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Research report

# Cytokine and endocrine parameters in mouse chronic social defeat: Implications for translational 'cross-domain' modeling of stress-related brain disorders

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## HIGHLIGHTS

- C57BL/6J male mice were exposed to the chronic social defeat stress paradigm.
- Loser mice showed elevated serum pro-inflammatory cytokines IL-7 and VEGF.
- Winner mice showed elevated levels of an anti-inflammatory cytokine IL-10.
- IL-2, IL-4, IL-1a, MCP-1 and corticosterone levels were unaltered in all groups.
- Our results implicate selected cytokines in chronic social stress in mouse models.

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## ABSTRACT

Mounting clinical and experimental evidence implicates various cytokines in stress-related affective brain disorders. Here, we analyze behavioral phenotypes in C57BL/6J male mice following the chronic social defeat stress paradigm, and examine their serum cytokines and corticosterone levels. Loser mice experiencing 20 days of daily 15-min social confrontations demonstrate elevated levels of pro-inflammatory cytokines interleukin IL-7 and vascular endothelial growth factor (VEGF), as well as a trend to increase IL-6 and IL-15. We also found higher levels of an anti-inflammatory cytokine IL-10 in the winner mice, with unaltered serum IL-2, IL-4, IL-1a, MCP-1 and corticosterone levels between the groups. Overall, our results suggest that animal affective-like states correlate with specific cytokine profiles, including some cytokines (e.g., VEGF, IL-7 or IL-15) whose role in neuropsychiatric disorders is only beginning to emerge. This study emphasizes the importance of integrative analyses of neural and immune phenotypes in stress and stress-related neurobehavioral disorders. These findings may also help foster the search for new therapeutic and preventative strategies that target selected cytokines and their signaling pathways.



## 1. Introduction

Although affective pathogenesis is commonly triggered by various stressors [1,2], the exact biological mechanisms of common stress-related neurobehavioral disorders, such as anxiety and depression, remain unclear [3–6]. Emphasizing the importance of neuro-immune interplay in CNS pathogenesis [4,7–12], recent studies directly link affective disorders with various cytokines, including monocyte chemotactic protein-1 (MCP-1) [13–16], pro-inflammatory interleukins (IL) IL-1α, IL-1β and IL-6, tumor necrosis factor-α (TNF-α) [7,17–20] and vascular endothelial growth factor (VEGF) [16]. For example, circulating levels of IL-1, IL-6, VEGF and TNF-α correlate with post-traumatic stress, anxiety and depression symptoms in humans [3–7,10,16,21–24]. In animals, IL-1, IL-2, and IL-6 levels correlate with higher release and turnover of brain monoamines, as well as with "depressive-like" behavior [9,12,17,25]. Importantly, the peripheral and central cytokine systems often operate in parallel, representing a continuum of neuro-immune interactions, where peripheral stimuli

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can induce expression of brain inflammatory cytokines, and brain inflammation activates peripheral inflammatory cytokines [3,26]. Collectively, this suggests the potential of peripheral cytokines as translationally relevant biomarkers for stress responses mediated by both central and peripheral mechanisms.

In addition to neuro–immune interactions, the neuroendocrine pathways also interact with stress-activated cytokine systems [6,27–31]. For example, altered cytokine levels modulate the hypothalamic–pituitary–adrenal axis (HPA) [3,32,33], while glucocorticoid 'stress' hormones increase susceptibility to both inflammatory and behavioral symptoms of anxiety or depression [3,32–34]. Therefore, an in-depth assessment of both cytokine and endocrine markers of stress may be necessary in order to better understand the neurobiological mechanisms of affective pathogenesis.

Social factors play an important role in human stress-related affective disorders [23,35–37] and their neural and immune mechanisms [31,38]. Social stress is also widely used to model neuropsychiatric disorders in various experimental (animal) models [39–45]. For example, chronic social defeat stress models available for biopsychiatry research [39,46–48] typically involve social confrontations between two conspecifics, in which the winner (dominant) and the loser (subordinate) animals emerge at the end of the social interaction [37,41,49,50]. While acute social stress occurs after a single confrontation [46,51–54], chronic social stress requires an extended period of days or weeks [36,50,55,56]. Usually combined with chronic exposure to sensory stimuli from aggressive mice [47,49], this induces a variety of physiological and behavioral symptoms, ranging from anxiety to 'anhedonic' depression, immune deficits, and altered expression of key brain genes [55,57–62].

Analyzing this problem, here we applied the sensory contact model (as a widely accepted model of social defeat stress [49,63,64]) to examine the link between behavioral and selected immunological and endocrine correlates of chronic social stress in mice. Targeting both behavioral and physiological phenotypes, this approach is also consistent with the growing recognition of the spectrum nature of brain disorders and the importance of integrative multi-domain modeling of human brain disorders [65–67].

## 2. Methods

### 2.1. Animals and housing

Thirty adult male C57BL/6J mice used in this study were obtained from the Jackson Laboratory (Bar Harbor, ME) and acclimated for 4–6 weeks prior to testing. The C57BL/6J strain was selected for the social defeat paradigm based on previous literature, recommendations of the developers of the chronic defeat protocol (N. Kudryavtseva's group), and our own experience with this mouse model (see [68] for details). At the beginning of the experiment, mice were 3–4 months old, and weighed 22–26 g. The animals were housed 4–5 mice/cage in the Tulane University School of Medicine Vivarium in Plexiglas cages (27.5 cm length, 21.5 cm height, 16.5 cm width) with standard bedding, as well as ad libitum access to food and water. Illumination (1170 ± 67 lx) was provided by ceiling-mounted fluorescent light tubes on a 12:12 light/dark cycle (on: 6.00 h, off: 18.00 h) for the duration of the study. At the beginning of the experiment, each mouse was paired with a conspecific, and housed in pairs in larger Plexiglas cages (23 cm height, 23 cm width, 44 cm length) containing a partition in the middle of the cage lengthwise. Each partition was made from 0.5-cm thick transparent Plexiglas with 30 small 0.7-cm holes which allowed constant sensory contact, but prevented physical contact. To maximize mouse aggression, we avoided social confrontations between cagemates housed together originally, i.e., prior to the beginning of the study. The housing of mice during the social stress protocol was re-arranged every 2–3 days in accordance with their social status (determined by previous social defeat testing), to avoid the reduction in winners' aggression due to the establishment of social hierarchy in the pair. Mice that were considered dominant received new partners with a subordinate social status (losers), to solidify their social dominance. Mice failing to show a clear social status by Day 5 of the experiment were (approximately 30% of animals) excluded from the study. As recommended in the original protocol [68] and by its developers (Prof. N. Kudryavtseva, personal communications, 2009–2011), control mice ($n = 10$) were individually housed in standard Plexiglas cages for 5 days, in order to minimize the potentially confounding effects of both the group housing and social isolation (Fig. 1A). Animal protocols reported here have been approved by the Institutional Animal Use and Care Committee, and adhered to the Institutional and National guidelines on animal experimentation.

### 2.2. Social defeat stress

The chronic social stress procedure was performed between 12.00 and 16.00 h. Fig. 1A illustrates the general study design. Partitioned cages with mouse pairs were taken from the holding room and placed in the testing room for 15 min of acclimation. Water bottles and plastic cage tops were promptly removed, and one or two wide black lines were put on the tails of mice with a marker to enable their recognition by the observers. Following this "behavioral activation" period, the divider was removed for 15 min to allow for interaction between the two mice [68]. Agonistic behaviors were scored by two trained observers (inter-rater reliability >0.85) based on latency (s) and frequency of sniffing, biting, dominant hetero-grooming, chasing, and touching. Jointly initiated behaviors were also scored in this study; part of neurobehavioral data collected during this project was used in a parallel study on an unrelated topic (stress-evoked grooming behavior) [68]. In order to create a lingering scent of the opponent after the social conflict, bedding from each cage side was mixed prior to returning the partition to its original position. Tampering with their homes in this way served to irritate the mice and reinforce the induction of the social stress. After each day of interaction, a clear dominant winner mouse would typically emerge [68]. In contrast, a passive mouse would typically display defensive behaviors, such as sideways or upright submissive postures, withdrawal, fleeing, lying on its back, or freezing. Winners ($n = 10$) were defined as mice that were dominant in ≥60% of their social encounters during the entire 20-day duration of social defeat paradigm. Losers ($n = 10$) were defined as animals experiencing ≤20% of victories during the entire duration of the experiment (Fig. 1A). To confirm the categorization of the social status of the mice, an alternative "point system" method was also employed, which assigned a value to each mouse depending on whether they had been judged to win, lose, or tie each social defeat encounter. Briefly, after each of the 20 daily 15-min trials, winner mice were given three points, each mouse involved in a 'tie' was given two points, and losers were given one point. Points were totaled cumulatively at the end of the 20-day chronic social stress period [68].

### 2.3. Apparatus and behavioral testing

Behavioral testing was performed between 13.00 and 16.00 h. The testing occurred one day after the last (20th) social defeat trial, in order to assess a global, long-term impact of social stress battery (rather than the immediate effects of acute stress produced by the last social confrontation). Mice were transferred for acclimation to an adjacent room 1 h prior to testing. The open field test was