# THE ROLE OF BRAIN INTERLEUKIN-1 IN STRESS-ENHANCED FEAR LEARNING

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ABSTRACT

MEGHAN E JONES: The role of brain interleukin-1 in stress-enhanced fear learning
(Under the direction of Donald T. Lysle)

Post-Traumatic Stress Disorder (PTSD) has been shown to be associated with pro-inflammatory markers, including elevated plasma levels of interleukin-1β (IL-1β). However, the precise role of neuroinflammation and central immune signaling on the development of this debilitating psychological disorder is not known. Here, we employed stress-enhanced fear learning, an animal model of the disorder, to examine the role of central IL-1β in PTSD. The results show that the severe stressor in SEFL induces a time-dependent increase in IL-1β immunoreactivity and mRNA expression within the dentate gyrus of the dorsal hippocampus. There was no increase in IL-1β in the basolateral amygdala or the perirhinal cortex. Moreover, blocking the action of IL-1β following the severe stressor with IL-1 receptor antagonist (10 µg, i.c.v., 24 and 48 hours after the stressor) prevented the development of SEFL. To provide further support for the role of IL-1β in the development of SEFL, we show that systemic morphine, a treatment which is known to reduce both PTSD and SEFL, also reduces IL-1β expression in the dorsal hippocampus induced by the severe stressor. These studies provide the first evidence that IL-1 is involved SEFL, and suggest that IL-1 signaling in the brain may play a critical role in the development of PTSD.

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# CHAPTER I
# INTRODUCTION

Psychopathology and disease states involving depression and anxiety have been associated with pro-inflammatory markers in both human populations and preclinical rodent models (Spivak, Shohat et al. 1997; Gill, Saligan et al. 2009; Stepanichev, Dygalo et al. 2014). However, the precise role of neuroinflammation and immune signaling in this context remains unclear. Cytokines, traditionally known for their role in the periphery in defense against infection and disease, have recently been revealed as important signaling molecules in neuro-glial communication pathways. For example, transgenic mouse lines deficient in central signaling involving pro-inflammtaory cytokines, such as interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α), consistently exhibit altered anxiety behavior in the elevated plus maze or open field test. (Silverman, Macdougall et al. 2007; Koo and Duman 2009). Further, an infusion of IL-1β into the lateral ventricle is sufficient to induce anxiety like behavior in the elevated plus maze (Koo and Duman 2009). Thus, the immune system has the capability to modulate central signaling and play an important role in behavior and the development of psychopathology.

IL-1β has been implicated in a wide range of behaviors. Early studies implicated this cytokine in a specific behavioral complex, sickness behavior, which is characterized by sleep disorders, anxiety, and diminished social interactions (Dantzer 2001). Importantly, IL-1β signaling is also critical for normal learning and memory processing. Accumulating evidence suggests that while a basal level of IL-1β is required for memory formation, both excessive

and insufficient amounts of IL-1β impair memory formation (Goshen, Kreisel et al. 2007). Further, converging evidence suggests that many of the neurobiological parallels between a disease state and a state of stress, both of which are often characterized by sickness behavior-like phenotypes, may be explained by IL-1β signaling (Maier 2003). There is evidence that IL-1β is up regulated throughout the brain after stress exposure (Nguyen, Deak et al. 1998; O'Connor, Johnson et al. 2003) and is important in stress-induced Hypothalamic-Pituitary-Adrenal (HPA) axis regulatory responses (Goshen, Kreisel et al. 2008).

The overall goal of the present study was to examine the role of brain IL-1β in the development of stress-enhanced fear learning (SEFL), an animal model of Post-Traumatic Stress Disorder (PTSD) developed by Fanselow and colleagues (Rau, DeCola et al. 2005). In PTSD, a severe traumatic event leads to debilitating psychological and physiological consequences characterized by chronic or exaggerated fear and anxiety (Gill, Saligan et al. 2009). PTSD affects 7.7 million Americans (NIMH), including up to 17% of combat veterans (Richardson, Frueh et al. 2010). However, there is no consistently effective treatment. While several animal models of PTSD have been developed (van der Kolk 1987; Yamamoto, Morinobu et al. 2009; Kaouane, Porte et al. 2012), the stress-enhanced fear learning paradigm (SEFL) is an outstanding model that captures critical components of human PTSD, including hyperarousal and hypersensitivity to future fear learning (Rau, DeCola et al. 2005). In the SEFL paradigm, rats previously exposed to a severe stressor show an enhanced or exaggerated learned fear response to a later mild form of stress in a distinct context, a hallmark symptom of PTSD.

The present studies test the hypothesis that the severe stressor in SEFL is capable of inducing alterations in IL-1β expression in the brain and that this expression is functionally relevant to the development of enhanced fear learning. In Experiment 1, we examined the time course of IL-1β expression following the severe stressor in SEFL. Our analysis focused on the dorsal hippocampus (DH, including the dentate gyrus (DG), CA1, and CA3), basolateral amygdala (BLA), and perirhinal cortex (PrhC) - three regions that have been shown to be critical to fear conditioning (Anagnostaras, Gale et al. 2001; Kim, Loucks et al. 2011; Acheson, Gresack et al. 2012; Kent and Brown 2012). In Experiment 2, we tested whether blocking central IL-1β signaling with an infusion of interleukin-1 receptor antagonist (IL-1ra) in the 48 hours following the severe stressor prevented the development of SEFL. In Experiment 3 we tested whether morphine administration attenuated the induction of IL-1β following the severe stressor in SEFL. This third experiment is based upon prior studies in our laboratory using this model showing that morphine administered after the severe stressor is effective in preventing the development of SEFL (Szczytkowski-Thomson, Lebonville et al. 2013). Moreover, clinical studies also show that morphine administration in the hours after a combat injury or single event trauma requiring hospitalization was associated with a reduced risk of PTSD (Bryant, Creamer et al. 2009; Stoddard, Sorrentino et al. 2009; Holbrook, Galarneau et al. 2010; Nixon, Nehmy et al. 2010; Melcer, Walker et al. 2014). Thus, in this third experiment, we hypothesize that IL-1β signaling is altered by morphine and therefore may be one mechanism through which morphine alters SEFL.

# CHAPTER II
# METHODS AND MATERIALS

## METHODS

### Animals

Male Sprague-Dawley rats (250-400g, Charles River Laboratories, Raleigh, NC) were housed individually under a reversed light-dark (12-hour) cycle with ad libitum access to food and water. Animals were handled regularly during a one week acclimation period prior to experimentation. All procedures were conducted in accordance with and approval by the UNC Institutional Animal Care and Use Committee.

### Experiment 1 - Analysis of IL-1β immunoreactivity and mRNA expression following the severe stressor in SEFL

Animals were exposed to only the initial stressor of our typical SEFL model. The SEFL model we employ has been described previously (Szczytkowski-Thomson, Lebonville et al. 2013) and is based on the model of Fanselow and colleagues (Rau, DeCola et al. 2005). Briefly, in Experiments 1a and 1b, animals were placed into Context A for 90 minutes to receive 15 scrambled foot shocks (2 mA, 1s) on a 6-minute variable time schedule. Control animals were exposed to Context A for an equivalent amount of time without foot shocks. In Experiment 1a brain tissue was processed for immunohistochemical analysis. These animals (N = 36, n = 6) were assigned to either a *no foot shock* group (NS Control) or a *foot shock* group and one of five time points: 0, 6, 24, 48, or 72 hours after the stressor. Animals in the NS Control group were sacrificed 24 hours after removal from Context A. This time point was chosen for our control group because any changes in IL-1β induced by learning

from mere exposure to a novel context would be evident after 24 hours (Goshen and Yirmiya 2009). All other animals were exposed to Context A and sacrificed via transcardial perfusion at the appropriate times.

In Experiment 1b brain tissue was processed for quantitative polymerase chain reaction (qPCR) analysis of mRNA. These animals (N = 32, n = 8) were assigned to either a *no foot shock* group (NS Control) or a *foot shock* group and one of three time points, 0, 24, or 48 hours after the stressor. Again, animals in the NS control group were sacrificed 24 hours after Context A exposure and all other animals were sacrificed via cervical dislocation at the appropriate times.

**Experiment 2 - Effect of a central infusion of IL-1ra on the development of SEFL**
**Surgical Procedures**

Animals were surgically implanted with intracerebroventricular (i.c.v.) cannulae. Animals were anesthetized with a 1.0 mg/kg intraperitoneal injection of 9:1 (vol:vol) ketamine hydrochloride (100 mg/ml) mixed with xylazine (100 mg/ml). Guide cannulae (26 Gauge, Plastics One, Roanoke, VA) were directed at the right lateral ventricle (AP -0.9 mm, ML -1.5 mm, DV -3.4 mm, relative to bregma). Animals were given three weeks for post-operative recovery. Correct placements were verified and animals with incorrect placement were dropped from the analysis.

**Procedures**

Animals (N = 36, n = 9) were assigned to a Context A treatment (*foot shock* or *no foot shock*) and a drug treatment (IL-1ra or vehicle) and exposed to the SEFL paradigm. The first day of the SEFL paradigm is the foot shock treatment in Context A, as described in Experiment 1. Context A (BRS/LVE, Laurel, MD; 26.7×24.8×30.7) and Context B (Med Associates, St. Albans, VT) were housed in separate rooms and had distinct textile, olfactory,

and auditory characteristics, as described previously (Szczytkowski-Thomson, Lebonville et al. 2013). Context B was set-up to record the animals' behavior using a video recording system (Sony Video Camera Model HDR-CX150). Seven days after Context A exposure, animals were placed into Context B for 30 minutes. On Day 8, animals were placed into Context B for a single scrambled foot shock (1mA, 1s) at 3 minutes, 12 seconds. On days 9, 10, 15 and 23 (Test Days 1, 2, 7 and 14), animals were placed in Context B for 8 minutes, 32 seconds and behavior was recorded to measure freezing behavior, a measure of learned fear, defined as a lack of all movement except that required for breathing. No animals in any group demonstrated freezing behavior to Context B during habituation/prior to the single foot shock, suggesting that there was no generalization of fear between contexts. Thus, any differences observed between treatment groups would reflect altered learning to the single foot shock in Context B. Videos were analyzed by raters blind to treatment condition.

IL-1ra (GenScript, Piscataway, NJ) was reconstituted in sterile saline (2.5 µg/µl). Twenty-four *and* 48 hours after removal from Context A, animals were microinfused with 4 µl of either IL-1ra (10 µg) or sterile saline vehicle at a rate of 2 µl/min. Injectors were left in place for 4 minutes to allow for diffusion. These time points were based on our earlier finding that morphine administration prevents the development of SEFL when administered 48 hours after, but not immediately after, Context A (Szczytkowski-Thomson, Lebonville et al. 2013).

**Experiment 3 - Analysis of IL-1β immunoreactivity following the severe stressor of SEFL in combination with morphine treatment**

Animals (N = 28, n = 7) were assigned to a Context A treatment (*no foot shock* or *foot shock*) and to a morphine treatment (morphine or vehicle). As described in Experiment 1, animals were only exposed to the initial stressor of the SEFL paradigm. Morphine was

obtained from the National Institute on Drug Abuse (NIDA) and dissolved in sterile saline (7.5 mg/ml). Immediately, 24, and 48 hours after removal from Context A, animals were administered either 7.5 mg/kg morphine or saline vehicle subcutaneously. One hour after the final injection, animals were sacrificed via transcardial perfusion.

**Immunohistochemical Analysis**

In Experiments 1a and 3, animals were deeply anesthetized with 9:1 (vol:vol) ketamine hydrochloride (100 mg/ml) mixed with xylazine (100 mg/ml) and transcardially perfused with cold phosphate buffer (PB; pH = 7.4) followed by 4% paraformaldehyde in 0.1 M PB. Brains were placed in 30% sucrose for cryoprotection and sliced into 40 µm sections. Tissue sections were washed for 15 minutes with 0.1 M PB, and incubated for 30 minutes at 80°C in sodium citrate (pH = 8.5) for antigen retrieval. Subsequently, sections were incubated for 30 minutes with streptavidin and biotin blocks (Vector Laboratories, Burlingame, CA, USA) and pre-incubated for 60 minutes with 3% normal goat serum and 0.3% Triton X-100 in 0.1 M PB. Sections were incubated overnight with rabbit anti-IL-1β (1:1000; Abcam, Cambridge, Ma) in 0.1 M PB with 3% normal goat serum and 0.3% Tween20. Similar to Johnson & Kan (Johnson and Kan 2010), sections were then incubated for 60 minutes with biotinylated goat anti-rabbit IgG (1:1000, Vector Laboratories, Burlingame, CA) in 1% normal goat serum and a streptavidin- Alexafluor488-conjugated tertiary antibody (1:1000, Life Technologies, Grand Island, NY) was used for visualization. Tissue sections labeled with only secondary and tertiary antibodies were used as secondary controls to ensure specificity of our primary antibody. Sections were mounted onto SuperFrost Plus slides (Fisher Scientific, Pittsburgh, PA) using Vectashield with DAPI hardset mounting medium (Vector Laboratories, Burlingame, CA).

Color images were captured through a digital camera (Roper Scientific), mounted on an optical microscope (BX-51, Olympus), and positive fluorescence was quantified using ImageJ (NIH). Images captured were between -2.76 mm and -4.2 mm relative to bregma (Paxinos and Watson, 1998). Three to six sections were analyzed per animal for each brain region and values were averaged and expressed as positive stain per 5 $\mu m^2$. Images were normalized to background fluorescence using a manual thresholding procedure such that the binary overlay completely covered all positive stain (similar to (Sugama, Takenouchi et al. 2011)). In addition, the number of IL-1β-positive cells in the all images taken from the dentate gyrus of the DH was counted manually. Images with high background that resulted from poor perfusion were dropped from the analysis. This decision was made at the time of thresholding and was made blind to the treatment condition. All thresholding and counting was conducted blind to the treatment condition. Publication images were compiled with Adobe Photoshop CS software. Color levels and background were reduced for optimal representation with levels and curves tools. Images from all experimental groups were treated equally.

**Quantitative Polymerase Chain Reaction (qPCR) analysis**

Following sacrifice, brains were extracted and the hippocampus was immediately dissected out on a cold plate. The dorsal third of the hippocampus was stored in 5X (vol/wt) RNAlater (Qiagen) and immediately flash frozen. Tissue was processed by the UNC Animal Clinical Chemistry and Gene Expression Laboratories according to protocols previously reported (Kim, Lee et al. 2002). Briefly, brain tissue was homogenized in RNAlysis buffer (PE Biosystems, Foster City, CA) with $Ca_2$ and $Mg_2$- free PB using a Fast Prep 120 mixer (QBIOgene, Vista, CA). RNA isolations were purified using the ABI Prism 6700 automated

nucleic acid workstation (PE Biosystems) according to the manufacturer's protocol. Real-time RT-PCR reactions were performed in the ABI Prism 7700 sequence detector (PE Biosystems) in a total volume of 30 µl (10 µl RNA, 20 µl reaction mixture). Each qPCR amplification was performed in duplicate: 30 min at 48°C for the RT reaction and 10 min at 94°C followed by 40 temperature cycles (15 sec at 94°C and 1 min at 60°C). Signal intensity was normalized to β-actin as an endogenous control. The nucleotide sequences of the PCR primers and fluorogenic probes used for the IL-1β and β-actin genes were as follows: IL-1β forward: 5'-GCC TCA AGG GGA AGA ATC TA-3', reverse: 5'-ATC CAC ACT CTC CAG CTG C-3', probe: 5'-FCT GTG TAA TGA AAG ACG GCA CAC CCA CQ-3'; β-actin forward: 5'-TGC CTG ACG GTC AGG TCA-3', reverse: 5'-CAG GAA GGA AGG CTG GAA G-3', probe: 5'-FCA CTA TCG GCA ATG AGC GGT TCC GQ-3'.

**Statistical Analysis**

For Experiments 1a and 1b, positive fluorescence and cell counts were analyzed using a one-way ANOVA with treatment group as the between subjects factor. For Experiment 2, a 2 x 2 ANOVA with Context A treatment and drug treatment as between subjects factors was used to analyze baseline freezing behavior. A 2 x 2 x 4 ANOVA with Context A treatment and drug treatment as between subjects factors and test day as a within subjects factor was used to analyze freezing behavior across test days. Lastly, for Experiment 3, positive fluorescence and cell counts were analyzed using a 2 x 2 ANOVA with Context A treatment and morphine treatment as between subjects factors. Significant interactions were examined using Fisher's least significant difference (LSD) post-hoc tests.

CHAPTER III
RESULTS

## RESULTS

### Experiment 1: Severe stressor enhances IL-1β immunoreactivity and mRNA expression in the dorsal hippocampus but not basolateral amygdala or perirhinal cortex

In Experiment 1a, IL-1β immunoreactivity was significantly enhanced by exposure to the severe stressor in a time-sensitive manner such that an increase was observed beginning 6 hours after the severe stressor and persisted through 72 hours (Figure 1). Severe stress significantly enhanced IL-1β immunoreactivity in the dentate gyrus (DG) both in terms of the percent area of positive fluorescence, $F(5, 25) = 6.472$, $p < .01$, and in terms of IL-1β-positive cell counts, $F(5, 16) = 13.544$, $p < .01$ . LSD post hoc tests revealed that compared to the *no foot shock* group, IL-1β expression was significantly enhanced at 6, 24, 48, and 72 hours ($p < .01$). In contrast, IL-1β expression in CA1, CA3, the BLA and the PrhC was not altered by the severe stressor at any of the time points (Figure 1).

In Experiment 1b, exposure to the severe stressor significantly enhanced IL-1β mRNA expression in the dorsal hippocampus in a time-dependent manner, $F(3, 26) = 6.927$, $p < .001$ (Figure 1). LSD post hoc tests revealed that, compared to the *no foot shock* group, IL-1β mRNA expression was significantly enhanced at 48 hours ($p < .01$). While mRNA and protein reflect distinct points of the IL-1 signal, the discrepancy in the time course of the effect between Experiments 1a and 1b may reflect the observation in Experiment 1 that the effect is specific to the DG. The tissue processed for qPCR included whole DH samples, and thus the time course of the effect may be different from the immunohistochemical analysis of

the DH since the CA1/3 did not achieve significance. Still, Experiment 1b further confirms an increase in IL-1β on a delayed timeline in the DH following foot shock.

**Experiment 2: IL-1ra prevents the development of stress-enhanced fear learning**

There was no effect of Context A treatment or drug treatment on baseline freezing in Context B, indicating no generalization of fear between contexts, $F(3, 38) = 1.91$, $p > .05$. A 2 x 2 x 4 ANOVA revealed a significant main effect of Context A treatment, $F(1, 36) = 11.08$, $p < .01$, and a significant main effect of drug treatment, $F(1, 36) = 12.99$, $p < .01$. There was also a significant effect of test day, indicating that conditioned fear diminished over time, $F(3, 34) = 21.76$, $p < .01$. Most interestingly, there was a significant Context A treatment by drug treatment interaction, $F(1, 36) = 6.497$, $p < .05$. LSD post hoc comparisons showed that the animals that received foot shock followed by vehicle exhibited significantly more freezing behavior compared to animals that received no foot shock in Context A followed by vehicle ($p < .01$), confirming a significant stress-enhanced fear learning effect. The animals that received foot shock in Context A followed by IL-1ra and the animals that received no foot shock in Context A followed by IL-1ra did not differ in freezing behavior exhibited in Context B, $p > .05$. Thus, these results show that stress-enhanced fear learning was prevented by an i.c.v. administration of IL-1ra (Figure 2).

**Experiment 3: Morphine administration attenuates severe stressor-induced IL-1β immunoreactivity in the dorsal hippocampus**

Severe stress significantly enhanced IL-1β expression within vehicle treated animals in the dentate gyrus of the DH, and this enhancement was significantly attenuated in animals that received foot shock followed by the morphine treatment (Figure 3). A 2 x 2 ANOVA revealed a main effect of Context A treatment, $F(1, 19) = 78.164$, $p < .001$, and a main effect of morphine treatment, $F(1, 19) = 13.651$, $p < .01$. Importantly, there was a significant

Context A treatment by morphine treatment interaction, $F(1, 19) = 31.050$, $p < .01$. LSD post hoc comparisons revealed that animals that received foot shock in Context A followed by vehicle treatment exhibited significantly enhanced IL-1β expression compared to animals that received no foot shock in Context A followed by vehicle treatment ($p < .001$), replicating the effect observed in Experiment 1a. Animals that received no foot shock in Context A followed by morphine treatment were not significantly different from animals that received no foot shock in Context A followed by vehicle. However, animals that received foot shock in Context A followed by the morphine treatment exhibited significantly less IL-1β expression compared to the group that received foot shock in Context A followed by vehicle, $p < .001$. Similar to Experiment 1a, all statistical analyses were confirmed by data obtained from counts of IL-1β-positive cells (Figure 3).

# CHAPTER IV
# DISCUSSION

The current studies demonstrate for the first time that exposure to a severe stressor that is capable of producing a PTSD-like phenotype in rats induces a time-dependent increase in IL-1β in the DH. Furthermore, central administration of IL-1ra prevents subsequent effects of that stressor, namely, the development of SEFL. Previously our laboratory established that morphine following the severe stressor also blocks the development of SEFL (Szczytkowski-Thomson, Lebonville et al. 2013). Here, we show that the same systemic morphine treatment also attenuates stress-induced IL-1β expression in the DH. These studies suggest that morphine may act through an IL-1β-dependent mechanism to alter SEFL. Collectively, the experiments provide new evidence that the expression of IL-1β in the brain following a severe stressor is a critical component of the development of SEFL, a model of PTSD.

In the present study, IL-1β was detected in three brain regions known to be important in PTSD and fear acquisition and expression—the hippocampus, amygdala, and perirhinal cortex (Kim, Loucks et al. 2011; Acheson, Gresack et al. 2012). These structures are extensively interconnected with multiple pathways involving bidirectional synaptic plasticity (Kent and Brown 2012). However, the effect of foot shock on IL-1β levels was observed only in the DH, with no change in immunoreactivity observed in the BLA or PrhC. More specifically, within the DH, the effect was most pronounced in the DG with much less expression that was not statistically altered by foot shock in CA1 and CA3. There may be other areas yet to be identified that also play an important role. For example, the ventral

hippocampus is thought to be important in the emotional and affective component of memory (Fanselow and Dong 2010) but it is not known what role IL-1 might have in the ventral hippocampus.

Several cell types are capable of producing IL-1β, and both neurons and glia express IL-1 receptors (Yabuuchi, Minami et al. 1994; Zhang, Sun et al. 2010). In astrocytes, IL-1β initiates the formation of IL-1β signaling intermediates, such as IL-1 receptor-associated kinase (IRAK; (Ringwood and Li 2008; Flannery and Bowie 2010)) and the subsequent activation of p38, extracellular signal-regulated kinase (ERK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB; (Guasch, Blanco et al. 2007; Huang, Smith et al. 2011)). Conversely, in hippocampal neurons, IL-1β activates p38 and ERK, but not NFkB (Huang, Smith et al. 2011). It should also be acknowledged that IL-1ra blocks multiple forms of IL-1 including IL-1α as well as IL-1β. The relative contributions of these different forms of IL-1 should be considered when determining the cellular sources of IL-1.

The mechanism through which IL-1β induces alterations in SEFL is not known. There are studies showing that neuroinflammation modulates synaptic plasticity in the hippocampus by influencing long term potentiation (LTP), a process thought to underlie some forms of learning. For example, TNF-α concentrations have been found to influence LTP in a dose-dependent manner such that extremely high TNF-α concentrations severely inhibited LTP in hippocampal neurons (Tancredi, D'Arcangelo et al. 1992). Furthermore, Schneider and colleagues found that tetanic stimulation used to induce LTP induced an increase in IL-1β mRNA expression specifically in hippocampal slices (Schneider, Pitossi et al. 1998) and that IL-1ra impaired the maintenance of LTP. Similarly, IL-1 receptor knockout mice did not exhibit LTP in the dentate gyrus (Avital, Goshen et al. 2003). Thus, there are a

number of studies indicating the cytokines are involved in both short- and long-term changes in synaptic plasticity.

Physiological evidence that cytokines influence synaptic plasticity is also supported by behavioral evidence that central cytokine signaling influences learning in several paradigms, including fear conditioning (Yirmiya and Goshen 2011; Szczytkowski, Lebonville et al. 2013). For example, acute administration of either TNF-α or IL-1β and chronic administration of interleukin-6 (IL-6) enhanced memory performance in a passive avoidance paradigm (Matsuda, Wen et al. 1996; Yirmiya, Winocur et al. 2002; Brennan, Beck et al. 2004). Furthermore, there is evidence for an effect of IL-1ra in step-down passive avoidance learning, but some report memory improvement (Depino, Alonso et al. 2004) while others have reported memory impairment(Yirmiya, Winocur et al. 2002). Early studies have also shown that morphine administration, which we show here to reduce hippocampal IL-1β, reduces memory for step down avoidance(Izquierdo 1979). Thus, it is clear that IL-1β can alter learning and memory, but the direction of the effects may depend upon such factors as the timing of the administration, the severity of stressor, and the type of learning being examined. The present findings are unique in that they begin to address the role of IL-1β in the neural plastic changes required to alter future learning, i.e., SEFL.

The present findings suggest that key neurobiological changes that render the animal hypersensitive to future fear learning occur on a delayed timeline, in concert with the timeline of some forms of memory consolidation. In support, Kozlovsky and colleagues (2012) found a similarly time-sensitive effect in the predator-scent stress paradigm, another animal model of PTSD (Kozlovsky, Zohar et al. 2012). An administration of corticotrophin-releasing hormone receptor antisense oligodeoxynucleotide into the DH 48 hours, but not

immediately, after severe stress significantly reduced the prevalence of the extreme behavioral response in the elevated plus maze and the acoustic startle response test, which is thought to reflect PTSD-like behavior (Kozlovsky, Zohar et al. 2012). This is consistent with prior data from our laboratory showing that the 48 hour time point is key (Szczytkowski-Thomson, Lebonville et al. 2013). Interestingly, the effect of morphine on the prognosis of PTSD in combat veterans was also time-dependent such that the relationship between morphine administration and a reduced likelihood of PTSD was strongest when it was administered in level 2 care, which occurs not immediately on the battlefield but within 72 hours of the initial trauma (Melcer, Walker et al. 2014). Frankland and Bontempi suggested a model of memory consolidation in which components are integrated in the hippocampus into a single memory trace which, *over time*, becomes consolidated to cortical structures and integrated with other experiences (termed *systems consolidation*) (Frankland and Bontempi 2005). One hypothesis is that SEFL (and PTSD) is (are) driven by an alteration in the later phases of memory consolidation following the trauma that results in the loss of contextual detail and renders the animal hypersensitive to future fear learning.

The present studies do not test whether morphine or IL-1ra impair contextual fear learning to Context A. We cannot assume that manipulations that attenuate SEFL, such as IL-1ra or morphine, also impair memory for Context A. Fanselow and colleagues (2005) showed that contextual fear learning to the context where the initial severe shock occurs is not critical to the expression of SEFL. They found that eliminating fear learning to Context A and extinction of Context A did not affect the expression of SEFL in Context B (Rau, DeCola et al. 2005). Taken together, it would be interesting to know how blocking IL-1

influences fear learning to Context A and further whether IL-1β expression is directly involved in the learning/enhanced learning to the subsequent foot shock in Context B.

PTSD is a prevalent problem in the United States with severe fiscal and emotional costs to society. The current findings provide strong evidence that the correlation between psychopathology and inflammatory dysregulation (e.g., (Bai, Su et al. 2014; Stepanichev, Dygalo et al. 2014) is important in behavioral outcomes. Moreover, Melcer and colleagues found that susceptibility to PTSD was related to Traumatic Brain Injury (TBI) severity in combat veterans (Melcer, Walker et al. 2014), further suggesting that central inflammation is related to the development of PTSD. The current studies show that altering central immune signaling has a direct causal effect on the development of a PTSD-like phenotype in rats. Further, we provide evidence that morphine, a treatment known to reduce the development of PTSD and SEFL, may act through an IL-1β-dependent mechanism, providing an exciting new target for the development of treatments for PTSD.

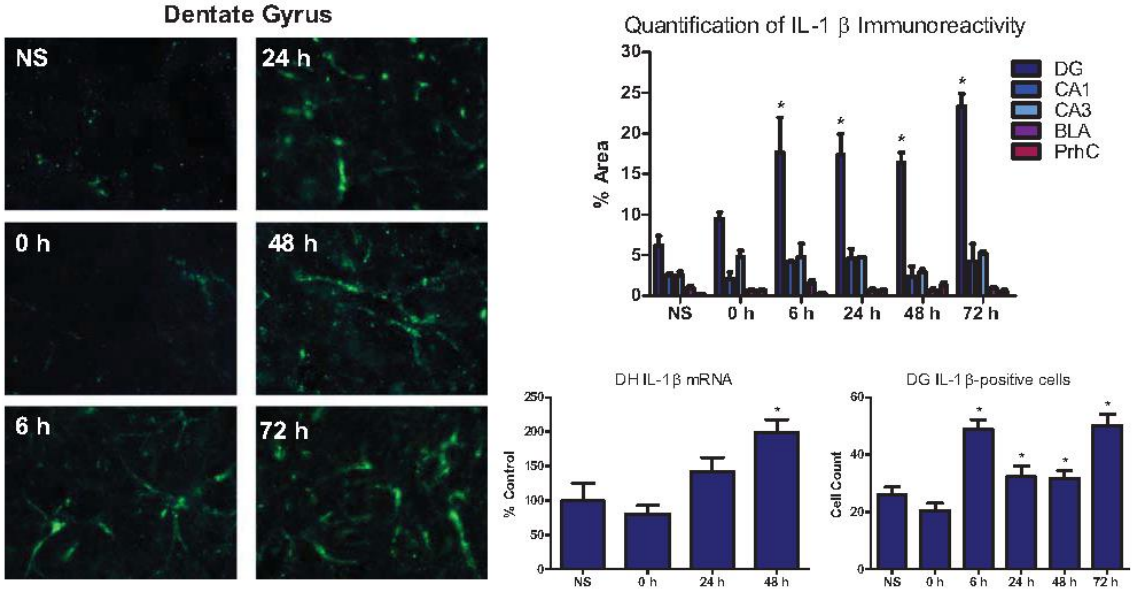


**Figure 1.** *Severe stress induced IL-1β in the dorsal hippocampus.* Representative images (20x) of IL-1β (green) in the dorsal hippocampus from animals in each of the six groups are shown on the left. The top right panel shows ImageJ analysis of positive fluorescence stain (top right) in the dentate gyrus, CA1, CA3, basolateral amygdala and perirhinal cortex. IL-1β immunoreactivity was significantly enhanced at 6 hours after (but not immediately after) severe stress only in the dentate gyrus of the dorsal hippocampus. This enhancement persisted through 72 hours (*$p < .05$ compared to NS control). qPCR analysis of mRNA expression confirmed the IL-1β increase in the dorsal hippocampus that we observed with immunohistochemistry (bottom right). IL-1β mRNA was enhanced at 48 hours following severe stress (*$p < .05$ compared to NS control). Further, IL-1β- positive cells were also counted in the dentate gyrus of the dorsal hippocampus (bottom right) and the same pattern was confirmed. (*$p < .05$ compared to 0 h). Error bars indicate SEM.

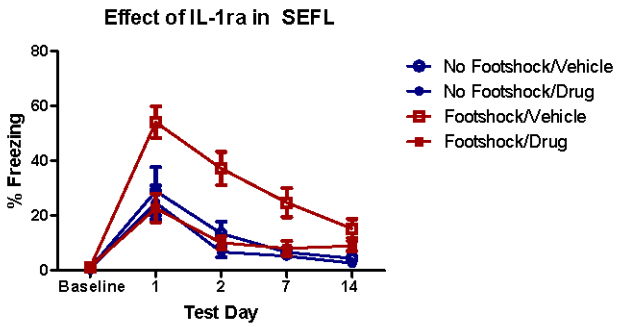


**Figure 2.** *IL-1ra prevents the development of SEFL.* IL-1ra infusion (i.c.v., 10 µg at 24 *and* 48 hours after removal from Context A) significantly reduced conditioned freezing behavior in Context B across test days. There were no significant differences between groups at baseline. Within the vehicle-treated groups, foot shock in Context A (open squares) significantly enhanced freezing behavior in Context B compared to animals that did not receive foot shock in Context A (open circles), demonstrating an SEFL effect. There was no effect of IL-1ra in the group that did not receive foot shock in Context A (closed circles). Most importantly, animals that received foot shock in Context A followed by IL-1ra infusion (closed squares) did not differ from the groups that did not receive foot shock in Context A. Error bars indicate SEM.

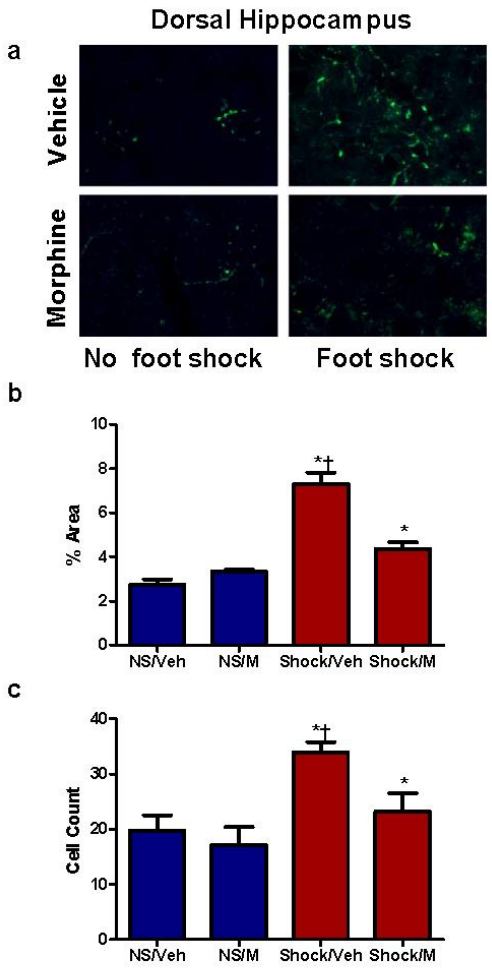


**Figure 3.** *Morphine attenuates stress-induced IL-1β in the dorsal hippocampus*. Representative images (20x) of IL-1β (green) in the dentate gyrus of the dorsal hippocampus from animals in each of the four groups are shown in (a). ImageJ analysis of positive fluorescence stain (b) revealed that in vehicle treated animals (NS/Veh and Shock/Veh), foot shock significantly enhanced IL-1β; however, morphine administration suppressed the induction of IL-1β in animals that received foot shock followed by morphine treatment (Shock/M). IL-1β- positive cells were also counted in the dentate gyrus of the dorsal hippocampus (c) and the same pattern was confirmed. (* greater than NS/Veh, $p < .05$; † greater than Shock/M, $p < .05$). Error bars indicate SEM.

WORKS CITED


1. Acheson, D. T., J. E. Gresack, et al. (2012). "Hippocampal dysfunction effects on context memory: possible etiology for posttraumatic stress disorder." *Neuropharmacology* **62**(2): 674-685.

2. Anagnostaras, S. G., G. D. Gale, et al. (2001). "Hippocampus and contextual fear conditioning: recent controversies and advances." *Hippocampus* **11**(1): 8-17.

3. Avital, A., I. Goshen, et al. (2003). "Impaired interleukin-1 signaling is associated with deficits in hippocampal memory processes and neural plasticity." *Hippocampus* **13**(7): 826-834.

4. Bai, Y. M., T. P. Su, et al. (2014). "Comparison of inflammatory cytokine levels among type I/type II and manic/hypomanic/euthymic/depressive states of bipolar disorder." *J Affect Disord* **166**: 187-192.

5. Brennan, F. X., K. D. Beck, et al. (2004). "Proinflammatory cytokines differentially affect leverpress avoidance acquisition in rats." *Behav Brain Res* **153**(2): 351-355.

6. Bryant, R. A., M. Creamer, et al. (2009). "A study of the protective function of acute morphine administration on subsequent posttraumatic stress disorder." *Biol Psychiatry* **65**(5): 438-440.

7. Dantzer, R. (2001). "Cytokine-induced sickness behavior: where do we stand?" *Brain Behav Immun* **15**(1): 7-24.

8. Depino, A. M., M. Alonso, et al. (2004). "Learning modulation by endogenous hippocampal IL-1: blockade of endogenous IL-1 facilitates memory formation." *Hippocampus* **14**(4): 526-535.

9. Fanselow, M. S. and H. W. Dong (2010). "Are the dorsal and ventral hippocampus functionally distinct structures?" *Neuron* **65**(1): 7-19.

10. Flannery, S. and A. G. Bowie (2010). "The interleukin-1 receptor-associated kinases: Critical regulators of innate immune signalling." *Biochemical Pharmacology* **80**(12): 1981-1991.

11. Frankland, P. W. and B. Bontempi (2005). "The organization of recent and remote memories." *Nat Rev Neurosci* **6**(2): 119-130.

12. Gill, J. M., L. Saligan, et al. (2009). "PTSD is associated with an excess of inflammatory immune activities." *Perspect Psychiatr Care* **45**(4): 262-277.

13. Goshen, I., T. Kreisel, et al. (2008). "Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression." *Mol Psychiatry* **13**(7): 717-728.

14. Goshen, I., T. Kreisel, et al. (2007). "A dual role for interleukin-1 in hippocampal-dependent memory processes." *Psychoneuroendocrinology* **32**(8-10): 1106-1115.

15. Goshen, I. and R. Yirmiya (2009). "Interleukin-1 (IL-1): a central regulator of stress responses." *Front Neuroendocrinol* **30**(1): 30-45.

16. Guasch, R. M., A. M. Blanco, et al. (2007). "RhoE participates in the stimulation of the inflammatory response induced by ethanol in astrocytes." *Exp Cell Res* **313**(17): 3779-3788.

17. Holbrook, T. L., M. R. Galarneau, et al. (2010). "Morphine use after combat injury in Iraq and post-traumatic stress disorder." *N Engl J Med* **362**(2): 110-117.

18. Huang, Y., D. E. Smith, et al. (2011). "Neuron-specific effects of interleukin-1beta are mediated by a novel isoform of the IL-1 receptor accessory protein." *J Neurosci* **31**(49): 18048-18059.

19. Izquierdo, I. (1979). "Effect of naloxone and morphine on various forms of memory in the rat: possible role of engogenous opiate mechanisms in memory consolidation." *Psychopharmacology (Berl)* **66**(2): 199-203.

20. Johnson, E. A. and R. K. Kan (2010). "The acute phase response and soman-induced status epilepticus: temporal, regional and cellular changes in rat brain cytokine concentrations." *J Neuroinflammation* **7**: 40.

21. Kaouane, N., Y. Porte, et al. (2012). "Glucocorticoids can induce PTSD-like memory impairments in mice." *Science* **335**(6075): 1510-1513.

22. Kent, B. A. and T. H. Brown (2012). "Dual functions of perirhinal cortex in fear conditioning." *Hippocampus* **22**(10): 2068-2079.

23. Kim, H. S., G. Lee, et al. (2002). "Molecular phenotyping for analyzing subtle genetic effects in mice: application to an angiotensinogen gene titration." *Proc Natl Acad Sci U S A* **99**(7): 4602-4607.

24. Kim, M. J., R. A. Loucks, et al. (2011). "The structural and functional connectivity of the amygdala: from normal emotion to pathological anxiety." *Behav Brain Res* **223**(2): 403-410.

25. Koo, J. W. and R. S. Duman (2009). "Interleukin-1 receptor null mutant mice show decreased anxiety-like behavior and enhanced fear memory." *Neurosci Lett* **456**(1): 39-43.

26. Kozlovsky, N., J. Zohar, et al. (2012). "Microinfusion of a corticotrophin-releasing hormone receptor 1 antisense oligodeoxynucleotide into the dorsal hippocampus attenuates stress responses at specific times after stress exposure." *J Neuroendocrinol* **24**(3): 489-503.

27. Maier, S. F. (2003). "Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition." *Brain Behav Immun* **17**(2): 69-85.

28. Matsuda, S., T. C. Wen, et al. (1996). "Interleukin-6 prevents ischemia-induced learning disability and neuronal and synaptic loss in gerbils." *Neurosci Lett* **204**(1-2): 109-112.

29. Melcer, T., J. Walker, et al. (2014). "Glasgow Coma Scores, early opioids, and posttraumatic stress disorder among combat amputees." *J Trauma Stress* **27**(2): 152-159.

30. Nguyen, K. T., T. Deak, et al. (1998). "Exposure to acute stress induces brain interleukin-1beta protein in the rat." *J Neurosci* **18**(6): 2239-2246.

31. Nixon, R. D., T. J. Nehmy, et al. (2010). "Predictors of posttraumatic stress in children following injury: The influence of appraisals, heart rate, and morphine use." *Behav Res Ther* **48**(8): 810-815.

32. O'Connor, K. A., J. D. Johnson, et al. (2003). "Peripheral and central proinflammatory cytokine response to a severe acute stressor." *Brain Res* **991**(1-2): 123-132.

33. Rau, V., J. P. DeCola, et al. (2005). "Stress-induced enhancement of fear learning: an animal model of posttraumatic stress disorder." *Neurosci Biobehav Rev* **29**(8): 1207-1223.

34. Richardson, L. K., B. C. Frueh, et al. (2010). "Prevalence estimates of combat-related post-traumatic stress disorder: critical review." *Aust N Z J Psychiatry* **44**(1): 4-19.

35. Ringwood, L. and L. Li (2008). "The involvement of the interleukin-1 receptor-associated kinases (IRAKs) in cellular signaling networks controlling inflammation." *Cytokine* **42**(1): 1-7.

36. Schneider, H., F. Pitossi, et al. (1998). "A neuromodulatory role of interleukin-1beta in the hippocampus." *Proc Natl Acad Sci U S A* **95**(13): 7778-7783.

37. Silverman, M. N., M. G. Macdougall, et al. (2007). "Endogenous glucocorticoids protect against TNF-alpha-induced increases in anxiety-like behavior in virally infected mice." *Mol Psychiatry* **12**(4): 408-417.

38. Spivak, B., B. Shohat, et al. (1997). "Elevated levels of serum interleukin-1 beta in combat-related posttraumatic stress disorder." *Biol Psychiatry* **42**(5): 345-348.

39. Stepanichev, M., N. N. Dygalo, et al. (2014). "Rodent Models of Depression: Neurotrophic and Neuroinflammatory Biomarkers." *Biomed Res Int* **2014**: 932757.

40. Stoddard, F. J., Jr., E. A. Sorrentino, et al. (2009). "Preliminary evidence for the effects of morphine on posttraumatic stress disorder symptoms in one- to four-year-olds with burns." *J Burn Care Res* **30**(5): 836-843.

41. Sugama, S., T. Takenouchi, et al. (2011). "Immunological responses of astroglia in the rat brain under acute stress: interleukin 1 beta co-localized in astroglia." *Neuroscience* **192**: 429-437.

42. Szczytkowski-Thomson, J. L., C. L. Lebonville, et al. (2013). "Morphine prevents the development of stress-enhanced fear learning." *Pharmacol Biochem Behav* **103**(3): 672-677.

43. Szczytkowski, J. L., C. Lebonville, et al. (2013). "Heroin-induced conditioned immunomodulation requires expression of IL-1beta in the dorsal hippocampus." *Brain Behav Immun* **30**: 95-102.

44. Tancredi, V., G. D'Arcangelo, et al. (1992). "Tumor necrosis factor alters synaptic transmission in rat hippocampal slices." *Neurosci Lett* **146**(2): 176-178.

45. van der Kolk, B. A. (1987). "The drug treatment of post-traumatic stress disorder." *J Affect Disord* **13**(2): 203-213.

46. Yabuuchi, K., M. Minami, et al. (1994). "Localization of Type-I Interleukin-1 Receptor Messenger-Rna in the Rat-Brain." *Molecular Brain Research* **27**(1): 27-36.

47. Yamamoto, S., S. Morinobu, et al. (2009). "Single Prolonged Stress: Toward an Animal Model of Posttraumatic Stress Disorder." *Depression and Anxiety* **26**(12): 1110-1117.

48. Yirmiya, R. and I. Goshen (2011). "Immune modulation of learning, memory, neural plasticity and neurogenesis." *Brain Behav Immun* **25**(2): 181-213.

49. Yirmiya, R., G. Winocur, et al. (2002). "Brain interleukin-1 is involved in spatial memory and passive avoidance conditioning." *Neurobiol Learn Mem* **78**(2): 379-389.

50. Zhang, R., L. Sun, et al. (2010). "Acute p38-mediated inhibition of NMDA-induced outward currents in hippocampal CA1 neurons by interleukin-1beta." *Neurobiol Dis* **38**(1): 68-77.