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The contribution of the locus coeruleus-norepinephrine system in the emergence of defeat-induced inflammatory priming

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Abstract

Exposure to psychosocial stress is known to precipitate the emergence of stress related psychiatric disorders such as depression and anxiety. While mechanisms by which this occurs remain largely unclear, recent evidence points towards a causative role for inflammation. Neurotransmitters, such as norepinephrine (NE) are capable of regulating expression of proinflammatory cytokines and thus may contribute to the emergence of stress-related disorders. The locus coeruleus (LC) is the major source of norepinephrine (NE) to the brain and therefore the current study utilized N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), an LC selective noradrenergic neurotoxin, to determine the discrete involvement of the LC-NE system in social defeat-induced inflammation in LC projection regions including the central amygdala (CeA), dorsal raphe (DR) and plasma. In the current study, rats were exposed to brief social defeat or control manipulations on 5 consecutive days. To determine whether a history of social defeat enhanced or “primed” the inflammatory response to a subsequent defeat exposure, all rats regardless of stress history were exposed to an acute social defeat challenge immediately prior to tissue collection. As anticipated, prior history of social defeat primed inflammatory responses in the plasma and CeA while neuroinflammation in the DR was markedly reduced. Notably, DSP-4 treatment suppressed stress-induced circulating inflammatory cytokines independent of prior stress history. In contrast, neuroinflammation in the CeA and DR were greatly augmented selectively in DSP-4 treated rats with a history of social defeat. Together these data highlight the dichotomous nature of NE in stress-induced inflammatory priming in the periphery and the brain and directly implicate the LC-NE system in these processes.

Keywords

Social defeat; resident intruder paradigm; DSP-4; central amygdala; dorsal raphe; locus coeruleus; norepinephrine; inflammation; neuroinflammatory priming; peripheral inflammatory priming

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1. Introduction

Exposure to psychosocial stress, such as bullying, abuse, or witnessing traumatic events are known to enhance the risk of developing depression and anxiety (Almeida, 2005). Depression is considered one of the most debilitating diseases in the U.S. (Vigo et al., 2016), affecting nearly 7% of adults and 11% of adolescents (National Institute of Health, 2013a, b). Historically, certain stress-related disorders including subtypes of depression have been associated with complex dysregulation of the noradrenergic system (Klimek et al., 1997; Moret and Briley; Ressler and Nemeroff, 2000; Serafini) and as such several lines of antidepressant therapies have been developed to modulate this catecholamine. However, these treatments require several weeks of daily use prior to producing anti-depressant effects. Therefore, it has been suggested that dysregulation of norepinephrine (NE) may not be the sole mechanism for the development of these disorders and may instead produce behavioral deficits by impacting secondary systems (Delgado and Moreno, 2000). In recent years, studies in animals and humans have indicated that depression is associated with enhanced inflammation in the periphery and the brain (Bharani et al.; Jardanhazi-Kurutz et al., 2011; Johnson et al., 2005; Miller et al., 2005; Setiawan et al., 2015). Importantly, it has been well recognized that NE can serve as a regulator of immune function by acting on α and β adrenergic receptors in the plasma and β adrenergic receptors in the brain (Johnson et al., 2005). Taken together these findings suggest that the inflammatory system may represent an important target by which NE may contribute to the emergence of depressive symptomatology.

The locus coeruleus (LC) is the major source of NE to the entire neuraxis and is poised to contribute to peripheral NE through descending projections to sympathetic spinal nerves (Bruinstroop et al., 2012; Samuels and Szabadi, 2008). A study utilizing non-social stressors in cats determined that stress-induced plasma NE was indeed associated with an increase in the spike frequency of the LC (Abercrombie and Jacobs, 1987). However, it has not been determined whether the LC system contributes to plasma NE content following social stress and was therefore an aim of the present study. Moreover, NE can directly impact inflammatory processes in both the brain and periphery. In the periphery, NE signals through β -adrenergic receptors on the cell surface of macrophages and neutrophils to stimulate the release of proinflammatory cytokines (Flierl et al., 2009; Li et al., 2015) and can augment the peripheral inflammatory response to an immune challenge (Grisanti et al., 2010; Kavelaars et al., 1997; Spengler et al., 1990). Although less is understood regarding the effects of NE on inflammation in the brain, studies have suggested that NE plays a crucial role in neuroinflammatory processes (Bharani et al.; Gyoneva and Traynelis, 2013a; Jardanhazi-Kurutz et al., 2011; Johnson et al., 2005). Jardanhazi-Kurutz et al. (Jardanhazi-Kurutz et al., 2011) identified that lesioned LC-NE projections induced astrogliosis and microgliosis in cortical and hippocampal structures thereby enhancing the inflammatory potential within these regions.

Notably, enhanced inflammation in the periphery and the brain has been reported to occur following repeated social stress exposure, producing enhanced cytokine concentrations in the plasma (Finnell et al., 2017a; Hodes et al., 2014; Powell et al., 2013; Wood et al., 2015) and distinct inflammatory profiles in the LC (Finnell et al., 2017a; Wood et al., 2015) and

LC projection regions including the dorsal raphe (DR) (Finnell et al., 2017a; Wood et al., 2015) and central amygdala (CeA) (Finnell et al., 2018; Muhie et al., 2017). Several of these studies further identified that stress-induced proinflammatory cytokine release is causal to this depressive-like phenotype as administration of an IL-1 receptor antagonist (Wood et al., 2015), resveratrol (Finnell et al., 2017a), a natural polyphenol with potent anti-inflammatory actions, or bone marrow transplant to reduce IL-6 (Hodes et al., 2014) could block the emergence of stress-induced behavioral dysfunction. While the role of inflammatory cytokines in depressive-like behaviors following social stress has been well established, it remains unclear if the LC-NE system plays a role in regulating defeat-induced inflammatory consequences. Based on these data, the current study utilized N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), a noradrenergic neurotoxin, to investigate the discrete contribution of the LC-NE system in the development of social defeat-induced cytokine release and inflammatory sensitization (priming) in LC projection regions.

2. Methods

An illustrated study timeline is presented in Figure 1.

2.1 Animals.

Male Sprague-Dawley rats (250–275g, intruders or controls) and Long-Evans retired breeders (600–800g, residents; Charles River, Durham, NC) were housed individually and maintained on a 12-hr light/dark cycle. Residents were screened for their territorial nature and level of aggression prior to inclusion in the study as previously published (Wood et al., 2010). Care and use of animals was approved by the University of South Carolina's IACUC and adhered to the NIH Guide for the Care and Use of Laboratory Animals.

2.2 Surgery.

Permanent guide cannulas (22 gauge; Plastics One, Roanoke, VA) were implanted into the right lateral ventricle (rostral-caudal: -0.8 mm relative to bregma; medial-lateral: -1.5 mm; dorsal-ventral: -4.2 mm from skull) of all Sprague Dawley males 10 days prior to the onset of stress or control manipulation as previously published (Wood et al., 2015). All rats received post-surgical analgesia (Flunazine 2.5 mg/kg) as well as supportive therapy of Bacon Softies (Bio-Serv, Flemington, NJ) daily for 48 hours.

2.3 Drug Preparation and Injection.

Seven days prior to the start of social defeat or control manipulation, male Sprague Dawley rats were randomly assigned into DSP-4 or vehicle treatment groups. DSP-4 (8 μ L infusion of 400 μ g/rat) or vehicle (8 μ L 1% ascorbic acid in phosphobuffered saline) were infused via the implanted cannula over a period of 3 minutes. This dose was selected based on its demonstrated efficacy to reduce NE content in the hippocampus by 70% (Sziray et al., 2010). Although DSP-4 is highly selective for LC-NE neurons, it can produce minor depleting effects on serotonin (5-HT) (Jonsson et al., 1981). To protect 5-HT neurons, selective 5-HT reuptake inhibitor (SSRI) citalopram (10 mg/kg intraperitoneal; AK Scientific, Union City, CA) was injected 30 minutes prior to DSP-4 or vehicle administration. Citalopram was utilized based on its notably high specificity for the 5-HT

transporter (Hiemke and Härtter, 2000) and based on the previous use of an SSRI combined with DSP-4 to protect 5-HT terminals (Howard et al., 2008). This low dose was selected based on studies demonstrating a lack of effects on rat behavior but successfully protecting against 5-HT depletion (Crowley et al., 2006; Howard et al., 2008; Tonissaar et al., 2008).

2.4 Social defeat/control.

Social defeat stress was conducted using a modification of the resident intruder paradigm originally developed by Miczek (Miczek, 1979) as we have previously published (Chaijale et al.; Finnell et al., 2017a; Wood et al.; Wood et al., 2010; Wood et al., 2015; Wood et al., 2013; Zitnik et al., 2016). Briefly, male Sprague Dawley rats of each treatment group were randomly assigned as “controls” or “intruders”. Intruders were placed into the home cage of a novel Long Evans retired breeder, termed the “resident”, for 30 minutes on 5 consecutive days. During this time, the intruder received multiple aggressive approaches and attacks from the resident, inducing behavioral submission. At the time of submission as noted by supine posture or after 15 minutes, whichever came first, intruders and residents were separated with a perforated Plexiglas partition to allow for continued olfactory, visual, and auditory communication with the resident for the remainder of the 30-minute stress period. Importantly, none of the animals included within this study sustained injuries as a result of defeat exposure. Control animals were placed behind the perforated partition in an empty novel cage for 30 minutes to match the novel environment of the intruders. Control animals were not present in the animal room during the social defeat exposures. Social defeat exposures were video recorded and later scored for intruder upright posture by an experimenter blinded to each animal’s treatment designation as previously reported (Finnell et al., 2017a; Wood et al., 2010).

2.5 Tissue collection.

2.5.1 Validation of study design.—To establish whether our study was in fact designed to detect stress-induced priming rather than simply reflecting an increase in resting levels, a separate subset of vehicle and DSP-4 treated rats with a history of control or defeat were generated according to the timeline in Figure 1. However, tissue from these animals was collected (as described below) under resting non-stress conditions on day 11. These data serve as a measure to identify the effect of stress and treatment on resting cytokine levels and as validation for our study design.

2.5.2 Social defeat-induced inflammatory sensitization.—To determine if a history of social defeat stress (with or without the LC lesion) produced inflammatory priming, all rats regardless of their stress history were subjected to a 30-minute social defeat 6–7 days after the 5th stress/control exposure followed immediately by euthanasia and tissue collection. Brains were blocked into anterior and posterior regions, flash frozen with ice cold 2-Methylbutane, and stored at -80°C . Trunk blood was centrifuged at 4°C for 15 minutes with a speed of 3200 rpm. Resulting plasma was collected and stored at -80°C .

2.6 CeA, Hippocampal, and DR collection and homogenization.

Brains were sliced coronally with a cryostat to the most rostral level of the CeA (Bregma -1.72 (Paxinos and Watson, 2007)) and hippocampus (Bregma -2.92 (Paxinos and Watson,

2007)) at which time 1×1 mm bilateral tissue punches were acquired using a 1 mm wide tissue biopsy punch (Harvard Apparatus, Holliston MA). The posterior brain was sliced to the most caudal level of the DR (Bregma -8.40 (Paxinos and Watson, 2007)) and a single DR punch was acquired at a depth of 2 mm using a 1 mm wide tissue biopsy punch. Histological verification of punch placement was conducted for each region using 30µm thick pre- and post-punch slices (Supplemental Figure 1) as previously published (Finnell et al., 2017a; Finnell et al., 2017b). Once histologically verified, tissue punches were homogenized as previously reported (Finnell et al., 2017a; Finnell et al., 2017b) using mechanical disruption with a bullet blender (Next Advance, Averill Park, NY) in a lab-made lysis buffer (500 mM NaCl, 50 mM Tris HCl pH 7.6, 10% NP-40, 70% glycerol) with 0.02% protease phosphatase inhibitor cocktail (Thermo Scientific, Waltham, MA). Protein concentration of each homogenate was determined using a Pierce BCA protein assay kit (Thermo Scientific, Waltham, MA) per manufacturer protocol.

2.7 High performance liquid chromatography (HPLC).

HPLC was conducted on plasma and hippocampal, CeA, and DR tissue homogenates as previously published (Finnell et al., 2017a; Finnell et al., 2017b). Briefly, all samples were spiked with 10nM dihydroxybenzylamine (DHBA, Thermo Fisher Scientific, Waltham, MD) to serve as an internal standard and filtered via centrifugation using Amicon Ultra 0.5 mL Ultracel 3kDa (tissue homogenates) or 50kDa (plasma) molecular weight cut off spin filters (Millipore, Billerica, MA) for 30 min at 4°C and 16,000xg. Flow through collected from the spin filters were used for HPLC analysis. All NE data were normalized to DHBA and tissue homogenates were further normalized to BCA protein concentrations. The hippocampus served as the NE lesion verification site as it exclusively receives NE innervation from the LC (Loy et al., 1980) and confirmed that DSP-4 treatment reduced NE by approximately 85% (Average Hippocampal NE pmol/ug protein ± SEM; Vehicle: 34.54 ± 6.4; DSP-4: 4.9 ± 1.5; $t_{(11)}=4.2$, $p<0.01$). These data are comparable to studies using the standard 50 mg/kg intraperitoneal dose (Cheetham et al., 1996; Harro et al., 1999; Harro et al., 1995; Kask et al., 2000) and direct ICV injection of 400µg of DSP-4 (Sziray et al., 2010) in which hippocampal NE was depleted by 70–100%.

2.8 Bio-Plex cytokine analysis.

Defeat-induced circulating (plasma) and brain region specific inflammatory effects were determined using Bio-Plex assays (BioRad, Hercules, CA). Homogenized CeA and DR tissue were loaded onto the plate at a concentration of 275µg/mL as previously published (Finnell et al., 2017a; Finnell et al., 2017b). Plasma samples were diluted 1:4 and all samples and standards were run in duplicate according to the manufacturer protocol. Plates were read using a Luminex Bio-Rad Bio-Plex system (Hercules, CA). To control for inter-kit variability, vehicle control rats were run on all plates and all Bio-Plex data were normalized to percent of vehicle control. Note that replicate Bio-Plex values resulting from the repeated sampling of vehicle controls were averaged following the percent of control calculation conducted across each plate. For certain cytokines this resulted in a vehicle control group average that was not equal to 100%.

2.9 Plasma corticosterone measurements.

To determine differences in circulating stress hormones following the acute defeat challenge, plasma corticosterone was measured using a Corticosterone ELISA (Enzo Life Sciences, Farmingdale, NY) as previously published (Finnell et al., 2017b). Briefly, plasma was diluted 1:40 and run according to manufacturer protocol. Plates were read using a Synergy 2 Multi-Mode plate reader (Bio Tek, Winooski, VT) with Gen5 software (Bio Tek, Winooski, VT).

2.10 Statistical analysis.

The statistical analysis used to analyze these data requires that data be free from statistical outliers (Lindsey et al., 2018). Two-times standard deviation calculations and the Modified Thompson Tau outlier test identified statistical outliers of raw values (or the average of duplicate values for the molecular analysis) in each data set (Finnell et al., 2017a; Finnell et al., 2017b). Values that exceeded two-times standard deviation were excluded from all group analyses. Values flagged by the Modified Thompson Tau analysis were further evaluated for the presence of false positive results. Any values that were deemed to have been marked from a false positive result were not excluded from group analyses as these values were not considered to be true outliers. Data sets comparing two groups (HPLC of hippocampal homogenates and during defeat behavioral data) were subjected to standard two tail t-tests ($\alpha=0.05$). To determine the effect of corticosterone and NE on social defeat-induced cytokine expression in the plasma and brain, two-tailed Spearman correlations ($\alpha=0.05$) were conducted. For comparisons of stimulated versus resting cytokine concentrations in the plasma and brain standard One-Way ANOVAs ($\alpha=0.05$) were conducted. All other data were subjected to a standard Two-Way ANOVA ($\alpha=0.05$) to determine main effects of DSP-4 treatment and stress history. All ANOVAs were followed by Holm-Sidak post hoc analysis to account for unequal sample sizes and variance. All statistical analyses were conducted using GraphPad Prism 6 software (La Jolla, CA).

3. Results

3.1 Behavior during social defeat.

To determine the effects of DSP-4 on intruder behavior during the 5 consecutive exposures to social defeat, upright postures exhibited by intruder rats were quantified from video recordings obtained on day 1 and 5 of social defeat. Comparison of upright postures on days 1 and 5 indicated that both DSP-4 and vehicle treated rats exhibited greater durations of upright postures on the 5th exposure (Effect of day: $F_{(1,16)}=6.0$, $p<0.05$; Effect of Treatment: $F_{(1,16)}=1.9$, $p=0.18$; Average $\pm$ SEM (sec); Vehicle: 199.2 ± 53.3 ; DSP-4: 120.4 ± 38.9) compared to the 1st exposure (Average $\pm$ SEM (sec); Vehicle: 94.2 ± 28.9 ; DSP-4: 26.6 ± 16.8). Subsequent post-hoc analyses indicated that there were no differences between treatment groups on either day 1 ($p=0.08$) or day 5 ($p=0.48$). In addition to quantifying upright postures, the amount of time required for a resident to induce behavioral submission (i.e. defeat latency) was also determined for each social defeat exposure. DSP-4 and vehicle treated animals exhibited similar defeat latencies across the 5-day social defeat period (Average $\pm$ SEM (sec); Vehicle: 215.8 ± 66.3 ; DSP-4: 270.7 ± 55.4 ; $t_{(10)}=0.6$, $p=0.6$), suggesting that DSP-4 treatment does not alter the submissive behavior of intruder rats to the

aggressive resident. Finally, analysis of social defeat episodes also confirmed that all rats experienced a similar number of attacks by the aggressive resident, confirming that effects of stress history or DSP-4 treatment were not confounded by differences in the intensity of the defeat (Effect of Treatment: $F_{(1,26)}=0.5$, $p=0.5$; Effect of stress history $F_{(1,26)}=0.1$, $p=0.8$; data not shown).

3.2 Validation of study design.

The time course of stress-induced (or endotoxin-induced) release of cytokines can vary depending on the rat's stress history and the cytokine being measured (Cheng et al., 2015; Johnson et al., 2002). Cytokine release in response to an initial acute stress exposure can require up to 4 hours to emerge. However upon subsequent exposure to the stressor, sensitized responses occur in the plasma and the brain, peaking as early as 1 hour following stress (Cheng et al., 2015; Johnson et al., 2002). Therefore, in the current study it was critical to verify that plasma and brain tissue collected immediately following the acute social defeat exposure (30 minutes after the start of social defeat) were reflective of a time point at which stress-induced elevations in cytokines were evident. To this end, two distinct preliminary studies were conducted in which plasma and brain tissue were collected under resting non-stress conditions from a subset of vehicle and DSP-4 treated rats that had a history of control or defeat.

3.2.1 Determination of resting inflammatory tone in CeA and DR following social defeat and DSP-4 treatment.—We have previously shown that plasma cytokine concentrations at rest do not differ between male Sprague Dawley rats with a history of social defeat or control (Finnell et al., 2017a). However, neuroinflammation at this same time point was determined to be significantly elevated in the locus coeruleus in male rats with a history of social defeat (Finnell et al., 2017a). Based on our previous findings, it was pertinent to determine if a history of social defeat or DSP-4 treatment produced long lasting increases in resting neuroinflammatory tone within the CeA and DR. To this end, vehicle and DSP-4 treated rats with a history of control or defeat were euthanized at rest 6 days after the final social defeat exposure. Subsequent Bio-Plex analysis determined that neither a history of social defeat nor DSP-4 treatment significantly altered cytokine concentrations within the CeA (Supplemental Table 1). In contrast, analysis of the DR indicated that DSP-4 treatment did indeed enhance neuroinflammatory tone at rest. Specifically, IL-1 β was significantly enhanced in all DSP-4 treated rats regardless of stress history (Supplemental Table 2). Similar effects were evident for IL-2, IL-4, and TNF- α (Supplemental Table 2). Importantly, although inflammatory tone was enhanced in DSP-4 treated rats at rest within the DR, subsequent post-hoc analyses did not identify significant differences between specific stress and treatment groups.

3.2.2 Verification of acute defeat-induced cytokine release.—Following determination of the effects of stress and DSP-4 treatment on resting inflammatory tone in the CeA and DR, a second validation study was conducted to confirm that stress-induced cytokine release could be detected at the chosen 30-minute post-stress time point. Cytokine levels from plasma, CeA and DR were compared between vehicle treated control rats collected on day 11 at rest or immediately following the acute social defeat challenge

(Supplemental Table 3). This comparison confirmed that cytokine expression following acute defeat was reflective of increased cytokine concentrations. However, the magnitude of stress-induced alterations in cytokines were largely cytokine specific. For example, plasma IL-1 β , IL-4, and IL-13 demonstrated robust increases following the acute defeat challenge, while IL-6 remained unchanged from vehicle treated rats euthanized under resting non-stress control conditions (Supplemental Table 3). Similarly, the acute defeat challenge also resulted in increased proinflammatory cytokine concentrations in the CeA as compared with control tissue collected under resting conditions (Supplemental Table 3). In contrast, the DR responded to acute defeat stress by a robust reduction in cytokines (Supplemental Table 3) as we have previously reported (Finnell et al., 2017a; Wood et al., 2015). These data verify that cytokine levels in the brain tissue and plasma collected 30 minutes post-defeat were indeed reflective of a state of stress-induced increases in cytokines above that of resting baseline levels. However, it is important to note that these likely do not reflect the peak cytokine concentrations induced by the acute defeat challenge as they were collected only 30 mins after stress initiation. More likely these levels reflect microglial release of microvesicles containing cytokine stores (Bianco et al., 2005) rather than direct increases in protein expression induced by the acute defeat challenge.

3.3 Effects of DSP-4 on release of social defeat-induced circulating cytokines in rats with a history of control or stress.

To determine whether a history of social defeat results in a primed (i.e., sensitized) peripheral inflammatory response to a subsequent stressor, all rats, regardless of stress history, were exposed to an acute social defeat challenge immediately prior to euthanasia. In vehicle treated rats, a history of social defeat produced significant facilitation of 3 out of 10 circulating stress-induced cytokines including IL-6 (Effect of Stress: $F_{(1,18)}=5.1$, $p<0.05$; Figure 2A), interferon (INF)- γ (Effect of Stress: $F_{(1,18)}=7.3$, $p<0.05$; Figure 2B), and TNF- α (Effect of Stress: $F_{(1,17)}=6.3$, $p<0.05$; Figure 2C). Consistent with NE's known facilitative effects on immune regulation, rats receiving the noradrenergic neurotoxin DSP-4 exhibited robust inhibition of stress-induced release of all 10 measured cytokines (Figure 2 and Table 1) including IL-1 β (Effect of Treatment: $F_{(1,18)}=28.3$, $p<0.0001$, Figure 2D), IL-4 (Effect of Treatment: $F_{(1,18)}=28.5$, $p<0.0001$, Figure 2E), and IL-13 (Effect of Treatment: $F_{(1,17)}=20.1$, $p<0.001$; Figure 2F). Moreover, DSP-4 blocked the stress-induced facilitation of IL-6 (Effect of Treatment: $F_{(1,18)}=28.3$, $p<0.0001$; Figure 2A), INF- γ (Effect of Treatment: $F_{(1,18)}=26.9$, $p<0.0001$; Figure 2B), and TNF- α (Effect of Treatment: $F_{(1,17)}=23.3$, $p<0.001$; Figure 2C). Taken together these data suggest that the LC-NE system may be a critical mediator of stress-induced cytokine release in the plasma and indicate that repeated stress exposure may not result in a general priming of the inflammatory system but rather produces cytokine specific sensitization.

3.4 DSP-4 treatment affects peripheral NE and corticosterone.

To confirm the contribution of the LC to circulating stress-induced NE levels in the plasma, HPLC detection of NE was conducted. These data confirmed that DSP-4 did in fact reduce the NE response to social defeat (Effect of Treatment: $F_{(1,20)}=8.9$, $p<0.01$; Figure 3A) that was independent of stress history. Moreover, NE concentration was positively correlated with expression of several plasma cytokines including IL-6 ($r=0.44$, $p=0.040$) and INF- γ

($r=0.43$, $p=0.047$). These data support previous reports that describe the proinflammatory potential of circulating NE (Grisanti et al., 2010; Johnson et al., 2005; Kavelaars et al., 1997; Spengler et al., 1990) and further suggest for the first time that the LC may be a significant source of social stress-induced NE in the periphery.

It is also recognized that NE can regulate a number of peripheral effectors including the anti-inflammatory stress hormone corticosterone. Therefore, these studies also quantified corticosterone in plasma. Interestingly, plasma corticosterone following the acute defeat challenge was opposite from findings in plasma NE and inflammation. Specifically, corticosterone concentrations were selectively blunted in vehicle treated rats with a history of defeat (Effect of Stress: $F_{(1,15)}=14.4$, $p<0.01$; Figure 3B). Treatment with DSP-4 blocked defeat-induced decreases in corticosterone in rats with a history of defeat (Effect of Treatment: $F_{(1,14)}=12.3$, $p<0.01$). Subsequent correlational analysis indicated that these acute defeat-induced plasma corticosterone concentrations were also related to the peripheral inflammatory response as 9 of the 10 measured cytokines including IL-1 β ($r=-0.58$, $p=0.016$), IL-6 ($r=-0.60$, $p=0.012$), and TNF- α ($r=-0.59$, $p=0.026$) were all negatively correlated with corticosterone. These data suggest that in addition to regulating peripheral inflammation through the enhancement of circulating NE, the LC-NE system may further impact peripheral inflammatory processes by indirectly modifying secondary systems that exhibit anti-inflammatory potential.

3.4 Stress-induced neuroinflammation is enhanced in the CeA of DSP-4 treated rats with a history of social defeat.

Social defeat-induced inflammatory priming was also evident in the CeA in response to the acute social defeat challenge. Specifically, vehicle treated rats with a history of social defeat exhibited modest yet consistent increases in CeA cytokines including IL-1 β (Stress x Treatment Interaction: $F_{(1,16)}=5.5$, $p<0.05$; Figure 4A), IL-6 (Stress x Treatment Interaction: $F_{(1,16)}=6.0$, $p<0.05$; Figure 4B), INF- γ (Effect of Stress: $F_{(1,17)}=11.5$, $p<0.01$; Effect of Treatment: $F_{(1,17)}=8.1$, $p<0.05$; Figure 4C), and TNF- α (Effect of Stress: $F_{(1,17)}=11.9$, $p<0.01$; Figure 4D) compared with vehicle treated rats with a history of control exposure. Importantly, this stress-induced neuroinflammatory priming was not just evident for pro-inflammatory cytokines as many of the anti-inflammatory cytokines analyzed including IL-1Ra (Effect of Stress: $F_{(1,17)}=9.8$, $p<0.01$; Effect of Treatment: $F_{(1,17)}=10.9$, $p<0.01$; Figure 4E) and IL-4 (Stress x Treatment Interaction: $F_{(1,16)}=6.0$, $p<0.05$; Figure 4F) were also elevated in vehicle treated rats with a history of defeat compared to vehicle treated rats with a history of control. Strikingly, treatment with DSP-4 sensitized neuroinflammatory responses to acute social defeat, but only in rats with a history of stress. Specifically, of the 22 measured cytokines (Figure 4, Table 2), 21 were significantly enhanced in DSP-4 treated rats with a history of social defeat compared to vehicle treated rats with a history of social defeat. Together these data suggest that the LC-NE system serves as a brake on neuroinflammation within the CeA and that this is sensitized in rats with a history of social defeat.

3.5 Acute defeat-induced neuroinflammation in the CeA is associated with loss of NE.

As expected, DSP-4 treatment functioned to reduce acute defeat-induced NE expression in the CeA. Interestingly, a history of social defeat alone also resulted in a significant reduction in NE within the CeA (Effect of Stress: $F_{(1,16)}=4.8$, $p<0.05$; Average $\pm$ SEM (pmol/ μ g protein); Vehicle-History of Control: 44.5 ± 31.2 , Vehicle-History of Defeat: 10.6 ± 11.4 , DSP-4-History of Control: 13.1 ± 16.2 , DSP-4-History of Defeat: 9.5 ± 6.0). Subsequent correlational analysis indicated that these acute defeat-induced NE concentrations were negatively associated with several cytokines. These effects were evident for both pro-inflammatory cytokines including TNF- α ($r=-0.53$, $p=0.019$) and INF- γ ($r=-0.48$, $p=0.036$) and anti-inflammatory cytokines such as IL-4 ($r=-0.51$, $p=0.026$). These data suggest that loss of NE within the CeA either in response to repeated stress exposure or DSP-4 treatment may contribute to the disinhibition of neuroinflammatory responses within this region.

3.6 A history of social defeat has opposing effects on acute defeat-neuroinflammation within the DR in vehicle versus DSP-4 treated rats.

In contrast to the pro-inflammatory effects of social defeat in the CeA, social defeat suppressed cytokine expression in the DR. For example, in rats with a history of defeat exposure, IL-1 β levels were markedly reduced in the DR following the acute defeat challenge compared to vehicle treated rats with a history of control (Effect of Stress: $F_{(1,17)}=5.3$, $p<0.05$; Figure 5A). Although a reduction of IL-1 β was also evident for DSP-4 treated rats with a history of defeat, this effect was not significant (Effect of Treatment: $F_{(1,17)}=4.6$, $p<0.05$; Figure 5A). Interestingly, a stress-induced reduction in cytokines was not evident for other pro-inflammatory cytokines. For example, acute defeat-induced TNF- α concentrations were not altered in vehicle treated rats with a history of defeat compared to vehicle treated rats with a history of control (Figure 5B). Moreover, treatment with DSP-4 functioned to enhance acute defeat-induced TNF- α (Effect of Treatment: $F_{(1,17)}=9.1$, $p<0.01$; Figure 5B) in all rats. Stress and DSP-4 also had effects on anti-inflammatory cytokines. Specifically, IL-2 (Stress x Treatment interaction: $F_{(1,17)}=13.1$, $p<0.01$; Figure 5C) and IL-4 (Effect of Treatment: $F_{(1,17)}=6.0$, $p<0.05$; Figure 5D) were both reduced in vehicle treated rats with a history of defeat. Moreover, DSP-4 treatment selectively enhanced these cytokines following the acute defeat challenge in rats with a history of defeat.

3.7 Acute defeat-induced cytokine concentrations are largely independent from NE content within the DR.

NE content in the DR following the acute defeat challenge was unaffected by stress history (Effect of Stress: $F_{(1,16)}=0.2$, $p=0.7$; Supplemental Figure 2) or treatment condition (Effect of Treatment: $F_{(1,16)}=0.02$, $p=0.9$; Supplemental Figure 2).

4. Discussion

Dysregulation of the noradrenergic system has been clearly associated with the pathogenesis of stress-related disorders. While noradrenergic reuptake inhibitors are effective in alleviating behavioral symptomatology in certain patient populations, these kinds of traditional anti-depressive therapies require weeks of continuous use and only produce

remission in approximately 28% of people (Fava, 2000; Trivedi, 2006). Moreover, noradrenergic depletion studies are largely ineffective in inducing or worsening symptoms of major depression (Delgado et al., 1993; Miller et al., 1996a; Miller et al., 1996b), suggesting that rather than a direct effect of NE, antidepressant therapies targeting NE may rely on changes within a secondary system that is modulated by NE. Stress-induced inflammation represents one such secondary system as it has been implicated in the emergence of depressive symptomatology and is under regulation by NE. The current study showed for the first time that the LC-NE system provides a significant source of circulating NE in the plasma in response to social defeat stress and supports the notion that the LC noradrenergic system facilitates inflammatory priming in the periphery. These findings are largely consistent with and extend findings of others confirming a pro-inflammatory role for NE at the level of the periphery (i.e. plasma; (Grisanti et al., 2010; Johnson et al., 2005; Kavelaars et al., 1997; Spengler et al., 1990); Figure 6). Moreover, this study was further extended to identify the novel findings that a history of repeated exposure to social defeat produced brain region specific alterations in cytokine expression that are distinctly regulated by NE (Figure 6).

It has long been recognized that the peripheral sympathetic nervous system is capable of regulating immunity by activating adrenergic receptors on the cell surface of macrophages, resulting in release of pro-inflammatory cytokines (Flierl et al., 2009; Li et al., 2015) and further augmenting inflammatory responses to an immune challenge (Grisanti et al., 2010; Kavelaars et al., 1997; Spengler et al., 1990). Until recently the contribution of central noradrenergic systems to these responses remained relatively unknown. Engler et al. (Engler et al., 2010) provided the first report of LC-NE involvement in peripheral inflammatory responses; loss of LC-NE by DSP-4 treatment inhibited lipopolysaccharide-induced cytokine release from splenocytes and reduced the percentage of natural killer cells within the spleen, suggesting that there are multiple intersecting mechanisms by which the LC-NE system can promote peripheral inflammation. In line with these findings, the current study determined that peripheral cytokine responses to social defeat were regulated by LC-NE projections as the DSP-4 lesion resulted in reduced circulating NE in plasma and inhibition of acute defeat-induced release of plasma cytokines. Beyond the acute effects of stress on cytokine release, a history of stress exposure has been shown to promote the priming of peripheral inflammatory responses such that a subsequent stress or immune challenge promotes a larger and faster inflammatory response. It has been shown that inflammatory priming results from enhanced secretion of inflammatory cells that have a greater migratory and pro-inflammatory capacity (Bierhaus et al., 2003; Grisanti et al., 2010; Nance and Sanders, 2007; Wohleb et al., 2015). The sympathetic nervous system is suggested to be critical in the production of these effects as it innervates a number of lymphoid tissues (Felten et al., 1985; Wohleb et al., 2015). While the current study did not directly assess the number and reactivity of inflammatory cell types, inflammatory priming was evident based on our findings that stress-induced cytokine release was augmented in rats with a history of stress compared with rats with a history of control. Moreover, the current studies provide direct support for the involvement of the LC-NE system in stress-induced peripheral inflammatory priming.

In addition to directly modifying inflammation by acting on inflammatory cell types in the periphery, central NE can regulate a number of effectors that also exhibit modulatory control over the inflammatory system. Corticosterone and corticosteroids have long been on the forefront as anti-inflammatory and immunosuppressive therapies (Coutinho and Chapman, 2011). There are a number of different mechanisms by which corticosterone has been suggested to exert its anti-inflammatory potency including interfering with actions of transcriptional regulators such as nuclear factor- κ B (NF κ B) and activator protein (AP)-1 (Heck et al., 1994; Jonat et al., 1990; Yang-Yen et al., 1990). In the context of acute stress exposure, corticosterone peaks at approximately 15 to 30 minutes and returns to baseline between 60 and 90 minutes following the stress exposure (Muir and Pfister, 1987; Wood et al., 2010). However, in the context of chronic stress and particularly homotypic stressors such as social defeat, corticosterone exhibits significant habituation (Grissom and Bhatnagar, 2009; Pitman et al., 1988; Wood et al., 2010). In line with these previous findings, the current study determined that acute defeat-induced corticosterone concentrations were significantly inhibited in vehicle treated rats with a history of social defeat. These data suggest that stress and LC-NE may contribute to the habituation of endogenous anti-inflammatory mechanisms such as corticosterone. However, the contribution of corticosterone to stress-induced inflammation remains unclear as DSP-4 and vehicle treated rats with a history of control exhibited nearly identical plasma corticosterone concentrations in response to the acute defeat challenge while simultaneously displaying vastly different plasma inflammatory responses.

Inflammatory priming is not isolated to cells in the periphery. In fact, there are several mechanisms by which stress can increase inflammatory responses in the brain. For example, stress and the associated increases in glucocorticoids have been shown to increase the number and activity of inflammatory competent cell types in the brain including microglia (Lehmann et al., 2016; Tynan et al., 2010; Wohleb and Delpech, 2017) and astrocytes (Lambert et al., 2000; Pearson-Leary et al., 2015). Moreover, exposure to repeated defeat has been shown to increase trafficking of peripheral inflammatory cells to the brain (Wohleb et al., 2012; Wohleb et al., 2014; Wohleb et al., 2013) presumably through alterations in the permeability of the blood brain barrier (Menard et al., 2017). Regardless of the mechanism, these effects are known to enhance the neuroinflammatory response to a subsequent stress or immune challenge. Importantly, stress-induced neuroinflammatory priming was also evident in socially defeated rats in the present studies, and in a region-specific manner.

The current study provides insight into a mechanism regulating defeat-induced neuroinflammatory priming within the CeA. Specifically, DSP-4 reduced NE levels in the CeA of rats regardless of stress history, however only rats with a history of stress demonstrated a robust 4–6-fold increase in cytokine expression. This suggests a dynamic stress-induced adaptation that may occur at the level of microglia and are in line with several studies that have suggested that central NE largely produces anti-inflammatory effects (Feinstein, 1998; Feinstein et al., 2002; Frohman et al., 1988; Hetier et al., 1991). However, much of what is understood about the impact of NE on neuroinflammation has been identified from experiments in cultured microglia. For example, studies conducted in cell culture have shown that activation of β 2-adrenergic receptors on the cell surface of microglia are capable of inhibiting pro-inflammatory cytokine release by enhancing cyclic adenosine

monophosphate (cAMP) (Mori et al., 2002). However, in a proinflammatory environment, as is seen after stress or an immune challenge, microglia are capable of shifting the expression of cell surface receptors from $\beta 2$ to a greater number of $\alpha 2$ -adrenergic receptors (Gyoneva and Traynelis, 2013b). In contrast to $\beta 2$ receptor stimulation, activation of $\alpha 2$ receptors on the cell surface of microglia fails to inhibit pro-inflammatory cytokine release and instead promotes the expression of catechol-O-methyl transferase (COMT) and Bcl-x1 (Mori et al., 2002). Taken together these data suggest that microglia present in proinflammatory environments recruit pathways that enhance microglial resilience to apoptotic events such as the generation of free radicals and facilitate the degradation of extracellular NE. Whether a shift from $\beta 2$ to $\alpha 2$ adrenergic receptors on the surface of microglia contributed to the exaggerated neuroinflammatory response to stress in DSP-4 treated rats with a history of stress has yet to be determined. However, DSP-4 has been shown to increase $\alpha 2$ adrenergic receptor expression in brain homogenates, highlighting the possibility of this mechanism (Szot et al., 2010). None-the-less, findings in the current study suggest that loss of NE either by stress or DSP-4 treatment results in disinhibition of inflammatory responses within the CeA.

Regardless of the putative mechanism by which NE may have disinhibited inflammatory responses in the CeA, inflammation in this brain area is known to lead to significant behavioral and cardiovascular disturbances. During stress, immune competent cells concentrated around the amygdala can release proinflammatory cytokines (Badowska-Szalewska et al., 2015) which in turn serve to increase activation of the CeA (Ming et al., 2013; Prager et al., 2013). Therefore, inflammation-induced activation of the CeA could not only mediate sensitized fear responses, but may also produce enhanced sympathetic activation that could lead to increases in blood pressure and heart rate (Iwata et al., 1987). The CeA is not the only brain area that displays enhanced activation to inflammatory stimuli. Inflammation-induced activation of the DR has also been shown to promote the emergence of anxiety-like behaviors (Yang et al., 2017). However, we have previously shown that in response to social defeat, inflammatory tone in the DR is actually suppressed (Wood et al., 2015). Similar inflammatory inhibition was noted in the current study. However, this inhibition was modest at best and may be due to a lack of changes in NE. Both the CeA and DR receive projections from a number of NE containing brain areas outside of the LC. For example, the DR receives NE projections from the lateral habenula (Aghajanian and Wang, 1977; Kalen et al., 1989) and the CeA receives NE projections from the ventromedial medulla (Zardetto-Smith and Gray, 1995) and the nucleus of the solitary tract (Daubert et al., 2012). However, findings from the current manuscript suggest that these NE inputs may differentially contribute to the NE content in the CeA and DR following stress and DSP-4 exposure. Specifically, stress and loss of LC-NE via DSP-4 significantly reduced NE content in the CeA. In contrast, NE content in the DR remained unchanged following stress or DSP-4 treatment. Taken together these data suggest that the lack of stress or treatment effects on DR NE could result from compensatory increases in the activity of these other NE containing brain regions while no such compensatory mechanisms were noted in the CeA.

5. Conclusion

Taken together the findings of the current study highlight the dichotomous effects of NE on stress-induced inflammatory processes in the periphery and the brain and further highlight the region specificity associated with social defeat-induced neuroinflammation. Most notably, a DSP-4 noradrenergic lesion of LC projections serves to robustly accelerate neuroinflammation in the CeA but only when combined with a history of stress.

6. Future Directions.

There are a number of important future studies that could be conducted to further elucidate the mechanistic basis by which the LC-NE system contributes to stress-induced inflammatory sensitization. For example, it will be critical to determine if stress-induced adaptations have occurred at the level of microglia and if the exaggerated increase in CeA inflammation could have altered CeA neuronal excitability. Finally, it will be critical to determine the contribution of the LC-NE system in stress-induced inflammatory responses in females. Women are not only more likely to develop depression and anxiety (Almeida, 2005; Feinstein et al., 2014; Nicodimos et al., 2009) but are also more sensitive to inflammatory related diseases (Kim et al., 2015). One of the major limitations in studying female stress sensitivity has been the inherent difficulty associated with using common social stress models, such as the social defeat paradigm described herein, with females (Finnell and Wood, 2018). We have recently validated a new social stress model in females which effectively combines the olfactory, auditory, and visual stimulus of social defeat with a witness component. Using this model of witness stress, we were able to show that intact cycling females were more sensitive to the emergence of stress-induced depressive-like behaviors (Finnell et al., 2018). Moreover, these behaviors were associated with significant enhancements in neuroinflammation in the CeA (Finnell et al., 2018). Importantly, data from these studies further suggested that witness stress may have altered the function of the LC-NE system as evidenced by significant reduced climbing behaviors in the forced swim test (Finnell et al., 2018), an effect known to be associated with reductions in NE (Cryan et al., 2002; Detke et al., 1995). Several review papers have been published discussing the contribution of sex in inflammation and depression (Bangasser and Valentino, 2014; Bekhbat and Neigh, 2018; Finnell and Wood, 2018; Lasselin et al., 2018), which further underscore the need for these studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Finnell et al Highlights

- Repeated social defeat exposure primes plasma and amygdala cytokine responses.
- In contrast, a history of social defeat blunts dorsal raphe cytokine release.
- Chemical lesion of the locus coeruleus (LC) inhibits stress-induced plasma cytokines.
- LC lesions disinhibited cytokine responsiveness to social defeat in the brain.
- Collectively these data directly implicate the LC in defeat-induced inflammation.

Figure 1. Study design and timeline for animals with a history of social defeat (A) or control (B). 10 days prior to the onset of stress all rats were implanted with intracerebroventricular (ICV) cannulas. Seven days prior to stress, DSP-4 or vehicle were administered. DSP-4 and vehicle treated rats were subjected to either 5 daily exposures to social defeat or control (daily between 0900–1130, Vehicle/Control N=8; DSP-4/Control N=7; Vehicle/Defeat N=7; DSP-4/Defeat N=7). Six days after the final social defeat/control exposure, all rats, regardless of stress history, were exposed to an acute social defeat challenge immediately prior to euthanasia and tissue collection. Two separate subsets of rats were exposed to 5 daily control sessions and euthanized 6 days later under resting, non-stress conditions (subset not represented in figure). These additional subsets were utilized for verification of study design.

Figure 2. DSP-4 treatment suppresses circulating cytokine release in response to an acute social defeat challenge regardless of stress history.

Following the acute social defeat challenge, vehicle treated animals with a history of social defeat exhibited enhanced (A) IL-6, (B) INF- γ , and (C) TNF- α , compared with vehicle treated controls consistent with stress-induced inflammatory priming. Strikingly, treatment with DSP-4 (panels A-F) suppressed stress-induced facilitation of IL-6, TNF- α , and INF- γ , as well as increases in (D) IL-1 β , (E) IL-4 and (F) IL-13, regardless of stress history.

* $p < 0.05$ vs. vehicle control; $^{\alpha}p < 0.05$, $^{\beta}p < 0.001$ vs. vehicle defeat.

Figure 3. DSP-4 treatment inhibits acute defeat-induced plasma NE and corticosterone habituation-induced by a history of social defeat.

(A) Following the acute social defeat challenge, vehicle treated rats with a history of social defeat exhibited a sensitized peripheral NE response. This defeat-induced sensitization was inhibited in DSP4 treated rats. (B) Vehicle treated rats with a history of social defeat selectively exhibit a habituated corticosterone response following the acute defeat challenge. In contrast, treatment with DSP-4 inhibited corticosterone habituation in rats with a history of defeat. * $p < 0.05$ vs. vehicle treated rats with a history of control; ^α $p < 0.05$ vs. vehicle treated rats with a history of defeat.

Figure 4. Treatment with DSP-4 exacerbates the acute defeat-induced neuroinflammation and priming in the CeA.

Following the acute social defeat challenge, vehicle treated rats with a history of social defeat exhibited moderate but consistent increases in pro-inflammatory cytokines (A) IL-1 β , (B) IL-6, (C) INF- γ , (D) TNF- α and anti-inflammatory (E) IL-1Ra and (F) IL-4 compared with vehicle treated rats with a history of control. Treatment with DSP-4 produced modest increases in stress-induced cytokines in rats with a history of control, while the LC-NE lesion robustly exacerbated the neuroinflammatory priming evident in rats with a history of social defeat exposure for all cytokines except TNF- α . * p <0.05 vs. vehicle treated rats with a history of control; f p =0.07, β p <0.001 vs. vehicle treated rats with a history of defeat; γ p =0.07 # p <0.05, ## p <0.01, ### p <0.001 vs. DSP-4 treated rats with a history of control. Dotted horizontal line indicates 100% of vehicle control.

Figure 5. A history of stress and DSP-4 treatment produce cytokine specific effects in the DR following acute defeat challenge.

Following the acute defeat challenge, vehicle treated rats generally exhibited a reduction of (A) IL-1 β , (B) TNF- α , (C) IL-2, and (D) IL-4. However, the magnitude of these reductions were largely cytokine dependent. While treatment with DSP-4 modestly enhanced IL-1 β independent of stress history, TNF- α , IL-2, and IL-4 were selectively enhanced in DSP-4 treated rats with a history of social defeat. [#] $p < 0.05$ vs. DSP-4 treated rats with a history of control; ^{β} $p < 0.05$ vs. vehicle treated rats with a history of defeat.

Figure 6. The contribution of the LC-NE system in peripheral and central social defeat-induced inflammation.

The LC serves as a significant source of NE to the brain, including regions such as the CeA and DR, as well as the periphery by acting on sympathetic spinal nerves located in the dorsal horn. In the brain NE can alter inflammatory tone by acting on microglia. Peripheral NE can similarly act upon circulating macrophages but also impacts the spleen to increase inflammatory tone in the periphery. Social defeat exposure in rats with functional LC projections produced enhanced peripheral inflammation and region-specific alterations of inflammatory tone within the brain (A). In contrast, defeat exposure in rats with chemical lesion of the LC-NE system resulted in the inhibition of peripheral inflammation but also produced a robust facilitation of neuroinflammation in both the CeA and DR (B).

Table 1.

DSP-4 treatment suppresses plasma cytokine and chemokine release in response to acute defeat challenge regardless of stress history.

Analyte	Vehicle		DSP-4		Main Effect of Treatment
	Control	Defeat	Control	Defeat	
IL-2	99.8± 11.1	137.1± 8.2	66.9± 19.2	54.5± 7.6 ^{β}	$F_{(1,17)}=17.3$, $p<0.001$
IL-10	100.2± 10.1	114.9± 9.3	58.4± 12.8 [*]	50.6± 7.4 ^{β}	$F_{(1,18)}=24.0$, $p<0.001$
GM-CSF	96.5± 9.4	110.8± 3.1	58.7± 3.9 ^{**}	49.5± 6.1 ^{γ}	$F_{(1,16)}=53.5$, $p<0.0001$
MCP-1	105.4± 12.8	135.1± 8.7	45.5± 8.8 ^{**}	54.7± 11.4 ^{γ}	$F_{(1,18)}=42.7$, $p<0.0001$

GM-CSF, granulocyte-macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein 1.

Data presented as average percent of vehicle control ± SEM.

p=0.06

*
p<0.05

**
p<0.01 vs. vehicle control

^{α}
p<0.01

^{β}
p<0.001

^{γ}
p<0.001 vs. vehicle defeat

Table 2.

DSP-4 treated rats with a history of stress exposure exhibit enhanced cytokine expression in the CeA following an acute social defeat challenge.

Analyte	Vehicle		DSP-4		Main Effect of Treatment (Tx)/Stress
	Control	Defeat	Control	Defeat	
IL-2	100.0± 11.8	193.9± 32.8	262.8± 84.7	701.6± 65.0 ^{γ**}	Stress x Tx: $F_{(1,16)}=6.0$, $p<0.05$
IL-5	100.0± 44.5	432.5± 223.9	658.3± 307.3	2346.9± 417.5 ^{β**}	Stress x Tx: $F_{(1,15)}=5.3$, $p<0.05$
IL-7	100.0± 33.7	762.0± 348.8	639.3± 265.5	2077.0± 183.0 ^{α*}	Stress: $F_{(1,17)}=13.2$, $p<0.01$ Tx: $F_{(1,17)}=10.3$, $p<0.01$
IL-10	100.0± 18.7	197.0± 36.4	175.2± 33.6	299.3± 34.0	Stress: $F_{(1,17)}=9.7$, $p<0.01$ Tx: $F_{(1,17)}=6.3$, $p<0.05$
IL-12 (p70)	100.0± 16.3	479.1± 236.2	601.3± 271.4	2140.6± 229.4 ^{β**}	Stress x Tx: $F_{(1,16)}=5.3$, $p<0.05$
IL-13	100.0± 15.2	286.9± 104.4	220.4± 70.8	543.6± 138.6	Stress x Tx: $F_{(1,16)}=5.1$, $p<0.05$
IL-17	100.0± 39.3	1145.5± 525.4	1435.7± 634.2	4540.3± 319.3 ^{β**}	Stress: $F_{(1,16)}=13.8$, $p<0.01$; Tx: $F_{(1,16)}=18.0$, $p<0.001$
IL-18	100.0± 2.6	114.9± 7.5	123.7± 12.2	172.5± 9.0 ^{β*}	Stress: $F_{(1,16)}=9.2$, $p<0.01$; Tx: $F_{(1,16)}=15.0$, $p<0.01$
EPO	100.0± 12.0	164.1± 24.4	152.0± 22.9	222.6± 22.1	Stress: $F_{(1,17)}=8.0$, $p<0.05$; Tx: $F_{(1,17)}=5.4$, $p<0.05$
GRO-KC	100.0± 14.6	198.6± 63.3	230.8± 71.4	658.3± 90.8 ^{β**}	Stress x Tx: $F_{(1,16)}=5.2$, $p<0.05$
GM-CSF	100.0± 26.2	437.9± 237.6	560.4± 249.0	1973.8± 204.3 ^{β**}	Stress x Tx: $F_{(1,16)}=5.2$, $p<0.05$
M-CSF	100.0± 17.2	180.2± 37.9	182.0± 43.3	462.3± 59.3 ^{β**}	Stress x Tx: $F_{(1,16)}=5.0$, $p<0.05$
MIP-1α	100.0± 30.6	731.1± 403.2	1090.7± 501.3	3958.0± 481.5 ^{β**}	Stress x Tx: $F_{(1,16)}=5.7$, $p<0.05$
MIP-3α	100.0± 11.4	132.0± 18.4	114.3± 12.3	231.2± 19.4 ^{β***}	Stress x Tx: $F_{(1,16)}=7.6$, $p<0.05$
RANTES	100.0± 18.2	400.0± 183.9	472.5± 190.3	1595.2± 141.8 ^{β**}	Stress x Tx: $F_{(1,16)}=5.3$, $p<0.05$
VEGF	100.0± 27.2	429.2± 166.7	307.0± 121.6	1078.1± 157.7 ^{α**}	Stress: $F_{(1,17)}=14.3$, $p<0.01$; Tx: $F_{(1,17)}=8.7$, $p<0.01$

EPO, erythropoietin; GRO-KC, chemokine ligand 1; GM-CSF, granulocyte macrophage colony stimulating factor; M-CSF, macrophage colony stimulating factor; MIP-1α, chemokine ligand 3; MIP-3α, chemokine ligand 20; RANTES, chemokine ligand 5; VEGF, vascular endothelial growth factor. Data presented as average percent of vehicle control ± SEM.

^α
p<0.05

^β
p<0.001

^γ
p<0.001 vs. vehicle treated rats with a history of defeat

*
p<0.05

**
p<0.01

p<0.01 vs. DSP-4 treated rats with a history of control.