PubMed: 15919760]
87. Godbout JP et al. Aging exacerbates depressive-like behavior in mice in response to activation of the peripheral innate immune system. Neuropsychopharmacology 33, 2341–2351 (2008). [PubMed: 18075491]
88. Norden DM, Trojanowski PJ, Villanueva E, Navarro E & Godbout JP Sequential activation of microglia and astrocyte cytokine expression precedes increased Iba-1 or GFAP immunoreactivity following systemic immune challenge. Glia 64, 300–316 (2016). [PubMed: 26470014]
89. Wohleb ES et al. Peripheral innate immune challenge exaggerated microglia activation, increased the number of inflammatory CNS macrophages, and prolonged social withdrawal in socially defeated mice. Psychoneuroendocrinology 37, 1491–1505 (2012). [PubMed: 22386198]
90. Iwata M et al. Psychological stress activates the inflammasome via release of adenosine triphosphate and stimulation of the purinergic type 2X7 receptor. Biol. Psychiatry 80, 12–22 (2016). [PubMed: 26831917]
91. Avitsur R, Stark JL, Dhabhar FS, Padgett DA & Sheridan JF Social disruption-induced glucocorticoid resistance: kinetics and site specificity. J. Neuroimmunol. 124, 54–61 (2002). [PubMed: 11958822]
92. Dutta P et al. Myocardial infarction accelerates atherosclerosis. Nature 487, 325–329 (2012). [PubMed: 22763456]
93. Shand FH et al. Tracking of intertissue migration reveals the origins of tumor-infiltrating monocytes. Proc. Natl Acad. Sci. USA 111, 7771–7776 (2014). [PubMed: 24825888]
94. Leuschner F et al. Rapid monocyte kinetics in acute myocardial infarction are sustained by extramedullary monocytopoiesis. J. Exp. Med. 209, 123–137 (2012). [PubMed: 22213805]
95. Weber GF et al. Interleukin-3 amplifies acute inflammation and is a potential therapeutic target in sepsis. Science 347, 1260–1265 (2015). [PubMed: 25766237]
96. Rauch PJ et al. Innate response activator B cells protect against microbial sepsis. Science 335, 597–601 (2012). [PubMed: 22245738]
97. Robbins CS et al. Extramedullary hematopoiesis generates Ly-6C^{high} monocytes that infiltrate atherosclerotic lesions. Circulation 125, 364–U415 (2012). [PubMed: 22144566]
98. Swirski FK et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science 325, 612–616 (2009). [PubMed: 19644120]
99. Feng X et al. Microglia mediate postoperative hippocampal inflammation and cognitive decline in mice. JCI Insight 2, e91229 (2017). [PubMed: 28405620]

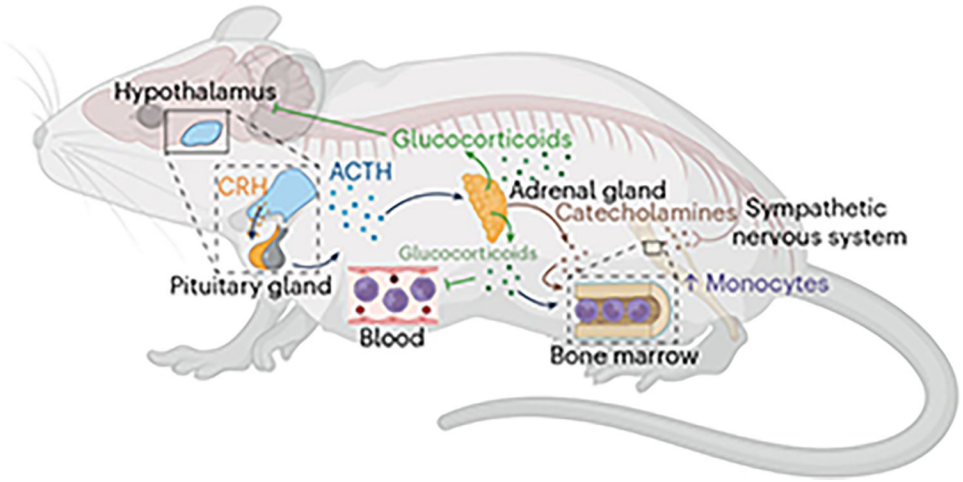

Fig. 1 |. Social defeat stress activates the hypothalamic pituitary adrenal axis and sympathetic nervous system to promote the release of monocytes into circulation.

Social defeat stress increases the release of corticotrophhin-releasing hormone (CRH) in the hypothalamus. The release of CRH prompts the pituitary gland to release adrenocorticotropic hormone (ACTH) into circulation. In turn, the adrenal glands respond and release both glucocorticoids and catecholamines. Glucocorticoids feed back to the hypothalamus to stop the production of CRH. Parallel to this, social defeat activates the SNS. SNS activation results in the release of catecholamines (for example, norepinephrine) that act directly on primary and secondary lymphoid tissues (for example, bone marrow). Both glucocorticoids and catecholamines converge to increase the production, maturation, and release of monocytes into circulation. Created with BioRender.com.

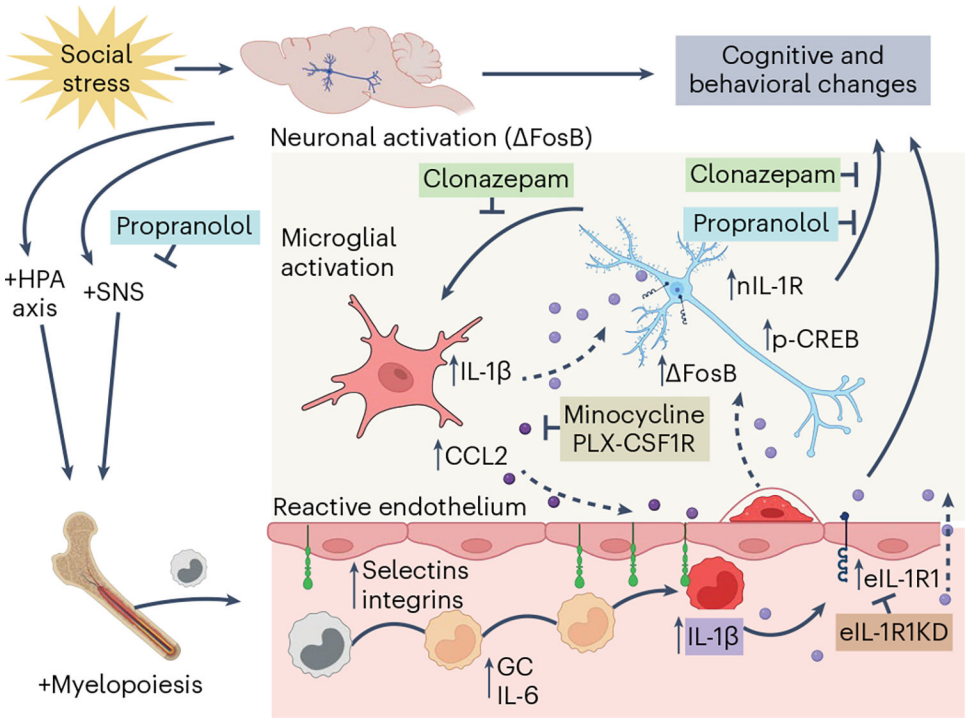

Fig. 2 |. Repeated social defeat induces neuronal activation that coordinates peripheral and central immune responses that influence behavior and cognition.

SDS increases neuronal activation (for example, ΔFosB and phosphorylation of cAMP-response element binding protein (p-CREB)) in the fear and threat areas of the brain and expression of neuronal interleukin-1 receptor-1 (nIL-1R1). This leads to activation of the SNS and HPA, which drives myelopoiesis within the bone marrow. As a result, monocytes are released into the circulation, where there are high concentrations of glucocorticoids (GC) and IL-6. These monocytes have an increased inflammatory profile and can traffic to tissues including the brain. Concomitant with this response is the activation of microglia and brain endothelia with RSD. A reactive endothelium includes increased cell adhesion molecules (selectins and integrins) and interleukin-1 receptor-1 (eIL-1R1). Microglia actively release chemokines (C–C motif ligand 2, CCL2) and cytokines (IL-1β). This microglia activation following RSD results in the increased recruitment of inflammatory monocytes to the brain vasculature and perivascular space. These monocytes provide IL-1β to the endothelia. This convergence of neuronal, endothelial, and microglial activation and monocyte recruitment contributes to cognitive and behavioral changes following stress (for example, anxiety, social avoidance, reduced working memory). This schematic also shows various interventions and their effect on this paradigm. Administration of propranolol, a beta-2 antagonist, prevents SNS activation, neuronal and microglial activation, recruitment of peripheral monocytes, and the development of anxiety-like behavior. Clonazepam, a benzodiazepine, blocks neuronal and microglial activation and social avoidance after social defeat. Additionally, minocycline and PLX5662, a colony stimulating factor-1 receptor (CSF1R) antagonist, prevents microglial activation and the development of anxiety following stress. Knocking down the IL-1R1 (eIL-1R1^{KD}) on endothelia reduces neuroinflammation and attenuates anxiety-like behavior following RSD. Created with BioRender.com.

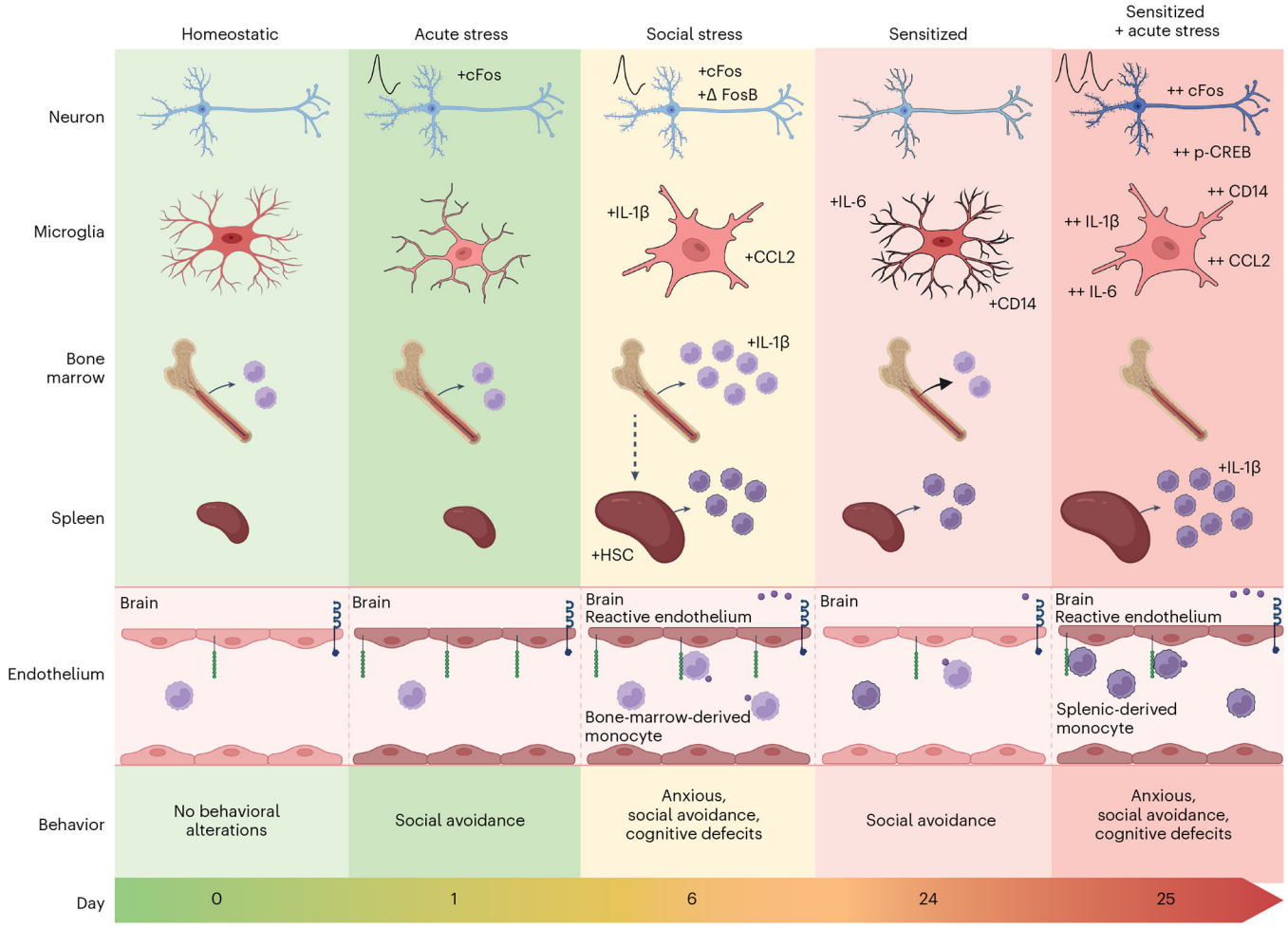

Fig. 3 |. Overview of repeated social defeat and stress sensitization of central and peripheral immune compartments.

The first box shows a 'homeostatic' state in the absence of stress. The second box shows acute stress with one cycle of social defeat (2 hours). This induced neuronal (cFos) activation in fear- and threat-appraisal regions and social avoidant behavior. In addition, microglia morphology in the amygdala was increased compared to controls. The third box shows RSD with six cycles of defeat. RSD promotes a reactive (+) endothelium, myelopoiesis in the BM, splenomegaly (HSCs), and neuronal (cFos, ΔFosB) and microglial (IL-1β and CCL2) activation. RSD also leads to behavioral deficits, including anxiety (open field, light dark), social avoidance, and cognitive deficits (Barnes maze, Morris water maze). After 24 days (fourth box), microglia remain in a primed pro-inflammatory state (IL-6 and CD14), and social avoidance behavior persists. The fifth box shows exacerbation (++) of immune responses and neuronal activation occur after an acute stress 24 days after the last cycle of RSD. The spleen becomes a reservoir for immune cells and releases monocytes into circulation following the acute defeat. These spleen-derived monocytes traffic to the reactive endothelium and can signal through IL-1R. Neurons show increased reactivity (increased p-CREB) and activation (cFos) in the fear- and threat-appraisal regions. Primed microglia are activated and release proinflammatory cytokines and chemokines (IL-1β, IL-6, CCL2).

Behavioral deficits, including anxiety (open field), social avoidance, and cognitive deficits (Y-maze), are evident after stress sensitization. Created with BioRender.com.

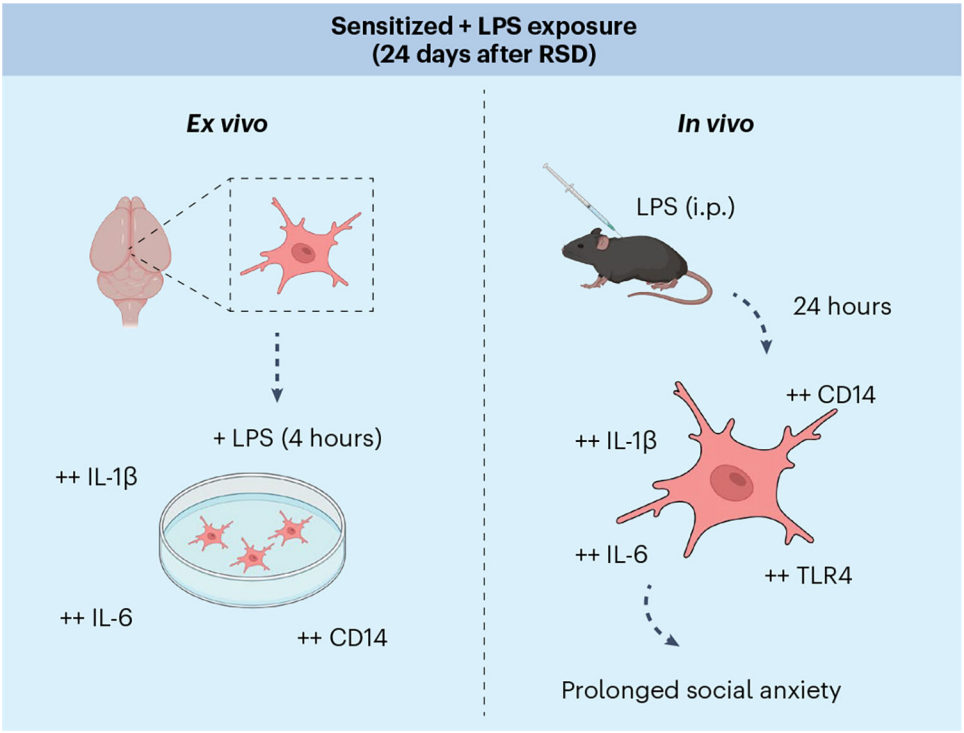

Fig. 4 |. Microglia are primed and more reactive to peripheral immune challenges after repeated social defeat.

This schematic shows the priming effect of social defeat stress on microglia. Left, microglia isolated (ex vivo) from RSD mice 24 days after the last cycle of RSD were stimulated with LPS for 4 hours. These microglia had increased pro-inflammatory gene expression compared with controls. In the right box, mice were injected with LPS 24 days after the last cycle of RSD. After 24 hours, microglia from LPS-injected RSD mice had exaggerated gene expression of pro-inflammatory (IL-1β) and pathogen-associated molecular patterns (TLR-4 and CD14) compared with microglia from saline-treated mice. Mice treated with LPS after stress had increased social anxiety in response to a juvenile and decreased exploratory behavior. Created with BioRender.com.