Interactive effects of a social stressor and Interleukin-6 injection on anxiety-related behaviors and expression of pro-inflammatory factors in male CD-1 mice

By

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Abstract

Stressful events induce a range of neurochemical and endocrine changes as part of the brain's adaptive response to such challenges. If the organism is repeatedly stressed, the development or exacerbation of psychopathologies, such as depression and anxiety, can ensue. A potential role for inflammatory factors in these disorders has emerged. In the present experiment, we investigated whether a challenge with interleukin (IL)-6, a pro-inflammatory cytokine commonly associated with depression, elicits anxiety-related behaviours and alters brain expression of inflammatory factors in CD-1 mice, and whether this differed based on the social stressor backdrop on which the cytokine was applied. The combination of an acute social stressor coupled with IL-6 injection enhanced expression of IL-1β, IL-6, and SOCS3 in a time- and brain region- dependant manner. The current results are consistent with the perspective that stress acts on the JAK/STAT pathway to enhance expression of certain pro-inflammatory factors, possibly leading to pathologies.

Acknowledgments

I would like to express my most sincere gratitude to Dr. Hymie Anisman for his patience, guidance, and wisdom throughout the years. Furthermore, I would like to give thanks to everyone in Dr. Anisman's laboratory, with special thanks to Marzena Sieczkos, Dr. Marie-Claude Audet, Dr. Shlomit Jacobson-Pick, and Dr. Jerzy Kulczycki for their constant assistance and encouragement. This research was supported by the Canadian Institutes of Health Research (CIHR).

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5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; BBB, blood brain barrier; BSA, Bovine Serum Albumin; CNS, central nervous system; COX, cyclooxygenase; CRH, corticotropin releasing hormone; CVOs, circumventricular organs; DA, dopamine; EPM, elevated plus maze; HPA, hypothalamic-pituitary-adrenal axis; ICV, intracerebroventricular; IFN, interferon; IL, interleukin; JAK, Janus kinase; KO, knockout; LPS, lipopolysaccharides; mPFC, medial prefrontal cortex; NE, norepinephrine; NSAID, non-steroidal anti-inflammatory drugs; OF, open field; PBS, phosphate buffered saline; PG, prostaglandins; PNMT, Phenylethanolamine N-methyltransferase; PTSD, post-traumatic stress disorder; RT-QPCR, reverse transcription-quantitative polymerase chain reaction; SEM, standard error of the mean; SNRI, selective norepinephrine reuptake inhibitor; SOCS, suppressors of cytokine signalling; SSRI, selective serotonin reuptake inhibitor; STAT, Signal Transducer and Activator of Transcription; TNF, tumor necrosis factor.

1. Introduction

Stressful events induce a range of neurochemical and endocrine changes as part of the brain's adaptive response to such challenges. While these changes are meant to prepare the organism to deal with the insult, if the organism is exposed to a stressor for a prolonged period or if it is repeatedly stressed, then adverse effects can arise, such as the development of affective disorders, such as anxiety and depression (Anisman et al., 2008).

Stressors, broadly defined, comprise anything that engenders a physiological response and is perceived as being stressful. Stress responses are the reaction to restore homeostasis, or allostasis (a response akin to that associated with homeostasis, but that occurs quickly in response to stressors), and are apparent under a wide variety of situations (McEwen, 2000). These include physical and psychological/social challenges, as well as immune insults that prompt a range of physiological reactions reminiscent of those provoked by other stressors (Anisman et al., 2008). Indeed, inflammatory immune activation is elicited by stressors, which then promote neurochemical changes that ultimately increase susceptibility to affective pathologies (Anisman et al., 2008). It has been suggested that the link between inflammation and depressive illness lies in cytokines, the signalling molecules of the immune system. Certainly, individuals suffering from chronic depression have higher levels of circulating pro-inflammatory cytokines (Anisman et al., 1999; Dowlati et al., 2010), while immunogenic challenges can elicit depressive-like behaviors (Reichenberg et al., 2001). As well, administration of the inflammatory cytokines IL-1β and TNF-α were found to promote alterations in monoamine utilization and turnover in certain areas of the rodent brain (Dunn, 2006; Hayley et al., 1999), as well as sickness behaviours that might reflect somatic aspects of depression (Dantzer et al., 2001).

In contrast to IL-1β and TNF-α, both of which have been extensively examined, there has been relatively little published research concerning the impact of IL-6 in depressive illness. This is puzzling given that depression was accompanied by elevated circulating IL-6 levels, which diminished with the abatement of symptoms following successful pharmacotherapy (Basterzi et al., 2005). Likewise, in humans, stressors, such as caregiving, were accompanied by higher levels of IL-6 (Dowlati et al., 2010; McDade et al., 2006). Accordingly, the present investigation was conducted to determine whether systemic administration of IL-6 influences levels of other pro-inflammatory factors, and whether this outcome varies with the social stressor backdrop upon which cytokine activation occurs.

Stressful events, as already mentioned, engender a broad range of biological responses that are thought to reflect adaptive changes to meet environmental demands. Activation of the autonomic nervous system, for example, is one of the immediate responses to challenges. Stressors also trigger activation of the hypothalamic-pituitary-adrenal (HPA) axis, which has been well described. Briefly, stressors cause elevations in levels of corticotropin releasing hormone (CRH) from the hypothalamus, which in turn, triggers release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. Consequently, the adrenal glands are stimulated by ACTH to release corticosteroid hormones into the blood stream (Sapolsky et al., 2000). These glucocorticoids serve many physiological and immune functions, such as the recruitment of substrates that increase energy metabolism from fats, carbohydrates, and proteins, as well as preventing excessive activation of the inflammatory and immune response, thereby limiting the potential for the development of autoimmune disorders, such as rheumatoid arthritis (Sapolsky et al., 2000). This reaction is adaptive and promotes recovery from stress in the short run, but it was not meant to deal with a chronic or repeated stressor. When the HPA system is

constantly activated, the price to maintain allostasis becomes greater, potentially leading to pathology (allostatic overload). It is important to consider that genetic and environmental factors also play a role in determining the organism's ability to cope with, or its "resilience" to, repeated stressors (Tannenbaum and Anisman, 2003).

The organism's response to stressors is dependent upon several factors, such as the chronicity, severity, and predictability of the stressor, as well as a history of early life stress (Anisman et al., 2003; Felitti et al. 1998). Although acute stressors typically have only transient effects on physiological processes (Sapolsky et al., 2000), these challenges can promote the sensitization of neuronal processes so that later insults may have more severe consequences (Anisman et al., 2003; Belda et al., 2008). The type of the stressor itself also plays a role in determining biochemical changes, as systemic stressors (being related to an internal body system, such as a cardiovascular or immune processes) involve signalling pathways that differ from those associated with processive stressors (involving higher processing of threats, such as a predator cues; Herman & Cullinan, 1997).

As indicated earlier, with repeated stressor experiences a series of adaptive changes occur, some of which involve elevated neurochemical activity. This, however, increases the likelihood that these systems will be overly taxed (allostatic overload), and might favor the development of pathology. For instance, the inability to shut down the stress response or over-activation of the HPA axis will cause an excess of corticosteroid hormones to be produced which has been associated with depressive illness (Holsboer, 2000). In contrast, the failure to sufficiently activate the stress response could lead to compensatory mechanisms being initiated, such as an increase in levels of immune factors and pro-inflammatory cytokines that are normally suppressed by glucocorticoids. Indeed, inbred rats genetically modified to have a

blunted HPA axis response show high susceptibility to autoimmune inflammatory disorders, such as arthritis, while those with a hyperactive HPA response display relatively high resistance to the same autoimmune diseases (Sternberg et al., 1989).

Inflammatory activation is among the many biological changes induced by stressors, and has been linked to depressive illness. In this regard, depression was associated with an increase of cell-mediated immunity, as seen by increased number of leukocytes, monocytes, and neopterin in depressed individuals (Kronfol and House, 1989; Muller et al., 1989; Dunbar et al., 1992) yet decreases in the number of lymphocytes and natural killer cells were also observed (Kronfol et al., 1983). While these observations are merely correlational, a causal link comes from the findings that individuals undergoing immunotherapy for hepatitis C and some types of cancer develop depressive-like symptoms (Valentine & Meyers; 2005) which were ameliorated when the treatment was discontinued (Loftis & Hauser, 2004). Similarly, treatment with antidepressants (Schramm et al., 2000) was also able to attenuate the depressive symptoms emerging from IFN-α immunotherapy.

It is now certain that communication occurs between the immune, the neuroendocrine, and the central nervous systems. For example, when healthy individuals were exposed to an immune challenge, such as treatment with an endotoxin, a behavioural and neuroendocrine profile emerged that was reminiscent of the outcomes seen in response to stressors, as well as resembling depressive-like symptoms (Reichenberg et al., 2001; Anisman & Merali, 1999). Endotoxins, such as lipopolysaccharides (LPS), cause an inflammatory response, and thus induce the synthesis of endogenous cytokines. It has been proposed that cytokines represent a possible link between immune dysregulation and depression. Indeed, injection of TNF-α is able to activate the HPA axis and induce sickness behaviours like those seen in response to LPS (Michie

et al., 1988; Bluthé et al., 2000a). Similarly, injection of IL-1β elicited a range of behavioural features reminiscent of depressive illness, such as reduced exploratory behaviours (Kent et al., 1992) as well as sickness and anhedonia (Dantzer et al., 2001; Kluger et al., 1998). Some of these behaviours were reduced upon antidepressant treatment (Merali et al., 2003). Interestingly, the IL-1β receptor antagonist IL-1RI prevented sickness behaviour upon IL-1β injection, but not LPS-induced sickness, suggesting that TNF-α simply takes the place of IL-1 in being the main mediator of these behaviours in the brain (Bluthé et al., 2000a). Furthermore, both cytokines increased the utilization and synthesis of monoamines in the hippocampus and other brain regions (Linthorst et al., 1995; Hayley et al., 1999). As expected, administering both IL-1β and TNF-α together causes an augmented effect on HPA activation and corticosterone levels in mice (Brebner et al., 2000).

The role of Interleukin-6 in depressive illness has not been as thoroughly examined as that of IL-1β and TNF-α, despite levels of circulating IL-6 being increased in depression sufferers, and antidepressant treatment being able to ameliorate this effect (Basterzi et al., 2005). A recent meta-analysis indeed found that depressed individuals display higher basal levels of the pro-inflammatory cytokines IL-6 and TNF-α compared to healthy controls (Dowlati et al., 2010). Higher levels of IL-6 were associated with lower adult hippocampal neurogenesis, a phenomena commonly seen in depressed individuals (Campbell et al., 2004; Marsland et al., 2008). Yet still, the few animal studies that have been conducted have largely pointed to IL-6 treatment having marginal effects despite this cytokine's potential involvement in depressive disorders. Accordingly, the present investigation was undertaken to determine whether: (a) a single systemic IL-6 injection influenced a range of pro-inflammatory factors and behavioural outputs. (b) If it has different effects depending on the backdrop of stress it is applied on (acute or

repeated), and (c) if these effects vary in a time-dependent manner. Thus, in Experiment 1, we assessed whether a single IL-6 injection applied in conjunction with a single psychosocial stressor event induced effects on levels of brain cytokines and other inflammatory factors similar to those seen with IFN-α treatment. In Experiment 2, we applied the same protocol with the goal of assessing the mice's performance in the open field (OF) and elevated plus maze (EPM) behavioural tests of anxiety 1.5 hr after injection. Finally, in Experiment 3 we observed whether a repeated social stressor interacted with a single IL-6 injection to influence molecular and inflammatory factors at 3 different time points (1.5 hr, 3 hrs, and 24 hrs after injection) to further examine the dynamic nature of the changes that occur in response to stressors.

2. Materials & Methods

2.1 Experimental Animals

Male CD-1 mice were purchased (Charles River, 6 - 8 weeks old) and individually housed in the vivarium; given a week to accumulate to the lab environment before the start of the experiment. Additionally, retired breeders (Charles River, 6 months old) were purchased to be used as part of the social stress procedure.

All the animals were housed in standard (27 x 21 x 14 cm^3) polypropylene cages in the vivarium. Moreover, the temperature in the animal rooms was maintained at around (20 ± 3) ^oC along with a 12-hour light cycle starting at 8:00 AM and ending at 8:00 PM. The mice had free access to food (standard mouse chow, Teklad 2014) and water was provided *ad libitum*. All procedures were conducted between 8:30 AM and 1:00 PM to minimize effects related to diurnal factors. All procedures were conducted In accordance with the guidelines set out by the Canadian Council on Animal Care and were approved by the Carleton University Animal Care Committee.

Procedures

2.2 Experiment 1: Effects of IL-6 and a social stressor on cytokine mRNA expression in the brain

Forty CD-1 mice were randomly divided into groups of stressed and non-stressed animals. On the day of the experiment, the mice in the stress group underwent a single stressful encounter with a retired breeder. This consisted of placing the retired breeder in the cage of the naïve mouse. In most resident-intruder paradigms the younger animal is placed in the cage of the retired breeder. However, as the CD-1 mouse is a fairly aggressive strain, the retired breeder was placed in the cage of the younger mouse in an effort to moderate the degree of aggression that

would occur. The two mice were allowed to interact until their first physical altercation, after which they were separated and the aggressor was returned to his home cage.

Mice (regardless of their stress condition) were injected intraperitoneally with either vehicle (0.3ml Bovine Serum Albumin, BSA. 0.1% in PBS) or murine interleukin-6 (4μg dissolved in 0.3 ml BSA). For the mice that were socially stressed, this occurred 15 minutes after the start of the interaction. After injection, all the animals were returned to their home cages, and then decapitated 1.5 hr afterward.

2.3 Experiment 2: Effects of IL-6 and a social stressor on anxiety-related behavioural tests

A second set of 40 CD-1 mice underwent an identical procedure to that of Experiment 1, with the exception that the mice were not decapitated, but were instead tested in two behavioral paradigms beginning 1.5hr after injection. The first test comprised brief open field assessments (5 min) in which mice were individually placed in a 45 × 45 cm open field, with an inner square of 21 × 24 × 24 cm. The time elapsed to enter the center area and how much time was spent there were both recorded by videotape. The second test, the elevated plus maze (EPM), was conducted one minute after the open field. The plus maze comprised two arms surrounded by 21cm high walls (closed arms), and two open arms with no walls (24.8 x 7.7 cm). Over a 5-minute period, the number of entries into open and closed arms, as well as the time spent there, were recorded (an entry was defined as the mouse putting all four paws into one of the arms).

2.4 Experiment 3: Effects of combining IL-6 with an acute or repeated social stressor on plasma corticosterone and cytokine mRNA expression in the brain at several different time points

Mice (N = 6/group) were subdivided so that they were exposed to one of three stressor condition (no stress, acute, or repeated), treatment with IL-6 vs. vehicle, and the time of decapitation (1.5 hr, 3 hr, or 24 hrs after injection). The repeated stressor procedure comprised

mice being exposed to an older, more aggressive retired breeder for a 15 min period. Following a single physical altercation a metal mesh divider separated the two mice for the remainder of the 15 min sessions after which the aggressor was returned to its home cage. On the 7th and final day, the naive mouse was injected after the end of the interaction and then decapitated at the specified decapitation time.

Mice that were in the acute stress condition were only allowed to interact with the retired breeders once, on the day of their injection. Mice that were in the non-stressed group were never exposed to the retired breeders.

2.5 Blood Collection and Corticosterone preparation

Trunk blood was collected (over 15-20s) in tubes containing 10mg EDTA and then centrifuged for 20 minutes at 2000g to isolate the blood serum. The plasma aliquots were stored at -80 ^{o}C, and the plasma corticosterone levels were assayed using a radioimmunoassay kit according to the manufacturer's protocol (ICN Biomedicals, CA, USA).

2.6 Brain Slice Preparation

Brains were rapidly removed and placed on a stainless steel brain matrix positioned on top of a block of ice. This brain blocker had a series of slots (spaced 500 µm apart) that served as guides for razor blades, to provide coronal brain sections. Areas of interest for cytokine determination included the medial prefrontal cortex (PFC) and the hippocampus (Hipp) as these regions have been implicated in major depressive illness. Tissue from these regions was collected by micro-punch using a hollow 20-gauge microdissection needle, following a published mouse atlas (Franklin & Paxinos, 1997).

2.7 *Reverse transcription-Quantitative polymerase chain reaction analysis (RT-QPCR)*

Total brain RNA was isolated and purified by standard methodologies employing Trizol

according to the manufacturer's protocol (Invitrogen; Burlington, ON, Canada). The total RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen; Burlington, Ontario), and aliquots of this reaction were used in simultaneous QPCR reactions.

A BIORAD MYIQ real time thermocycler was used to collect the data. For QPCR, SYBR green detection was used according to the manufacturer's protocol (Stratagene Brilliant QPCR kit). Each of the PCR primer pairs generated amplicons between 129 and 200 base pairs. Amplicon identity was checked by restriction analysis. The primer efficiency was determined from the slope relation between absolute copy number or RNA quantity and the cycle threshold using the BIORAD MYIQ software. All primer pairs had a minimum of 90% percent efficiency. Primers that amplify Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), Beta-Actin, and synaptophysin mRNA were used as a control to normalize the data. To compensate for inter-individual variability that ordinarily exists within the assay, the expression of each species was normalized by subtracting its C_t from the housekeeping C_t. Following the procedure described by Livak & Schmittgen, 2001, normalized brain mRNA expression values were converted to fold changes relative to controls. Primer sequences were as follows: Mus GAPDH, forward: GGT CGG TGT GAA CGG ATT TG, reverse: TGC CGT TGG AGT CAT ACT G. Mus β-actin, forward: GAA CCC TAA GGC CAA CCG TG, reverse: GGT ACG ACC AGA GGC ATA CAG G. Synaptophysin, forward: GGA CGT GGT GAA TCA GCT GG, reverse: GGC GAA GAT GGC AAA GAC C. Mus IL-1β, forward: TGT CTG AAG CAG CTA TGG CAA C, reverse: CTG CCT GAA GCT CTT GTT GAT G. Mus IL-1R1, forward: ATG AGT TAC CCG AGG TCC AGT G, reverse: TAC TCG TGT GAC CGG ATA TTG C. Mus TNF-α, forward: GTA GCC CAC GTC GTA GCA AA, reverse: GCT GGC ACC ACT AGT TGG TT. Mus TNFR, forward: CAG AAC ACC GTG TGT AAC TGC C, reverse: GGG TTT GTG ACA TTT

GCA AGC. Mus IL-6, forward: ACG GCC TTC CCT ACT TCA CA, reverse: TGC CAT TGC ACA ACT CTT TTC TC. Mus IL-6R, forward: CTC TCC AAC CAC GAA GGC TG, reverse: TGC AAC GCA CAG TGA CAC TAT G. Mus IL-10, forward: AGGCGCTGTCATCGATTTCTC, reverse: CATGGCCTTGTAGACACCTTGG. SOCS3, forward: GCG GGC ACC TTT CTT ATC C, reverse: TCC CCG ACT GGG TCT TGA C.

2.8 Statistical Analysis

For Experiments 1 and 2, a 2 (stressed vs. non-stressed) x 2 (IL-6 vs. vehicle) analysis of variance (ANOVA) was employed to determine statistical significance. For Experiment 3, a 3 (acute stressor vs. repeated stressor vs. non-stressed) x 2 (IL-6 vs. vehicle) x 3 (1.5 hr vs. 3 hr. vs. 24 hr). ANOVA was employed to determine statistical significance. Follow-up comparisons of the means comprising main effects or simple effects of significant interactions were conducted using t tests with Bonferonni corrections to maintain α at 0.05. Data are presented as mean ± SEM.

3. Results

3.1 Experiment 1: mRNA expression of pro-inflammatory cytokines in the prefrontal cortex (PFC)

Figure 1 shows the effects of a social stressor in the form of an aggressive encounter, and how this stressor interacts with an intraperitoneal injection of IL-6 to influence mRNA levels of the pro-inflammatory cytokine IL-1β. Examining fold changes of IL-1β mRNA in the prefrontal cortex, an interaction between the drugs and the stress condition was found, $F(1, 31) = 7.09$, $p < 0.05$. Follow up t tests with a Bonferonni correction indicated that although IL-6 injection did not alter expression of IL-1β in the prefrontal cortex of non-stressed mice, those mice that had been exposed to the aggression stressor exhibited higher levels of IL-1β mRNA when they were injected with IL-6 than when they were treated with vehicle ($p < 0.01$). Similarly, a Drug x Stressor interaction was also observed in the PFC levels of IL-1R1, $F(1, 31) = 12.74$, $p < 0.01$. The follow-up t tests showed that mice that experienced the aggressive bully encounter and the IL-6 injection displayed a more pronounced increase in PFC mRNA 1.5 hr after injection compared to all other groups ($p < 0.001$, see Figure 1).

The mRNA expression of IL-6 within the PFC varied as a function of the Drug, $F(1, 30) = 5.54$, $p < 0.05$, and the Stressor condition, $F(1, 30) = 9.356$, $p < 0.01$. Although the interaction between those two factors was just shy of significance, $F(1, 30) = 3.24$, $p = 0.08$, follow up tests were conducted to determine the simple effects comprising this interaction as a specific hypothesis had been made concerning this interaction. As seen in Figure 2, it was found that among mice that had been stressed and injected with IL-6, prefrontal cortical expression of IL-6 was greater than that of their vehicle injected counterparts ($p < 0.01$). Likewise, expression of the

receptor IL-6R was dependant on the Drug injection $F(1, 31) = 5.60$, $p < 0.05$, and the Stressor condition $F(1, 31) = 6.48$, $p < 0.05$, with the interaction between the two variables falling short of significance $F(1, 31) = 3.61$, $p = 0.06$. As seen in Figure 2, and determined by post-hoc tests, mice that were stressed and injected with IL-6 exhibited a significant increase of mRNA expression of IL-6R compared to controls ($p < 0.01$).

Unlike the pattern seen with the other pro-inflammatory cytokines, PFC expression of TNF-α was only influenced by the Stressor condition, $F(1, 31) = 9.93$, $p < 0.01$. As seen in Figure 3, the stressor caused reduced TNF-α response compared to the non-stressed animals. This pattern was also observed in the IL-6 injected mice, though it was not significant. Figure 3 also shows that mRNA levels of TNFR expression varied as a function of the Drug x Stressor interaction, $F(1, 31) = 4.17$, $p < 0.05$. Follow-up tests confirmed that 1.5 hr after IL-6 injection, stressed mice showed a marked increase of TNFR expression relative to the remaining groups groups ($p < 0.001$).

Finally, levels of the cytokine inhibitor SOCS3 in the PFC varied as a function of the Drug x Stressor interaction, $F(1, 33) = 6.41$, $p < 0.05$. Bonferroni corrected t tests showed that injection of IL-6 alone significantly increased SOCS3 mRNA expression ($p < 0.05$), but when the cytokine injection was combined with an aggressive encounter, the fold change increase was enhanced relative to that of mice that received only one of the treatments (see Figure 4) ($p < 0.001$). Indeed, as the stressor treatment alone had absolutely no effect at all, the combined effects of the stressor and the IL-6 treatment reflected a synergistic action of these treatments.

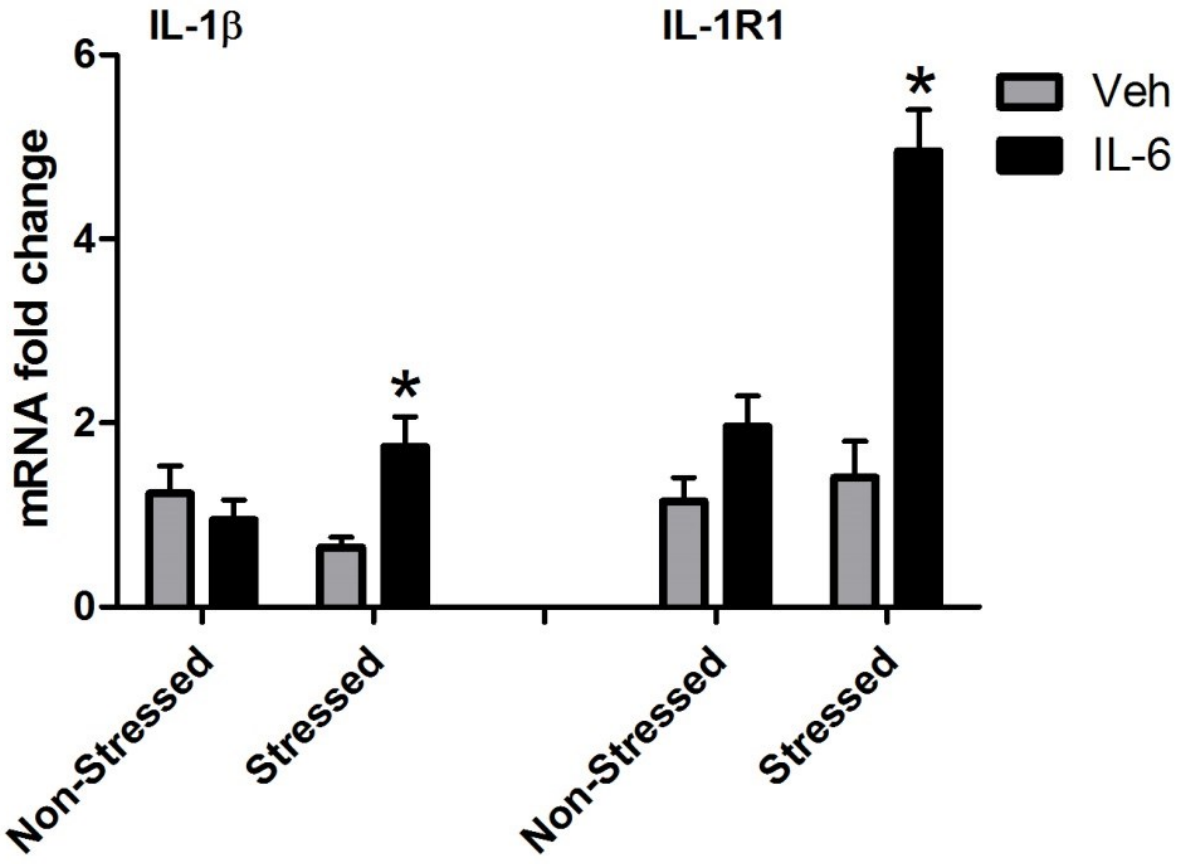

Figure 1. Prefrontal cortex (PFC) mRNA expression (mean fold changes ±SEM) of IL-1β and its receptor, IL1-R1, among CD-1 mice that had been exposed to an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4μg). * $p < 0.05$ enhanced response relative to vehicle treated mice and relative to non-stressed mice. N = 7-10/group.

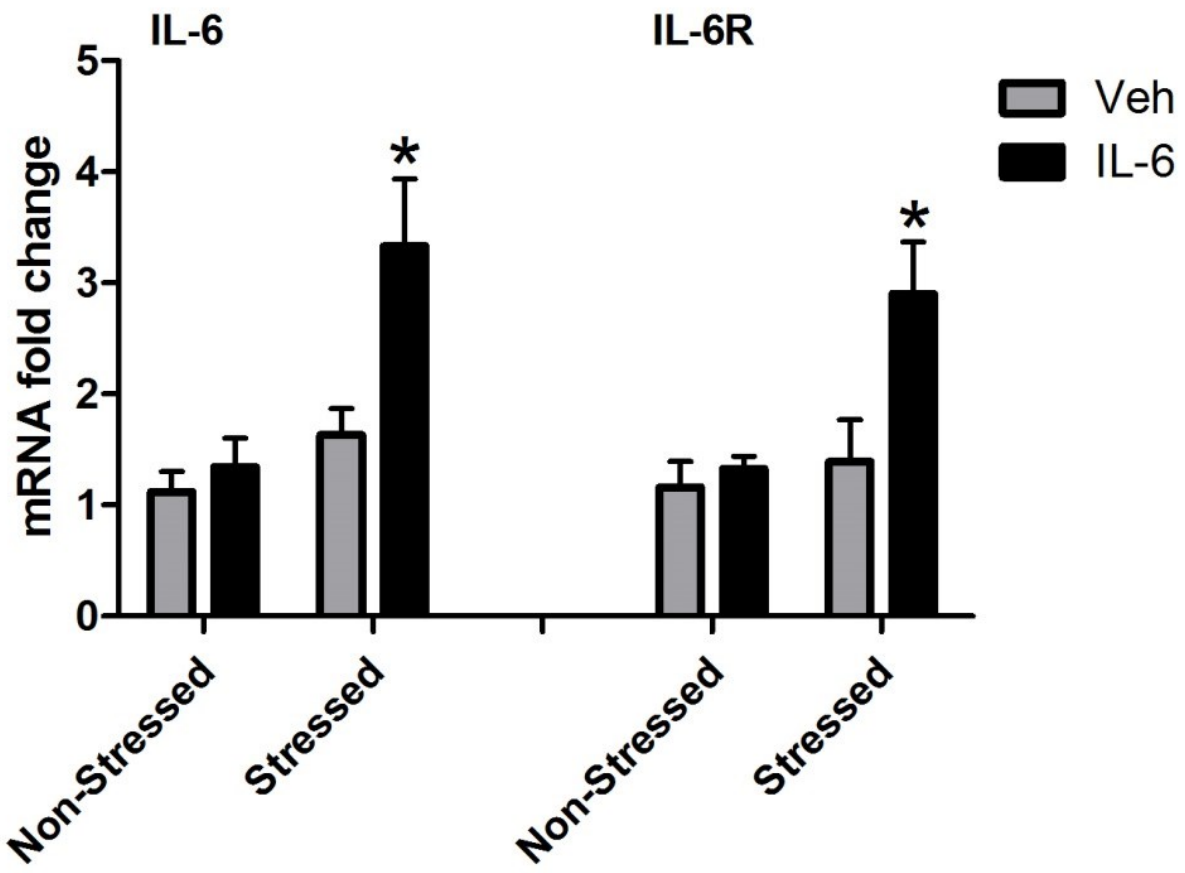

Figure 2. Prefrontal cortex (PFC) mRNA expression (mean fold changes ±SEM) of IL-6 and its receptor, IL-6R, among CD-1 mice that had been exposed to an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4μg). * $p < 0.05$ enhanced response relative to vehicle treated mice and relative to non-stressed mice. N = 7-10/group.

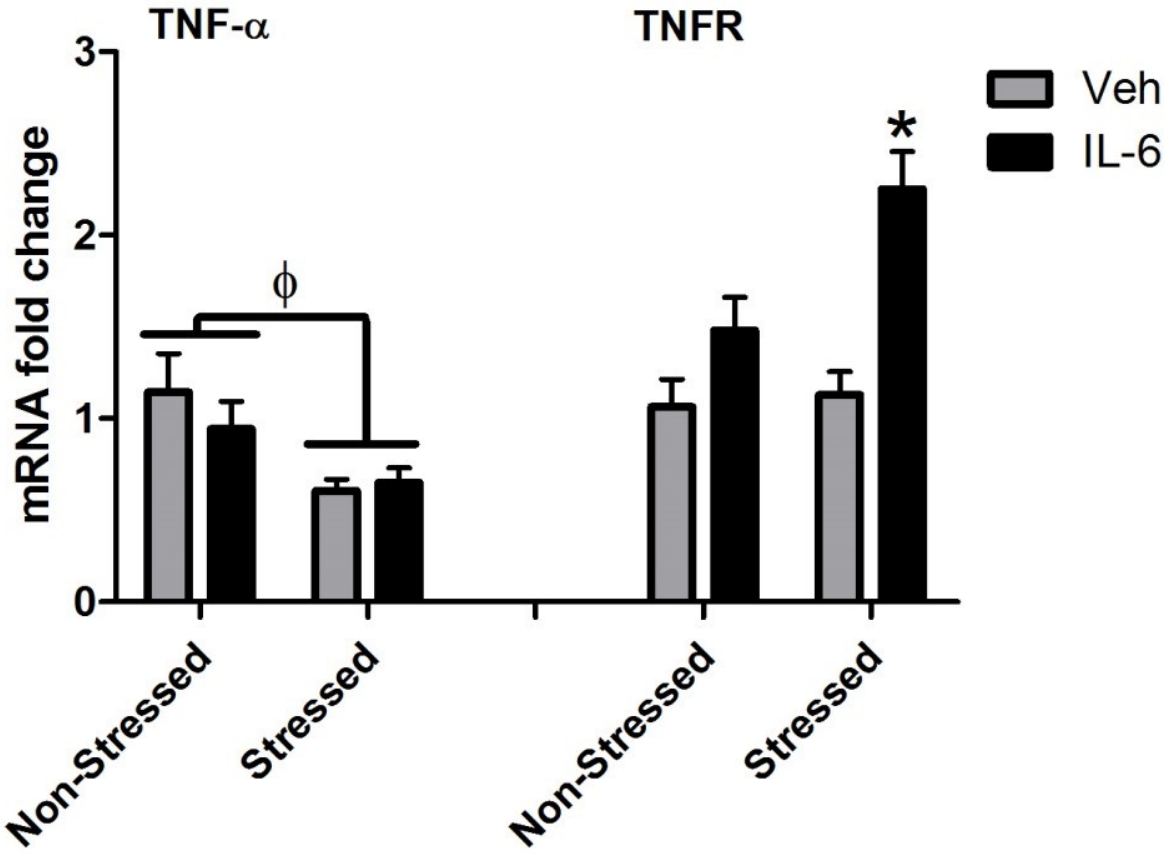

Figure 3. Prefrontal cortex (PFC) mRNA expression (mean fold changes ±SEM) of TNF-α and its receptor, TNFR, among CD-1 mice that had been exposed to an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4μg). * $p < 0.05$ enhanced response relative to vehicle treated mice and relative to non-stressed mice. Φ $p < 0.05$ diminished response relative to non-stressed mice. N = 7-10/group.

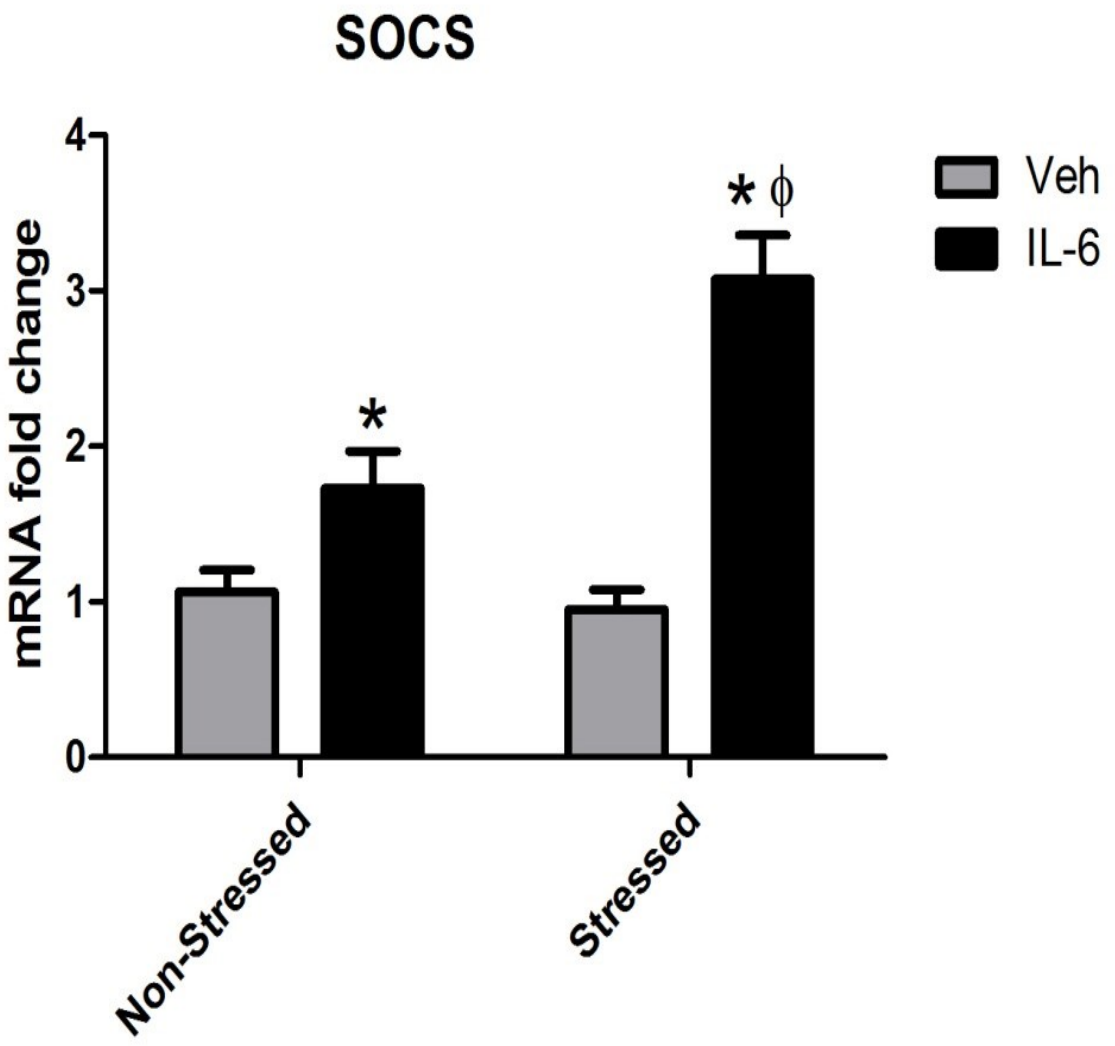

Figure 4. Prefrontal cortex (PFC) mRNA expression (mean fold changes ±SEM) of SOCS3 among CD-1 mice that had been exposed to an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4µg). * $p < 0.05$ enhanced response relative to vehicle treated mice; Φ $p < 0.05$ relative to non-stressed mice. N = 7-10/group.

3.2 Experiment 1: mRNA expression of pro-inflammatory cytokines in the hippocampus (Hipp)

Figure 5 shows the levels of IL-1β mRNA in the hippocampus. While a trend towards an increase in response to IL-6 injection alone was evident, there were no main effects of neither drugs nor the stressor condition. The IL-1R1 mRNA levels showed an interaction between Drugs x Stressors, $F(1, 27) = 7.111$, $p < 0.05$. Animals that were stressed showed higher levels of IL-1R1 than those that were not (Bonferroni corrected t tests $p = 0.051$).

IL-6 mRNA, seen in Figure 6, did not show any effects under ANOVA, while IL-6R levels varied as a function of the Drugs x Stressor interaction, $F(1, 28) = 5.170$, $p < 0.05$. The follow up t tests showed that stressing the mice or injecting them with IL-6 were both able to raise the mRNA of IL-6R beyond that of non-stressed controls.

Levels of TNF-α in the hippocampus varied as a function of the Drug x Stressor interaction, $F(1, 28) = 5.442$, $p < 0.05$. Follow up t tests with a Bonferroni correction revealed that the injection of IL-6 alone caused a visible increase in TNF-α mRNA which was not observed when the animals were stressed or when they were injected with vehicle (see Figure 7). The levels of TNFR varied as a function of the Drug treatment, $F(1, 27) = 6.647$, $p < 0.05$. As mice that were injected with IL-6 showed relatively higher levels of TNFR than their vehicle injected counterparts.

Finally, levels of the suppressors of cytokine signalling 3 (SOCS3) mRNA, seen in Figure 8, varied as a function of the Drug treatment used, $F(1, 28) = 58.41$, $p < 0.001$. Injection of IL-6 caused a significant increase in SOCS3 mRNA in the hippocampus regardless of the stressor backdrop on which it was applied.

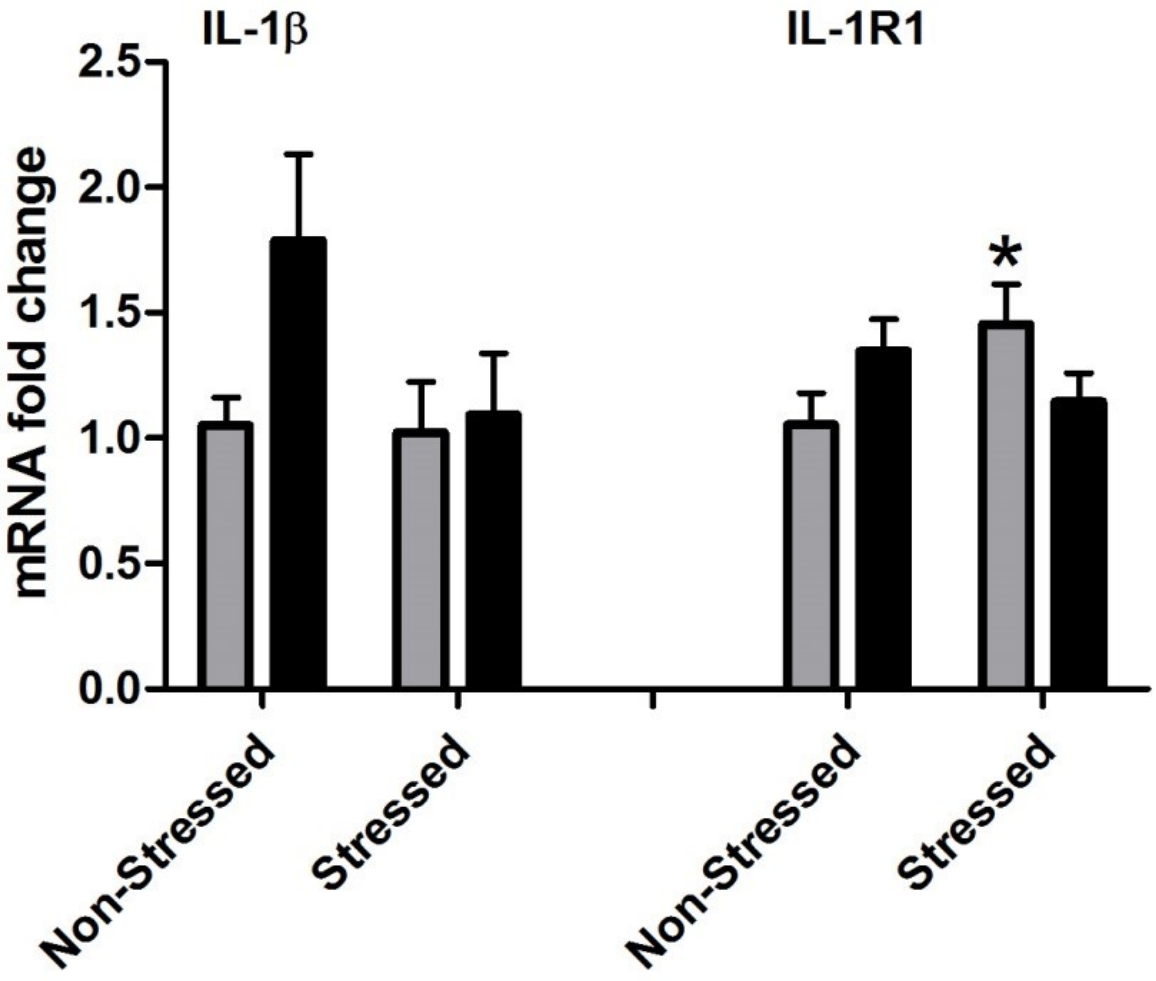

Figure 5. Hippocampus (Hipp) mRNA expression (mean fold changes ±SEM) of IL-1β and its receptor, IL1-R1, among CD-1 mice that had been exposed to an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4μg). * p = 0.051 enhanced response relative to non-stressed, vehicle treated mice. N = 7-10/group.

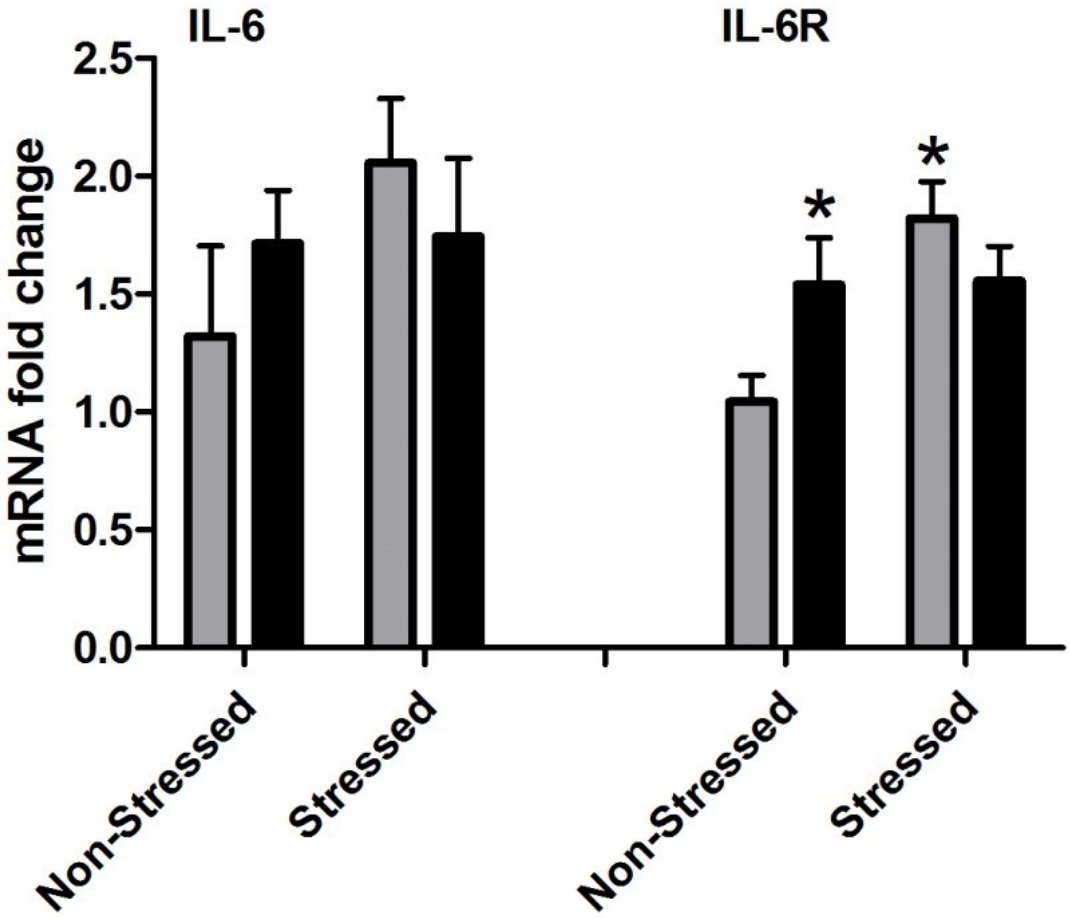

Figure 6. Hippocampus (Hipp) mRNA expression (mean fold changes ±SEM) of IL-6 and its receptor, IL6R, among CD-1 mice that had been exposed to an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4µg). * $p < 0.05$ enhanced response relative to non-stressed, vehicle treated mice. N = 7-10/group.

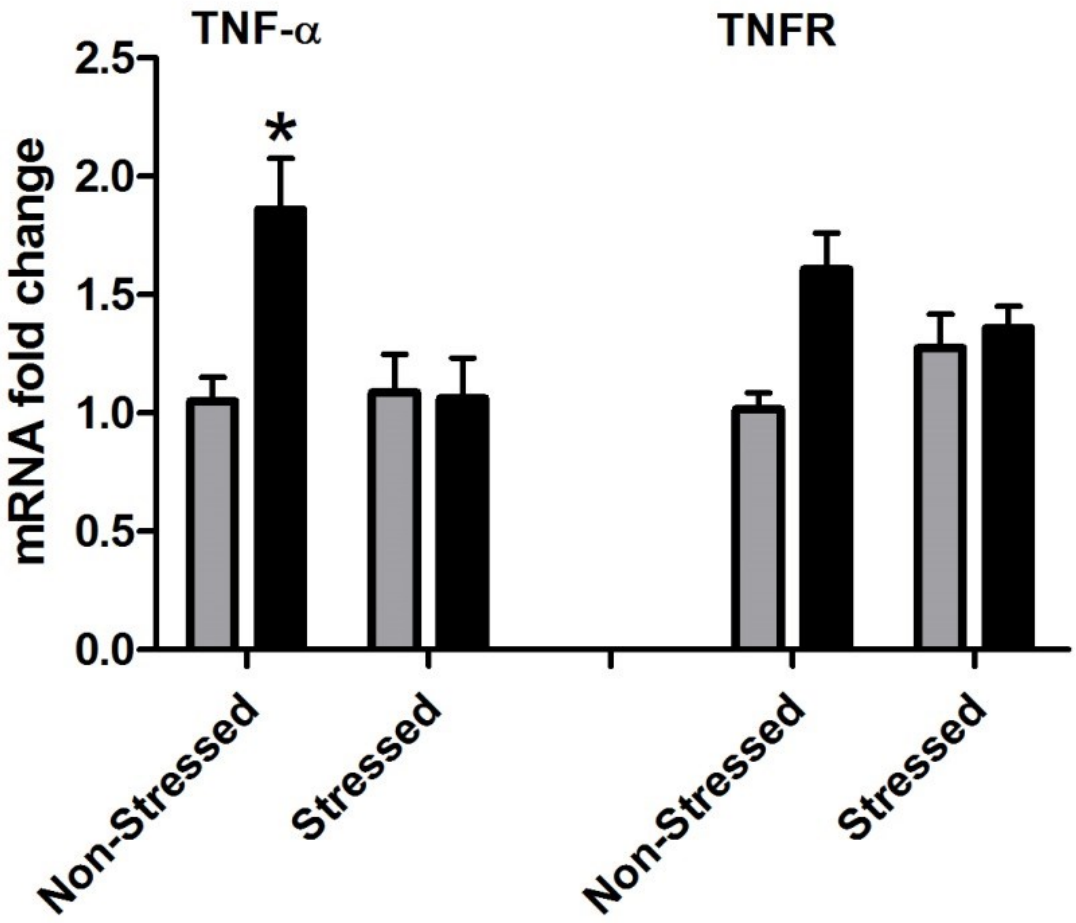

Figure 7. Hippocampus (Hipp) mRNA expression (mean fold changes ±SEM) of TNF-α and its receptor, TNFR, among CD-1 mice that had been exposed to an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4μg). * $p < 0.05$ enhanced response relative to non-stressed, vehicle treated mice and stressed, IL-6 injected mice. N = 7-10/group.

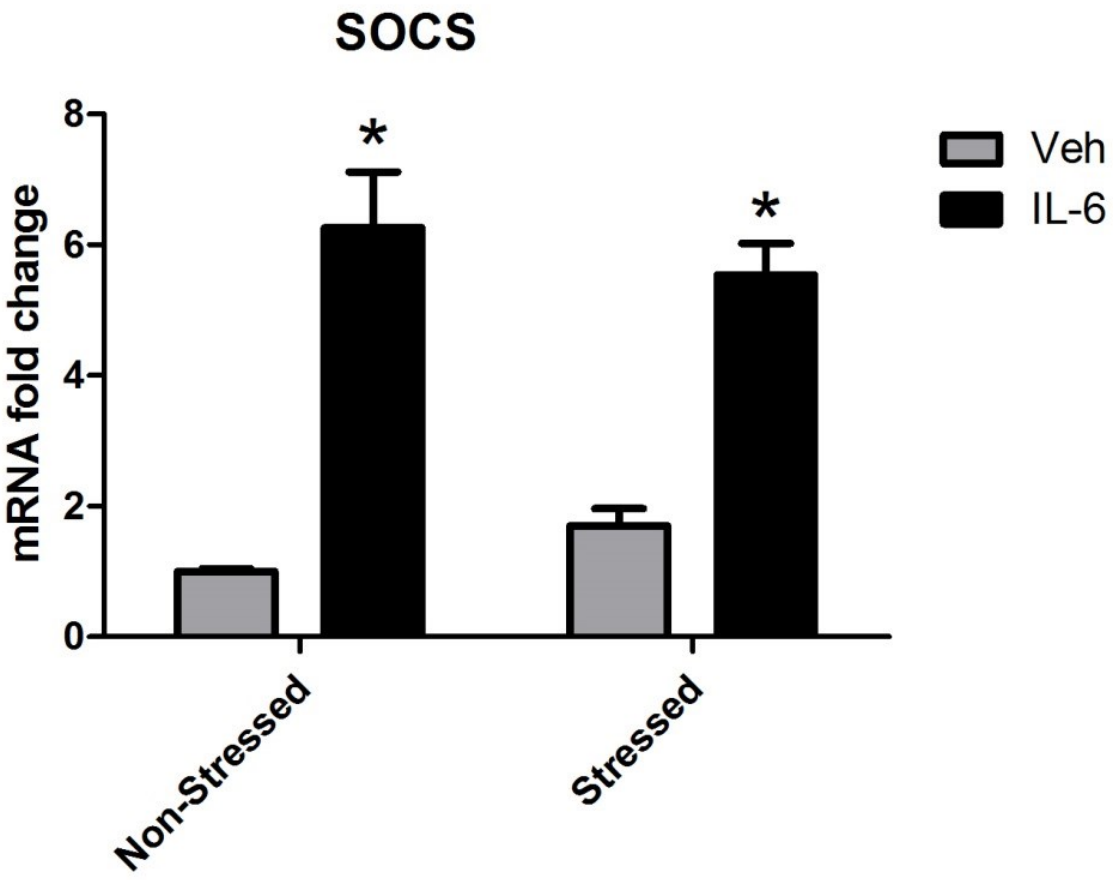

Figure 8. Hippocampus (Hipp) mRNA expression (mean fold changes ±SEM) of SOCS3 among CD-1 mice that had been exposed to an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4μg). * $p < 0.05$ enhanced response relative to vehicle treated counterparts. N = 7-10/group.

3.3 Experiment 2: Anxiety related behaviour as measured by the Open Field and Plus Maze

Figure 9 shows the latency to enter the open field 1.5 hr after stressed and non-stressed animals were injected with either the vehicle or IL-6. Mice that were injected with IL-6 entered the center of the open field sooner than their vehicle injected counterparts, $F(1, 33) = 6.84$, $p < 0.05$. The total time that the animals spent inside the inner square of the open field, however, was solely dependent on the stress condition, $F(1, 34) = 5.38$, $p < 0.05$. Mice that were stressed generally spent more time in the center square of the open field, as depicted in Figure 10.

Performance in the elevated plus maze varied as a function of the Stressor x Drug treatment interaction with regards to the number of entries into the open arms of the EPM, $F(1, 33) = 8.48$, $p < 0.01$. Follow-up tests confirmed that, amongst non-stressed mice, the IL-6 injection increased the number of entries into the open arms ($p < 0.05$). When the animals were stressed, their entries into the open arms were increased regardless of injection (see Figure 11). A Stressor x Drug treatment interaction was also found with regards to the time spent in the open arms of the EPM, $F(1, 32) = 4.58$. $p < 0.05$. As depicted in Figure 12, and corroborated by post-hoc tests, IL-6 injection alone did not influence the time spent in the open arms of the maze. However, when the animals were stressed, they spent more time in the open; this effect was ameliorated when the stressed mice were injected with IL-6 ($p < 0.05$). In contrast, neither the stress condition nor the drug treatment influenced closed arms behaviour, as both the entries into the closed arms and the time spent there did not significantly differ between groups (p's > 0.05, Figures 13 and 14).

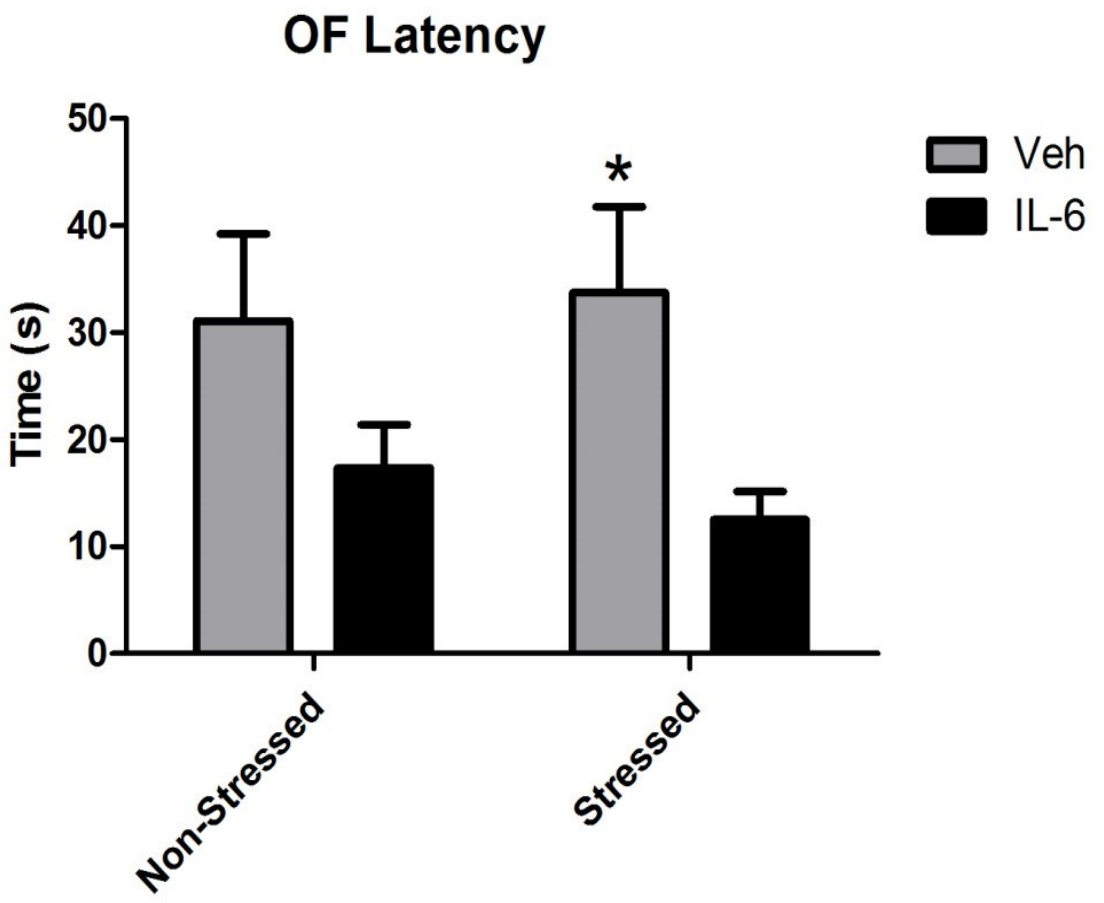

Figure 9. Latency (Mean time ±SEM) to enter the center square of the open field among CD-1 mice that had been exposed to either no stressor or an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4µg). * $p < 0.05$ relative to IL-6 injected counterparts. N = 10/group.

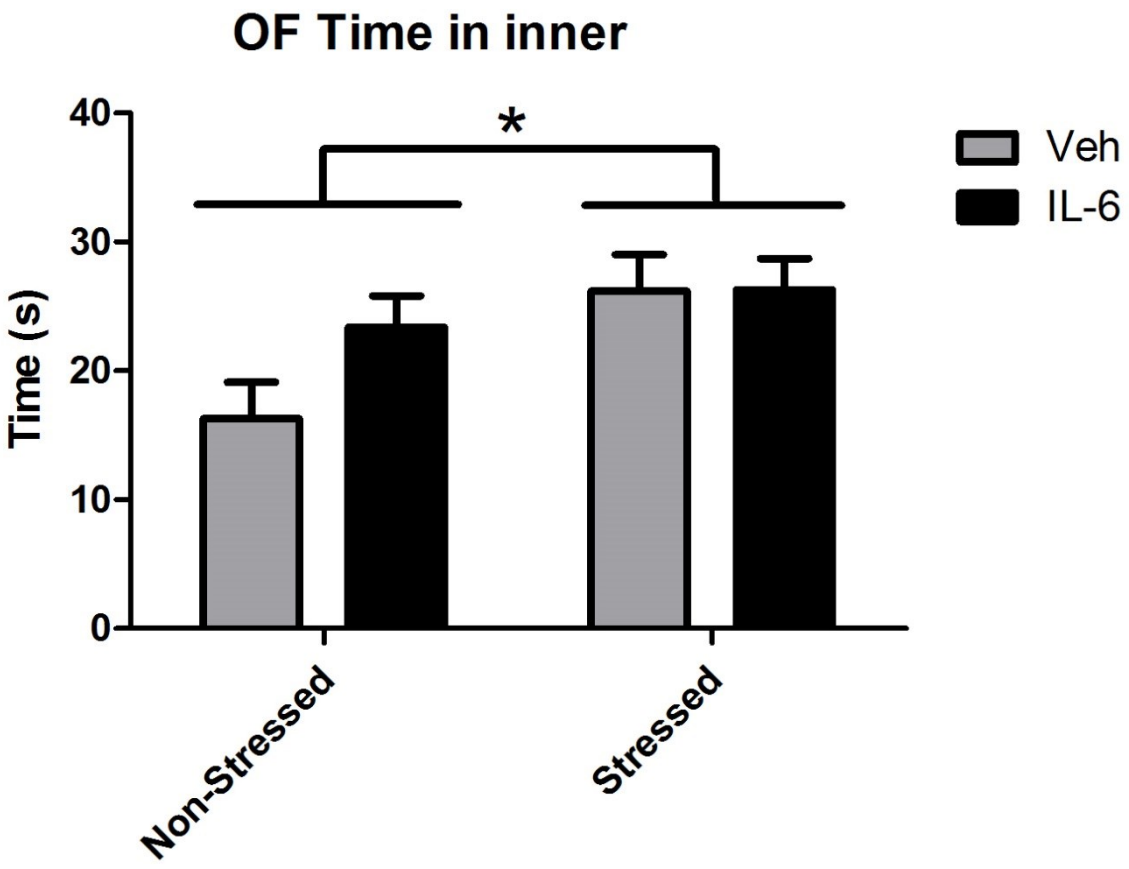

Figure 10. Total time (Mean ±SEM) spent in the inner (center) square of the open field among CD-1 mice that had been exposed to either no stressor or an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4µg). * $p < 0.05$ relative to non-stressed, vehicle injected animals. N = 10/group.

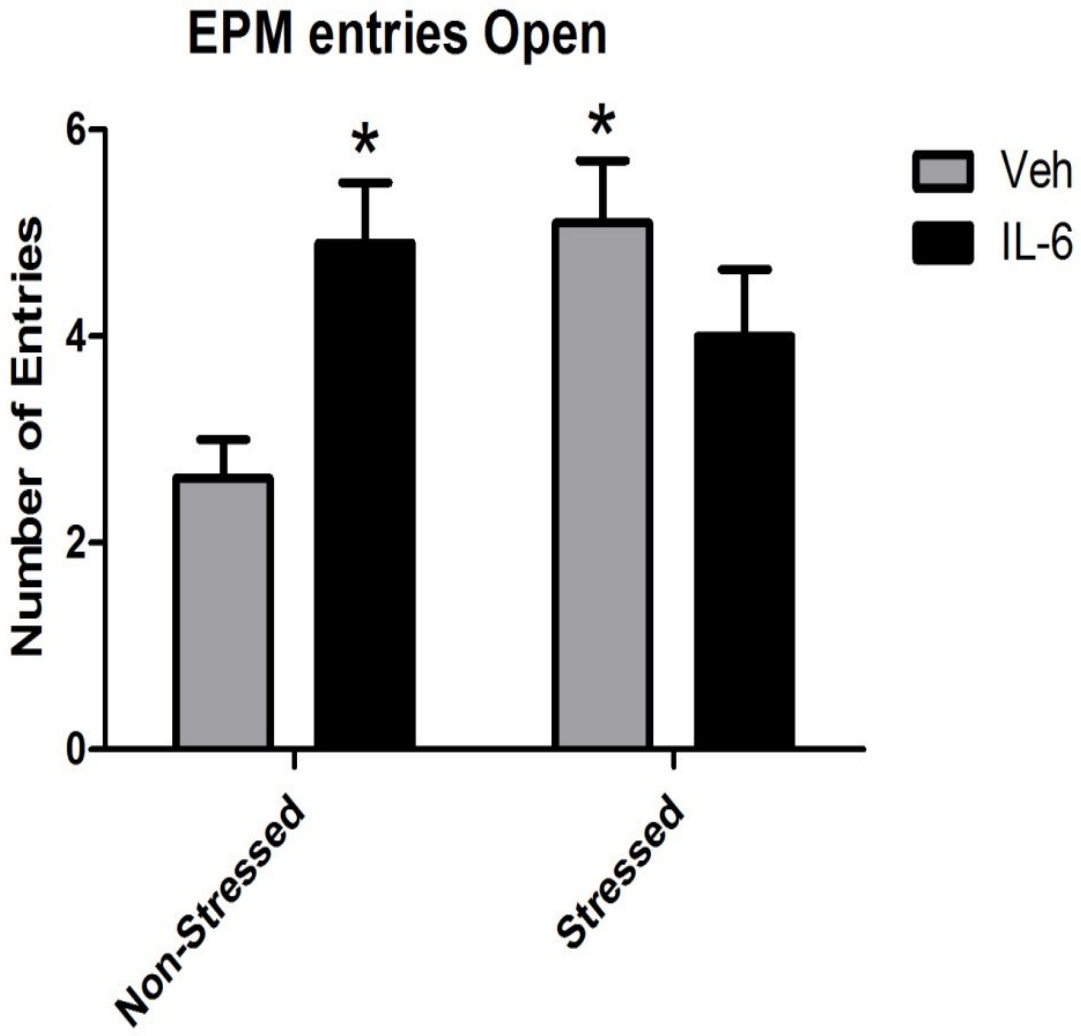

Figure 11. Number of entries (Mean ±SEM) into the open arms of the elevated plus maze among CD-1 mice that had been exposed to either no stressor or an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4µg). * $p < 0.05$ relative to non-stressed, vehicle injected animals. N = 10/group.

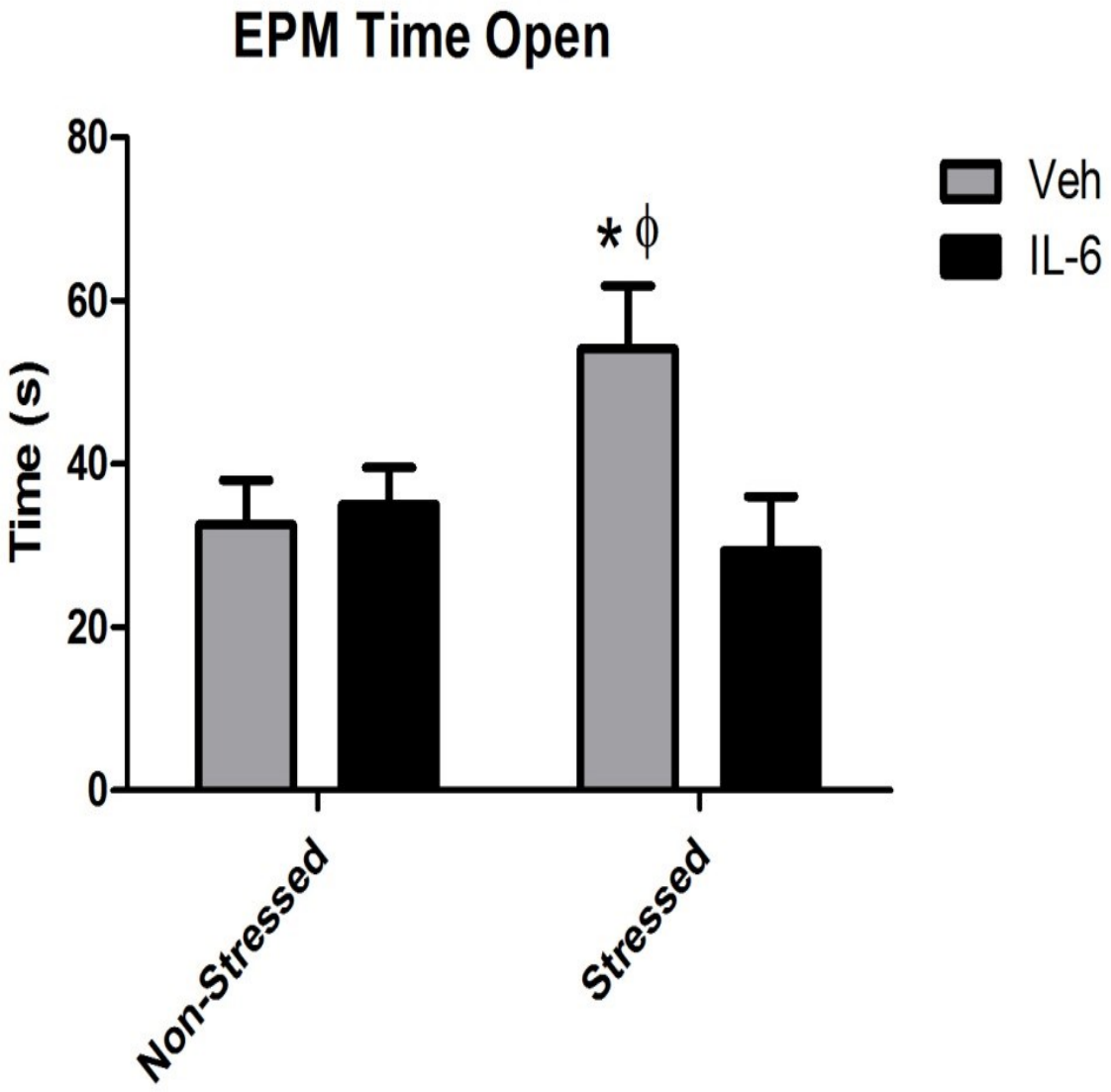

Figure 12. Total time (Mean ±SEM) spent in the open arms of the elevated plus maze among CD-1 mice that had been exposed to either no stressor or an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4μg). * $p < 0.05$ relative to non-stressed, vehicle injected animals. Φ $p < 0.05$ relative to IL-6 injected counterparts. N = 10/group.

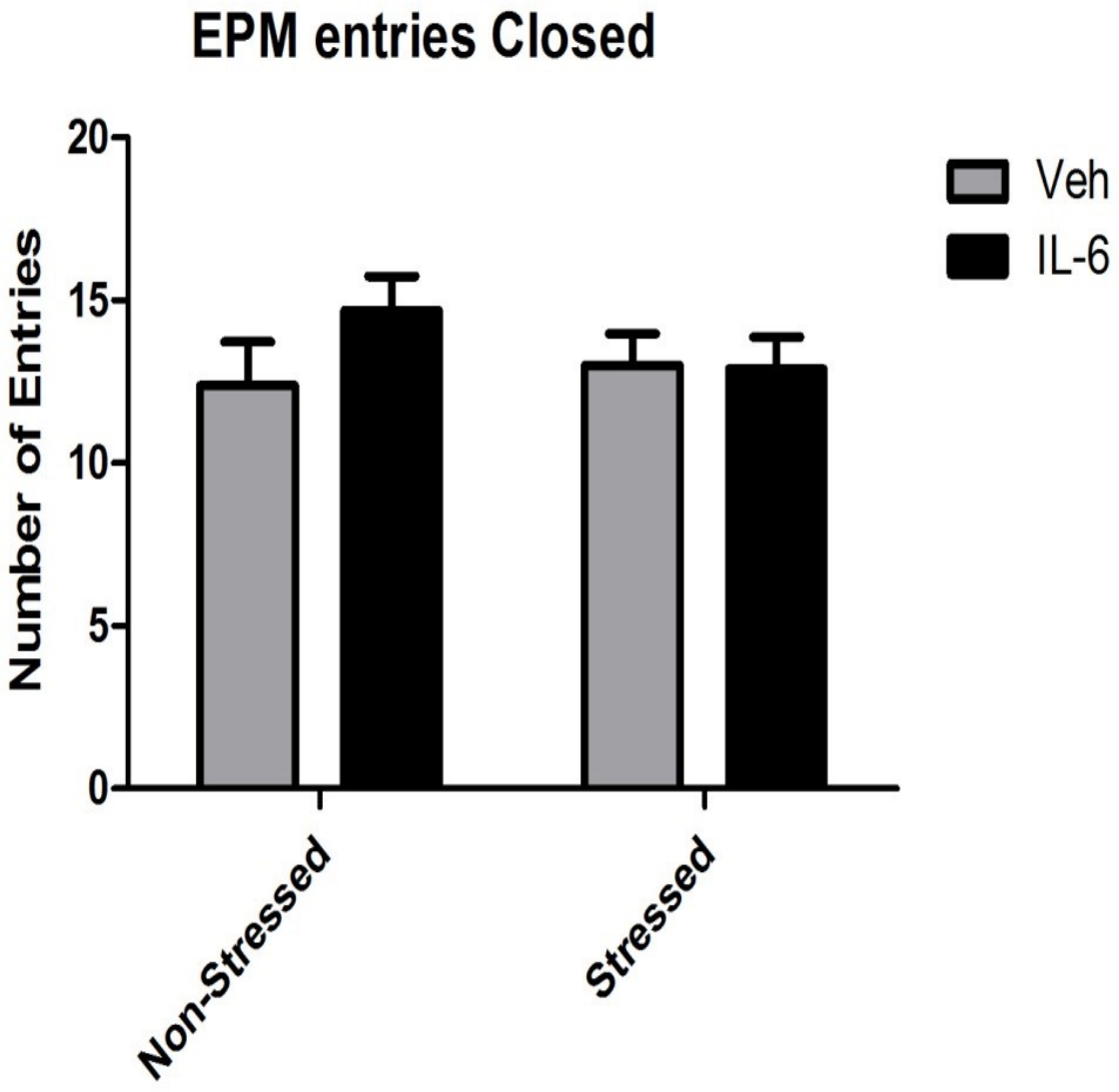

Figure 13. Number of entries (Mean ±SEM) into the closed arms of the elevated plus maze among CD-1 mice that had been exposed to either no stressor or an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4μg). N = 10/group.

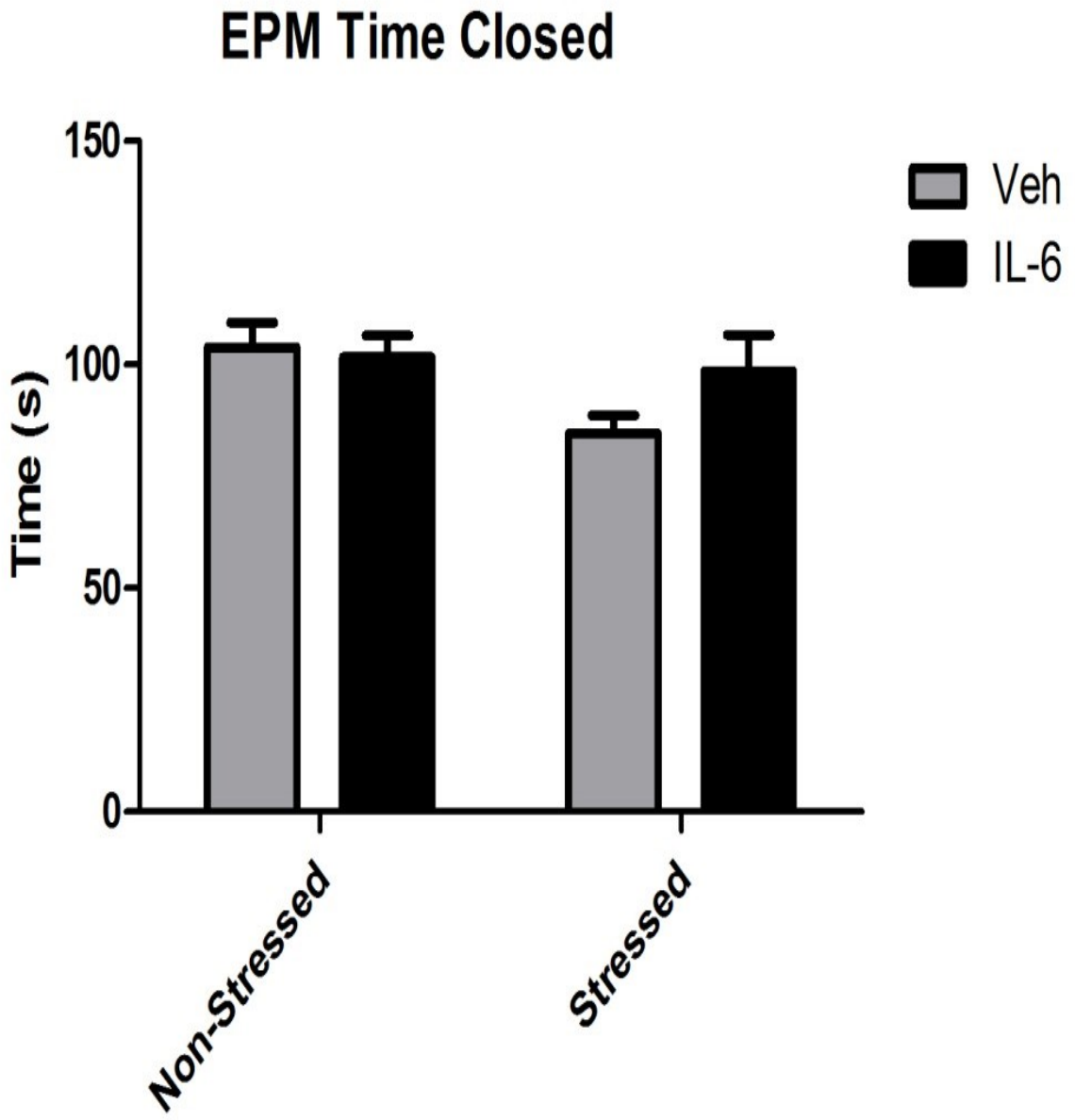

Figure 14. Total time (Mean ±SEM) spent in the closed arms of the elevated plus maze among CD-1 mice that had been exposed to either no stressor or an acute social stressor (retired breeder) followed by an injection of vehicle (0.3ml BSA) or IL-6 (4µg). N = 10/group.

3.4 Experiment 3: Plasma Corticosterone levels

Levels of plasma corticosterone for each of the different treatment groups are shown in Figure 15. Circulating corticosterone levels varied as a function of the stress x time interaction, $F(4, 83) = 2.77$, $p < 0.05$. Follow-up tests were conducted of the simple effects comprising this interaction. These tests revealed that in the absence of the IL-6 injection, corticosterone levels were elevated 1.5 hr after exposure to the acute social stressor, whereas at 3 and 24 hr levels of corticosterone were comparable to non-stressed controls. When the animals were injected with IL-6 after acute stressor exposure, an elevation was also evident a 1.5 hr later, and decreased at 3 hr, and was at baseline levels at 24 hrs. However, when the mice were repeatedly stressed for 7 days, no apparent increase in corticosterone levels was observed, regardless of the treatment received.

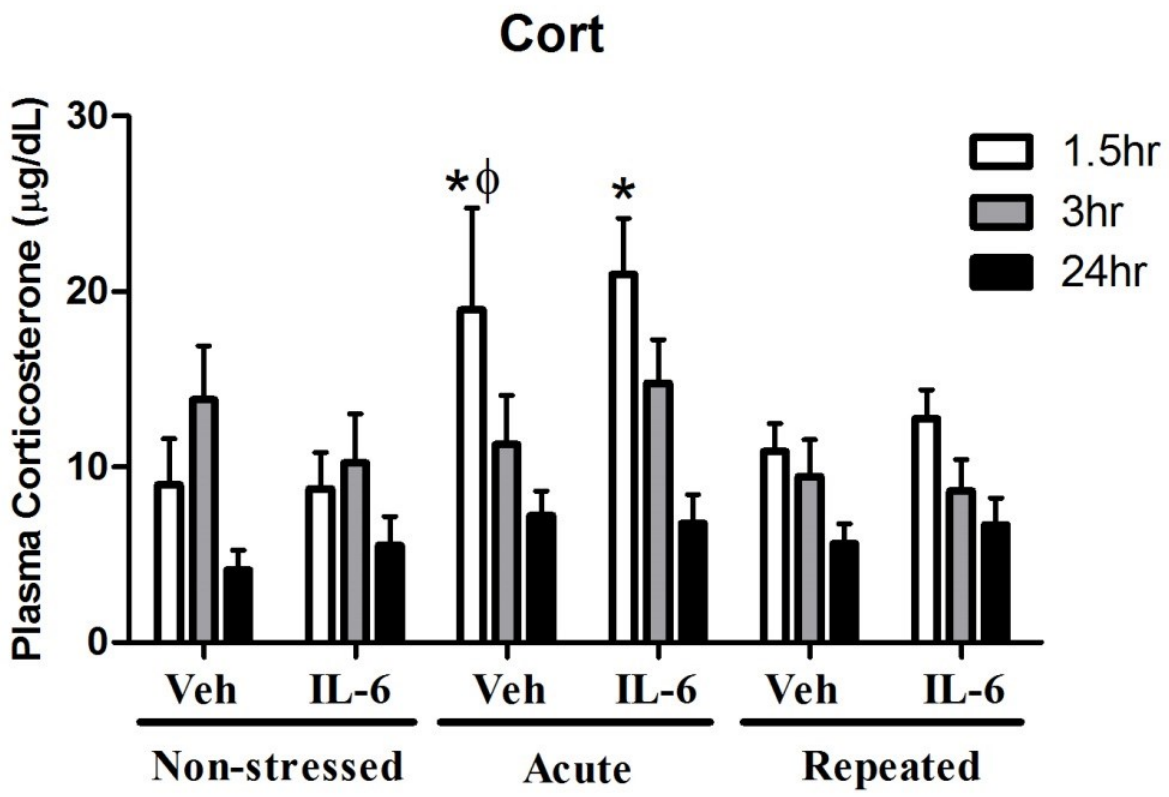

Figure 15. Mean (±SEM) concentrations of plasma corticosterone at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor (retired breeder exposure for 7 days). * $p < 0.05$ enhanced response relative to BSA treated mice in the non-stressed condition; ϕ $p < 0.05$ enhanced response relative to acutely stressed, vehicle treated mice at 3 hr. N = 6/group.

3.5 Experiment 3: mRNA expression of pro-inflammatory cytokines in the prefrontal cortex (PFC)

Within the PFC, levels of IL-1β mRNA varied as a function of the Stressor x Time, $F(4, 70) = 2.57$, $p < 0.05$, and Drug treatment x Time interactions, $F(2, 70) = 3.54$, $p < 0.05$. The follow-up t tests indicated that when mice were only injected with IL-6, levels of IL-1β were significantly higher 3 hr later compared to vehicle treated mice (see Figure 16). Among mice that had been exposed to an acute stressor, which itself did not influence IL-1β expression, the subsequent IL-6 treatment increased IL-1β measured 1.5 hr later. In contrast, the IL-1β elevation ordinarily evident at the 3 hr time point was no longer apparent. Essentially, the combined treatment resulted in a leftward shift of the time curve so that the IL-1β change occurred earlier, but also dissipated sooner as well.

The expression of IL-1R1 likewise varied as a function of Stressor x Time, $F(4, 71) = 2.90$, $p < 0.05$, and the Drug x Time interactions, $F(2, 71) = 5.62$, $p < 0.01$. Follow up t tests with a Bonferonni correction showed that, curiously, in the absence of the stressor, levels of the receptor's mRNA seemed to increase 3 hr after vehicle injection. Levels of the mRNA were also appreciably increased at 3 hr compared to 1.5 hr when the non-stressed mice were injected with IL-6 (see Figure 17). When the animals were exposed to a retired breeder on test day, however, IL-6 injected animals showed an increase in mRNA at the 1.5 hr time point that remained at the 3 hr point, returning to baseline levels by 24 hr. When the stressor was repeated for 7 days, the increase was only observed 3 hr after IL-6 injection, while the 1.5 hr effect was gone.

Levels of IL-6 mRNA in the PFC varied as a function of the Drug x Time interaction, $F(2, 67) = 5.12$, $p < 0.01$. As seen in Figure 18, and corroborated by follow up tests, when the injection was combined with an acute social stressor, the increase in IL-6 levels was significant

at 1.5 and 3 hr. When the stressor was repeated for 7 days, the increase was delayed so that it was only observed 3 hr after IL-6 injection. The mRNA expression of IL-6R also varied only as a function of the Drug x Time interaction, $F(2, 71) = 5.20$, $p < 0.01$. The follow-up t tests revealed that IL-6 injection caused an increase in the levels of the receptor in the non-stressed mice at the 3 hr time point, but was at control levels by 24 hr. When the animals were exposed to a single stressor before injection, there was a leftward shift wherein the increase in mRNA was observed at 1.5 hr first, continuing to 3 hr point, and then returning to vehicle levels by 24 hr, as seen in Figure 19. A similar pattern was seen in animals that were repeatedly stressed, though this effect failed to reach significance.

IL-10 expression within the PFC (Figure 20) was influenced by the Drug x Time interaction, $F(2, 58) = 7.69$, $p < 0.01$. The follow-up t tests revealed that, despite the high variability in the data, exposure to an acute social stressor caused a significant increase in IL-10 levels when the animals were sacrificed at 3 hrs as opposed to 1.5 hr after vehicle injection. When the mice were injected with IL-6 instead, the increase at 1.5 hr approached significance compared to vehicle treated counterparts ($p = 0.066$). When the IL-6 injection was combined with a repeated stressor, a significant increase was seen at 1.5 hr, which was gone by 3 and 24 hrs.

TNF-α expression within the PFC was influenced by the Stressor condition, $F(2,80) = 6.71$, $p < .003$ and by the Drug x Time interaction, $F(2, 80) = 3.46$, $p < 0.05$. As seen in Figure 21, IL-6 mRNA expression was reduced in mice that had been repeatedly stressed relative to nonstressed mice. As well, TNF-α mRNA expression 3 hr after IL-6 administration was elevated to that evident in nonstressed mice treated with IL-6. This interaction was not further moderated by the stressor treatment, but as a priori predictions had been made in this regard, follow-up

comparisons were made for the simple effects of this 3-way interaction. These comparisons confirmed that in nonstressed mice the expression of TNF-α was elevated by the IL-6 treatment, but this outcome was only apparent at the 3 hr time point, after which the levels of the cytokine were comparable across groups.

The expression of TNFR also varied with the Drug x Time interaction, $F(2, 72) = 4.28$, $p < 0.05$. As with the IL-1R1 receptor, there was a significant increase in mRNA levels 3 hr after vehicle injection. As seen in Figure 22, the injection of IL-6 alone did not have any significant effect, however when it was combined with an acute stressor an increase in PFC mRNA was observed 1.5 hr after injection, while the other time points remained unaffected. When the animals were repeatedly stressed, the observed increase in IL-6 injected mice was diminished.

The mRNA of cytokine inhibitor SOCS3 in the PFC varied with the Stressor x Drug x Time interaction, $F(4, 70) = 2.52$, $p < 0.05$ (see Figure 23). The follow-up t tests confirmed that the IL-6 treatment influenced SOCS3 expression, as drug injection caused an increase of SOCS3 mRNA at the 1.5 and 3 hr compared to vehicle treated mice. When the drug was applied upon a background of an acute stressor, the IL-6 injected animals showed a substantial increase at the 1.5 hr point compared to vehicle treated mice, but this outcome was not apparent at 3 and 24 hr. When the stressor was repeated and combined with IL-6, a similar increase was seen at 1.5 hr, although though it was not as substantial.

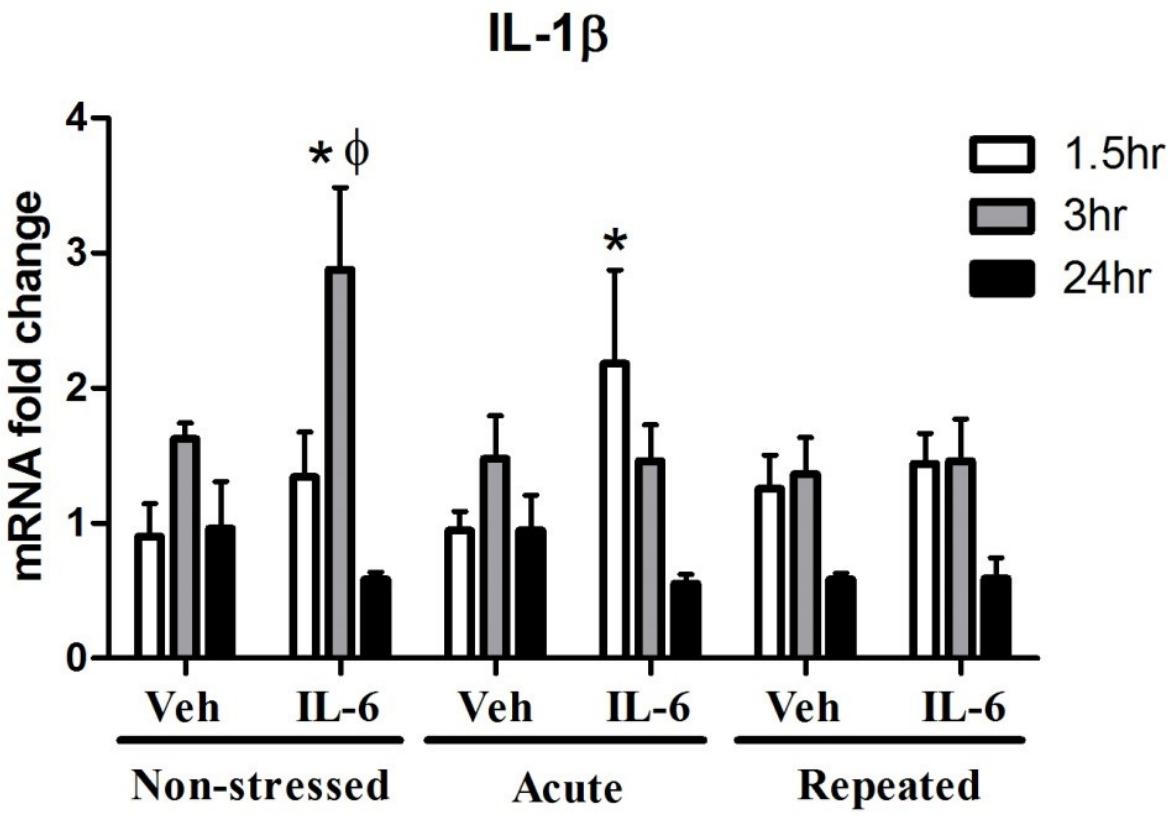

Figure 16. Mean (±SEM) mRNA expression of IL-1β within the prefrontal cortex (PFC) at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to vehicle treated counterparts; Φ $p < 0.05$ relative to 1.5 hr mice. N = 6/group.

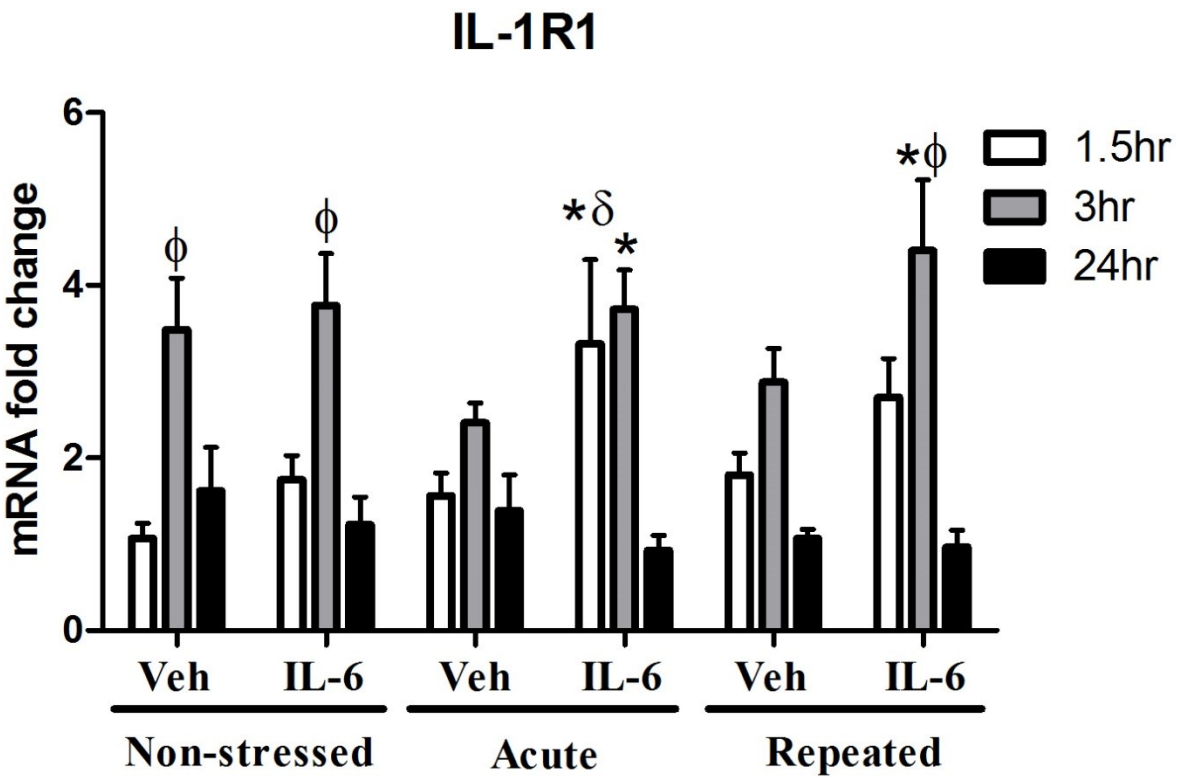

Figure 17. Mean (±SEM) mRNA expression of IL-1R1 within the prefrontal cortex (PFC) at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to vehicle treated counterparts; Φ $p < 0.05$ relative to 1.5 hr mice; δ $p < 0.05$ relative to non-stressed, IL-6 injected animals at the same time point. N = 6/group.

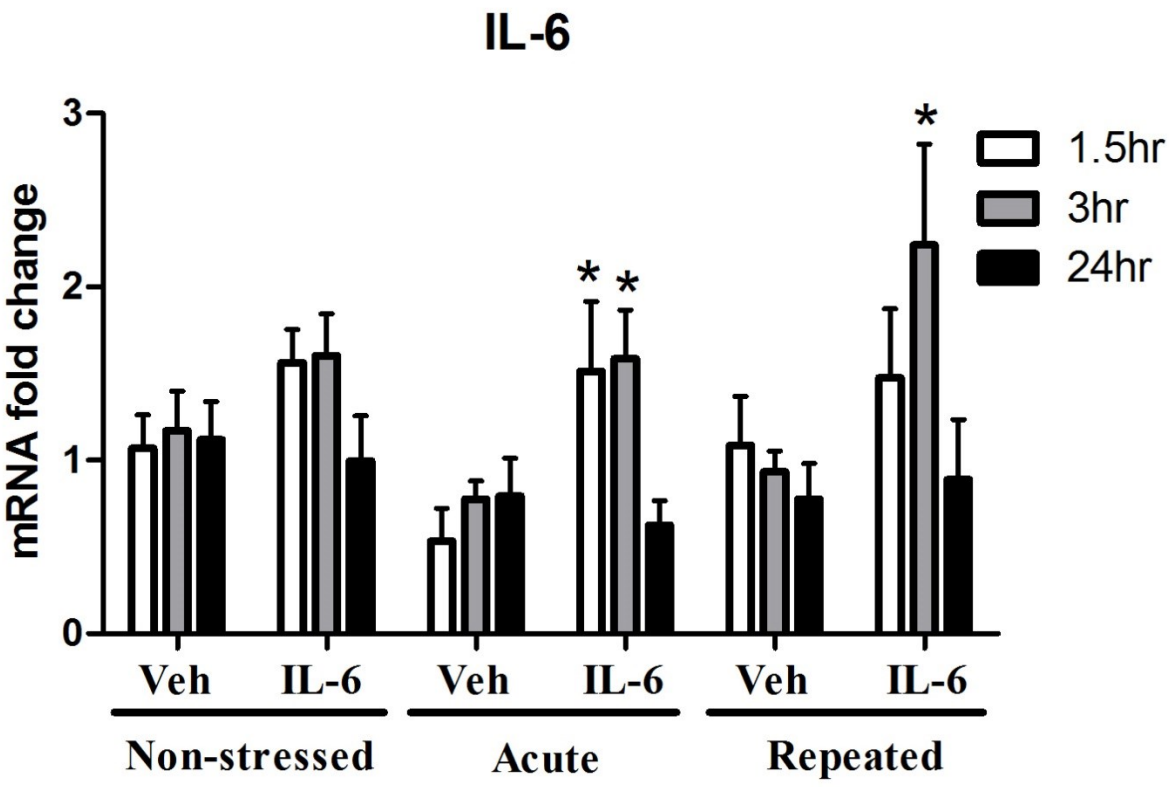

Figure 18. Mean (±SEM) mRNA expression of IL-6 within the prefrontal cortex (PFC) at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to vehicle treated counterparts. N = 6/group.

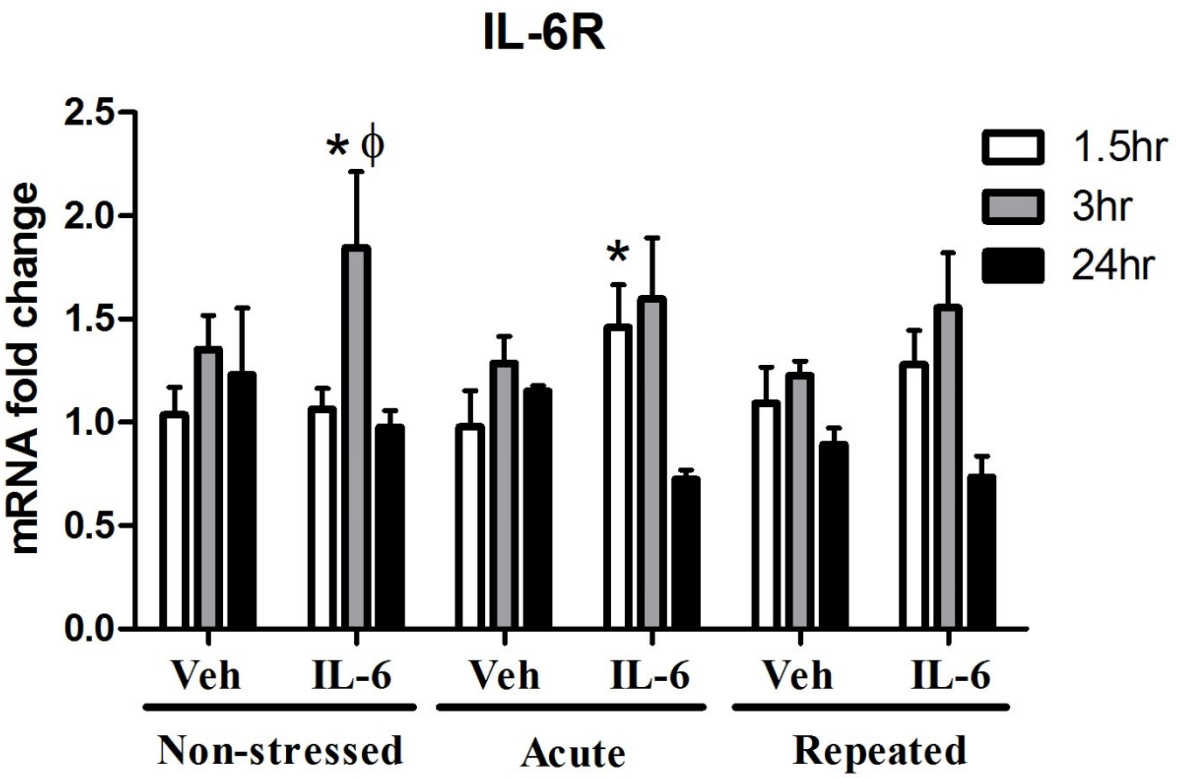

Figure 19. Mean (±SEM) mRNA expression of IL-6R within the prefrontal cortex (PFC) at 1.5, 3 or 24 hr following IL-6 (4 µg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to vehicle treated counterparts; Φ $p < 0.05$ relative to 1.5 hr mice. N = 6/group.

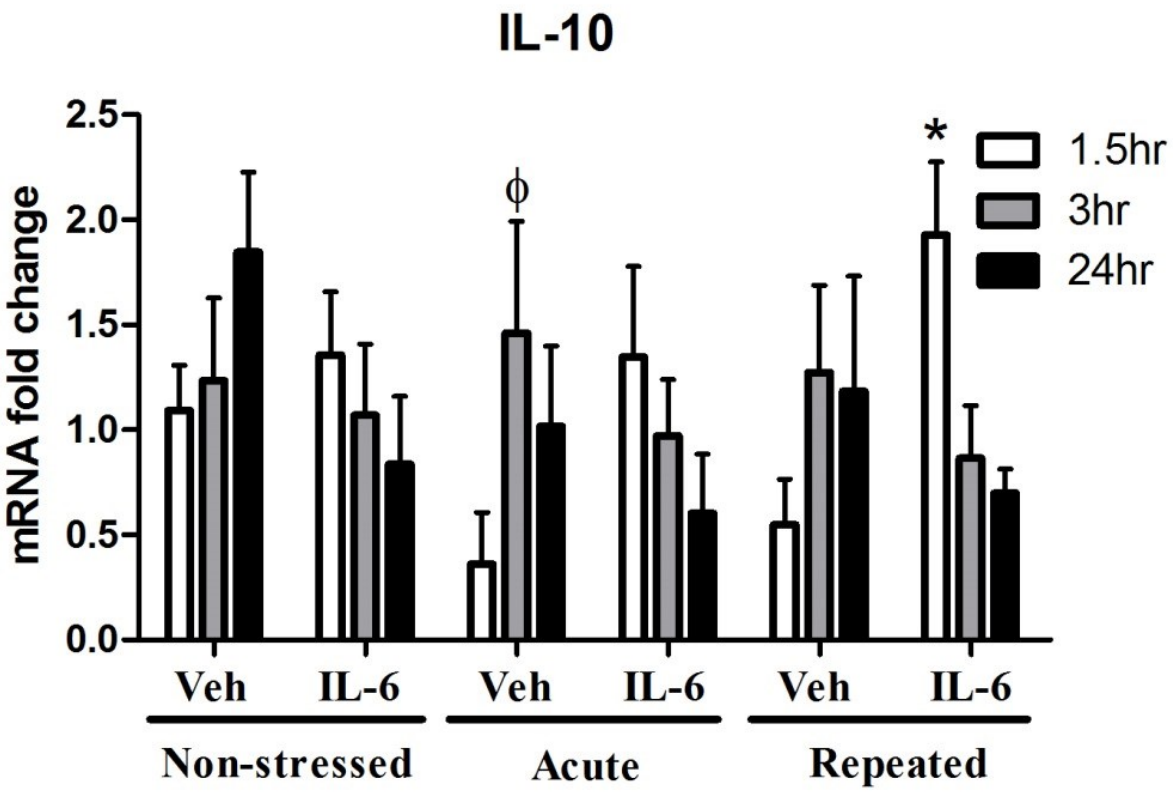

Figure 20. Mean (±SEM) mRNA expression of IL-10 within the prefrontal cortex (PFC) at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to vehicle treated counterparts and mice in the 3 hr group; Φ $p < 0.05$ enhanced response relative to mice in the 1.5 hr group. N = 6/group.

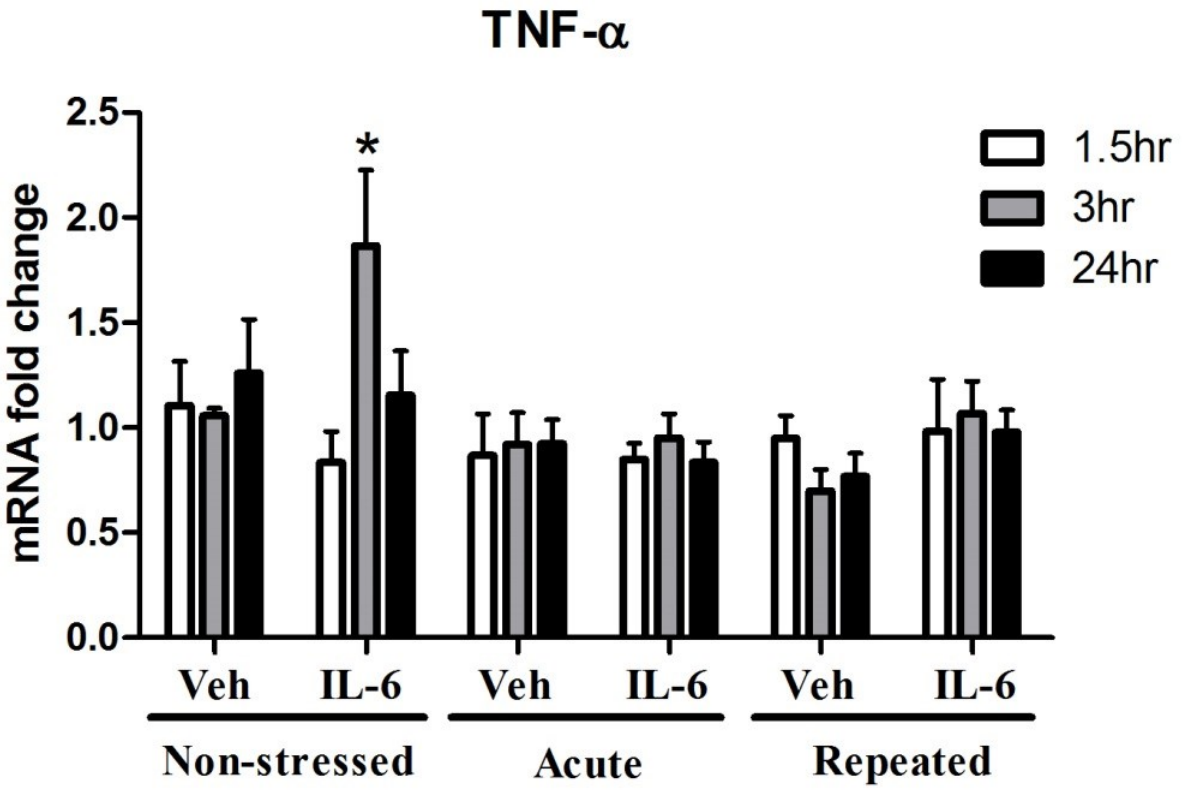

Figure 21. Mean (±SEM) mRNA expression of TNF-α within the prefrontal cortex (PFC) at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to vehicle treated counterparts and mice in the 1.5 hr group. N = 6/group.

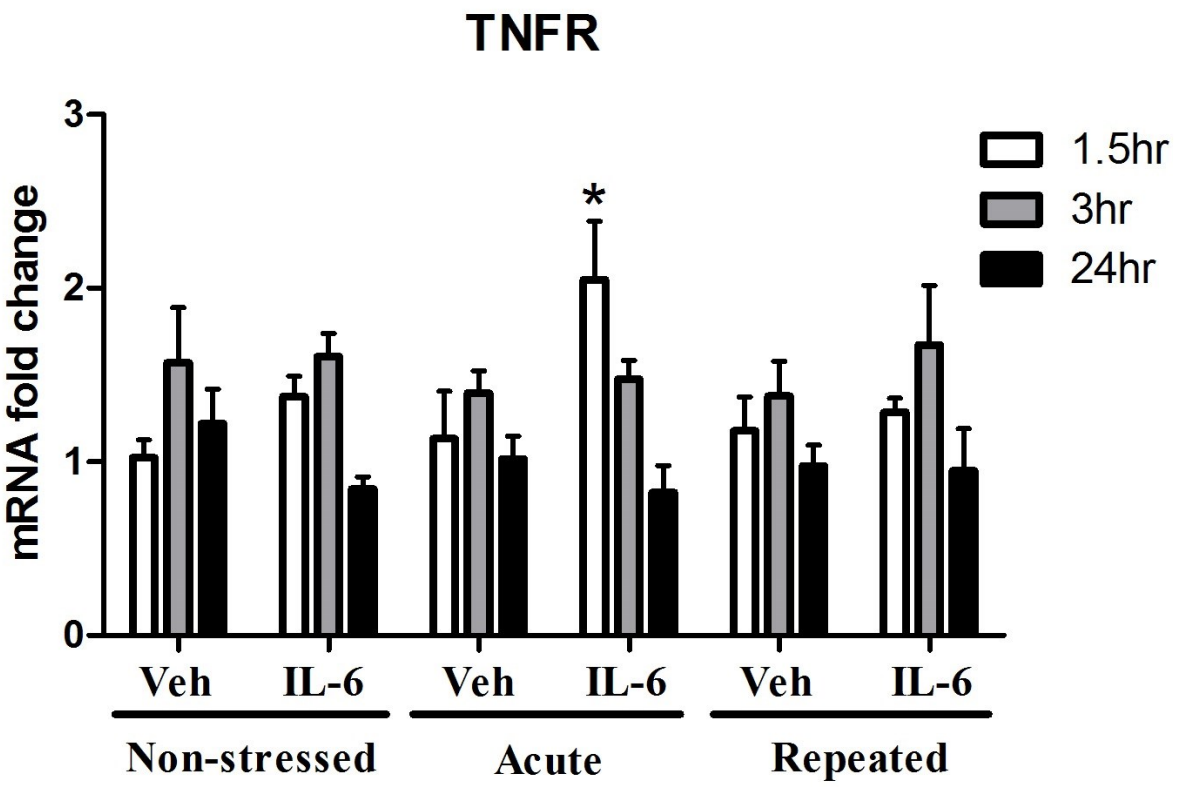

Figure 22. Mean (±SEM) mRNA expression of TNFR within the prefrontal cortex (PFC) at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to vehicle treated counterparts, non-stressed counterparts, and mice in the 3 hr group. N = 6/group.

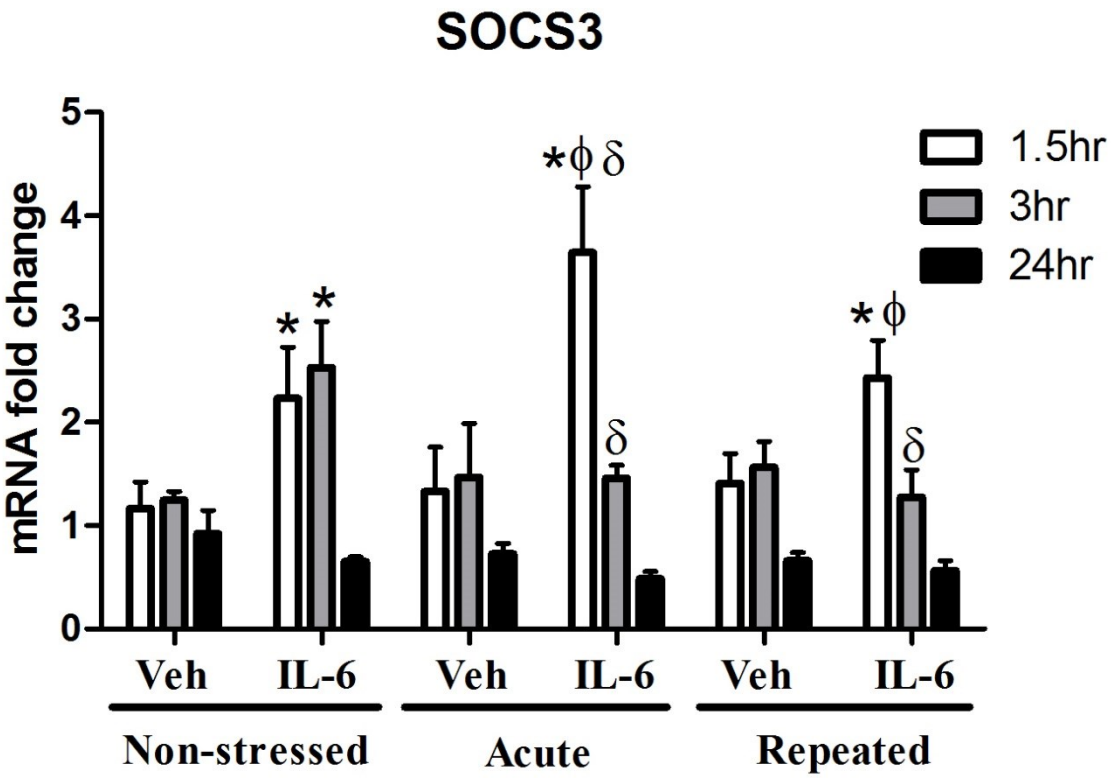

Figure 23. Mean (±SEM) mRNA expression of SOCS3 within the prefrontal cortex (PFC) at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to vehicle treated counterparts; Φ $p < 0.05$ relative to mice in the 3 hr group; δ $p < 0.05$ relative to non-stressed counterparts. N = 6/group.

3.6 Experiment 3: mRNA expression of pro-inflammatory cytokines in the hippocampus (Hipp)

Levels of IL-1β mRNA within the hippocampus varied as a function of the Stressor x Time and Drug x Time interactions, $F(2, 84) = 1.41$, $p < 0.05$, and $F(2, 84) = 4.77$, $p < 0.05$, respectively. The follow up t tests indicated that exposing the mice to an acute social stressor caused an increase of mRNA expression 3 hrs after the stressor, but this outcome was not apparent when the stressed animals were injected with IL-6. When the cytokine injection was combined with a repeated stressor, an increase of IL-1β was observed 1.5 hr after injection, which was not seen when the repeated stressor was applied alone (see Figure 24). Hippocampal expression of IL-1R1 varied with the Stressor condition, $F(2, 85) = 4.47$, $p < 0.05$, and the Time following treatment, $F(2, 85) = 14.47$, $p < 0.001$. A general pattern was observed wherein mice that were decapitated 1.5 and 3 hrs after injection showed higher IL-1R1 levels than the ones sacrificed after 24 hrs (Figure 25). Moreover, repeatedly stressed mice generally had higher IL-1R1 expression than their non-stressed counterparts.

Within the hippocampus, levels of IL-6 varied as a function of the Stressor x Time interaction, $F(4, 80) = 2.80$, $p < 0.05$. Follow up t tests with a Bonferonni correction revealed that when the mice were injected with IL-6, an increase of mRNA levels of the cytokine occurred 3 hr after injection. This effect was not evident when the injection was combined with an acute social stressor. As shown in Figure 26, when the mice were stressed repeatedly for 7 days, an increase of IL-6 occured1.5 hr later, but was absent at 3 and 24 hr. When the repeatedly stressed mice were injected with IL-6, a similar pattern was observed, though it was only on the cusp of significance ($p = 0.051$). Levels of the IL-6R mRNA only varied with Time following the treatment, $F(2, 89) = 4.89$. $p = 0.01$, with those mice sacrificed 3 hr after injection showing generally higher expression of IL-6R compared to their 24 hr counterparts (Figure 27).

The anti-inflammatory cytokine IL-10 in the hippocampus varied as a function of the interaction between the Stressor x Drug x Time conditions, $F(4, 84) = 3.28$, $p < 0.05$. As seen in Figure 28, and confirmed by follow-up tests, injection of IL-6 caused an increase of IL-10 mRNA that was evident 3 hr after the treatment, and was no longer apparent at 24 hr. A single social stressor episode provoked a significant increase of IL-10 mRNA that was similarly evident 3 hr later; however, in mice that have received both the stressor and the IL-6 treatment, the rise of IL-10 was no longer evident. When the injection was combined with a repeated stressor, an apparent increase was observed at both the 1.5 and the 3 hr time points, though the magnitude of the change s were modest and slightly shy of significance ($p = 0.068$ and 0.079 relative to vehicle injected counterparts, respectively).

Levels of TNF-α within the hippocampus, seen in Figure 29, showed a three-way interaction between Stressor x Drug x Time, $F(4, 86) = 2.44$, $p = 0.05$. Follow up tests of the simple effects comprising this interaction indicated that the only significant increase of hippocampal TNF-α mRNA occurred 3 hrs after IL-6 injection, and that this effect was prevented when the injection was combined with an acute or a repeated stressor. Levels of the receptor TNFR varied only as a function of the Time of sacrifice, $F(2, 85) = 5.15$, $p < 0.01$, in that mice that were sacrificed 3 hrs after injection had generally higher fold increases of TNFR mRNA compared to those sacrificed after 24 hrs (Figure 30).

The mRNA of the cytokine inhibitor SOCS3 in the hippocampus varied as a function of a Drug x Time interaction, $F(2, 86) = 10.57$, $p < 0.001$. Bonferonni corrected t tests revealed that when the mice were injected with IL-6, a significant increase in their SOCS3 levels was observed 1.5 and 3 hr after injection ($p = 0.006$ and 0.053, respectively). When the IL-6 injection was combined with an acute social stressor, the increase was only observed 1.5 hr after injection.

Similarly, as seen in Figure 31, when the stressor was repeated for 7 days, the increase observed in response to the IL-6 injection was more prominent at 1.5 hr than that seen in the comparable Acute and non-stressed groups,.

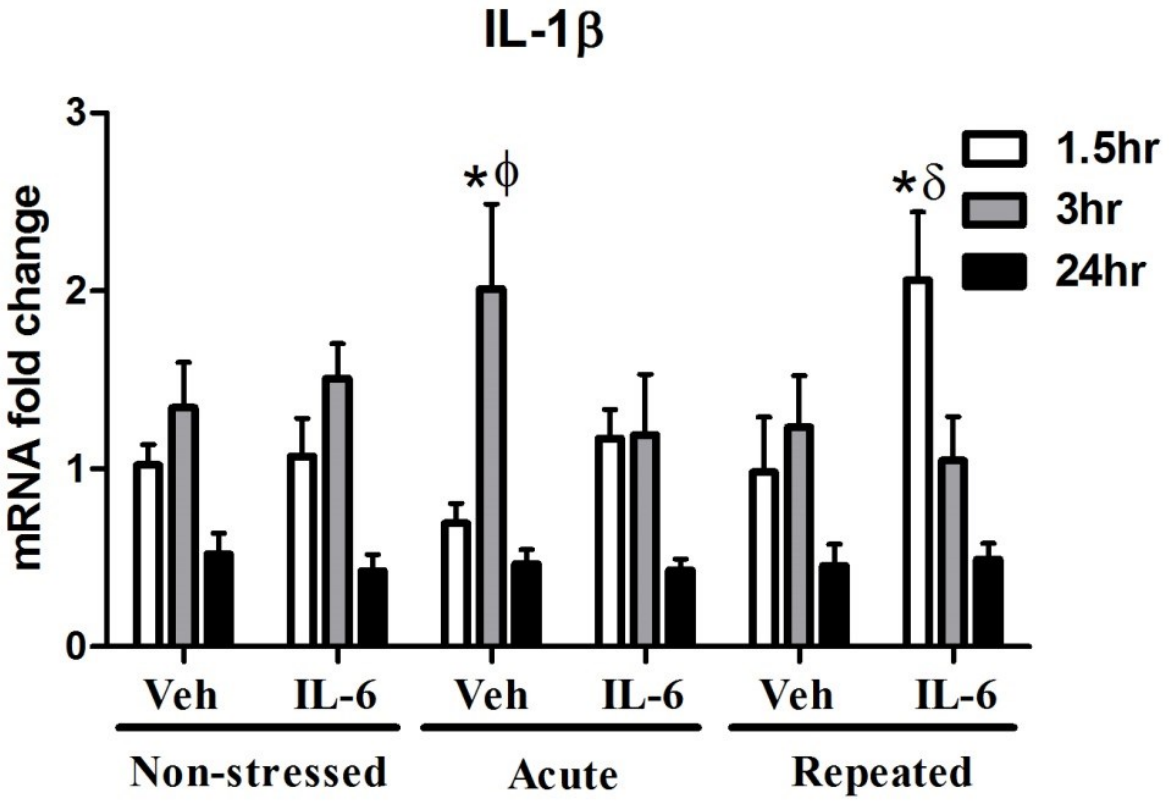

Figure 24. Mean (±SEM) mRNA expression of IL-1β within the hippocampus at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to non-stressed controls; Φ $p < 0.05$ relative to mice in the 1.5 hr group; δ $p < 0.05$ relative to mice in the 3 hr group. N = 6/group.

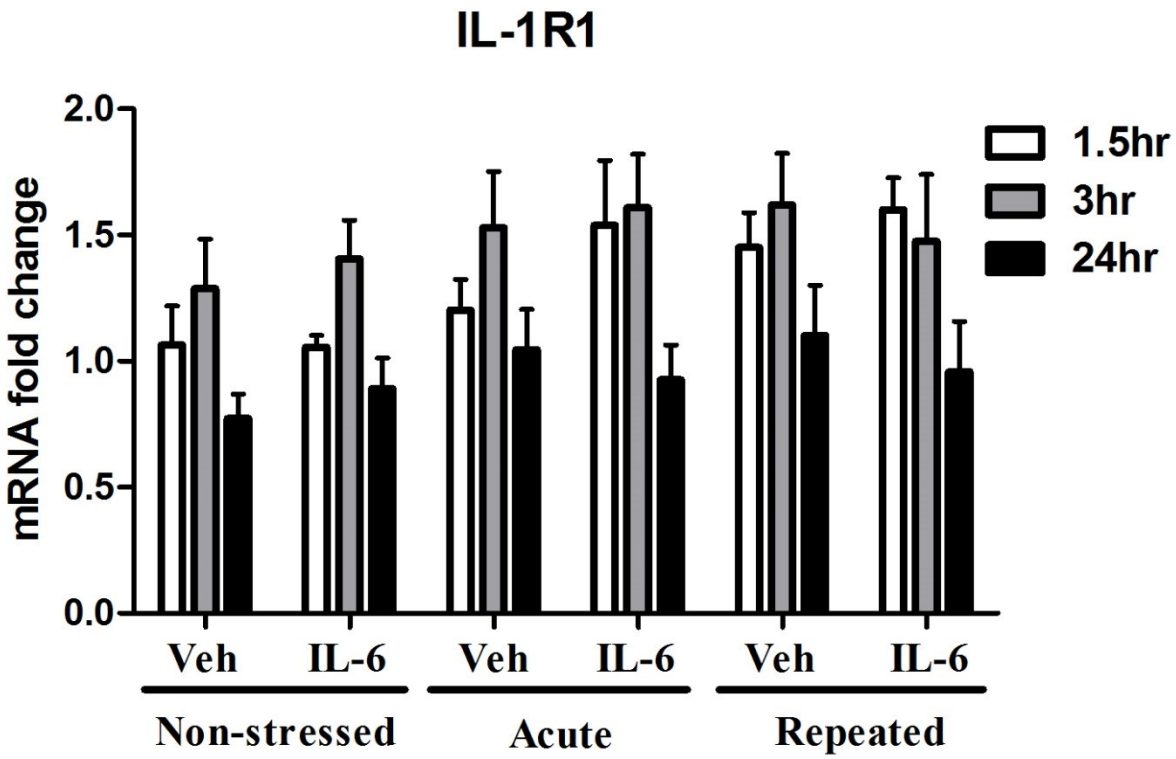

Figure 25. Mean (±SEM) mRNA expression of IL-1R1 within the hippocampus at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). N = 6/group.

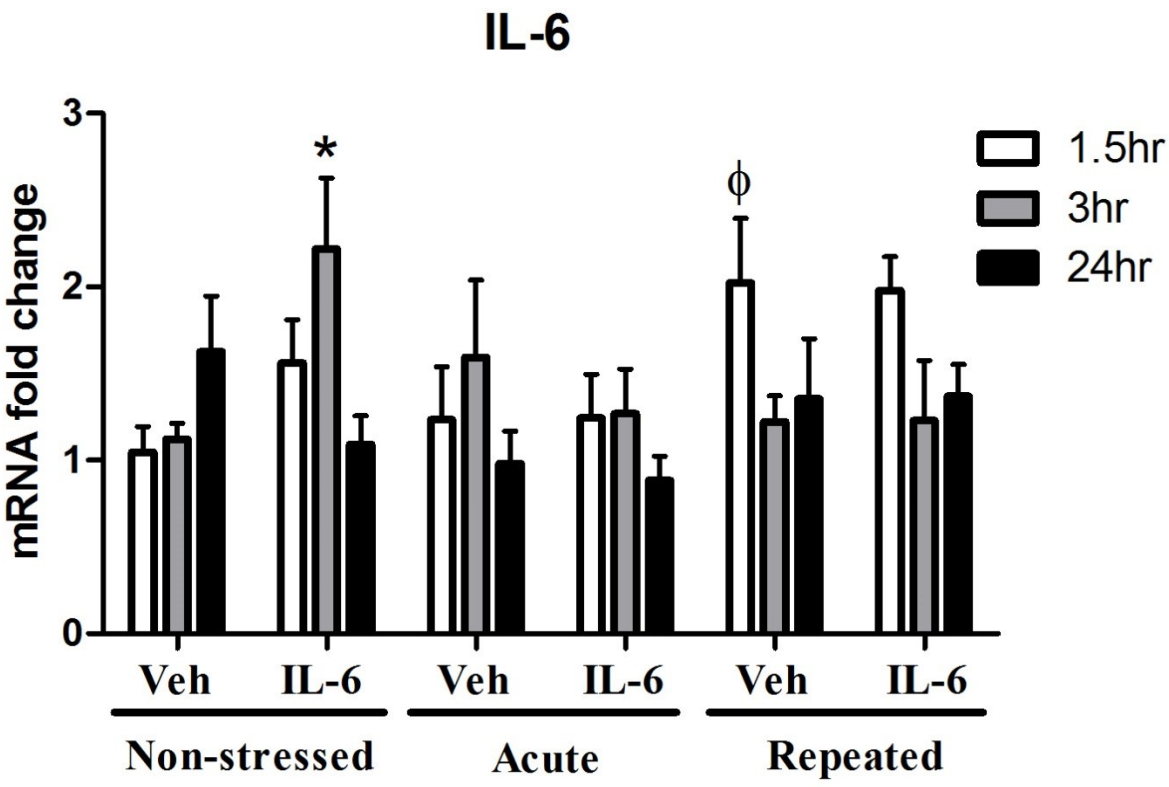

Figure 26. Mean (±SEM) mRNA expression of IL-6 within the hippocampus at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to non-stressed controls; Φ $p < 0.05$ relative to non-stressed controls and mice in the 3 hr group. N = 6/group.

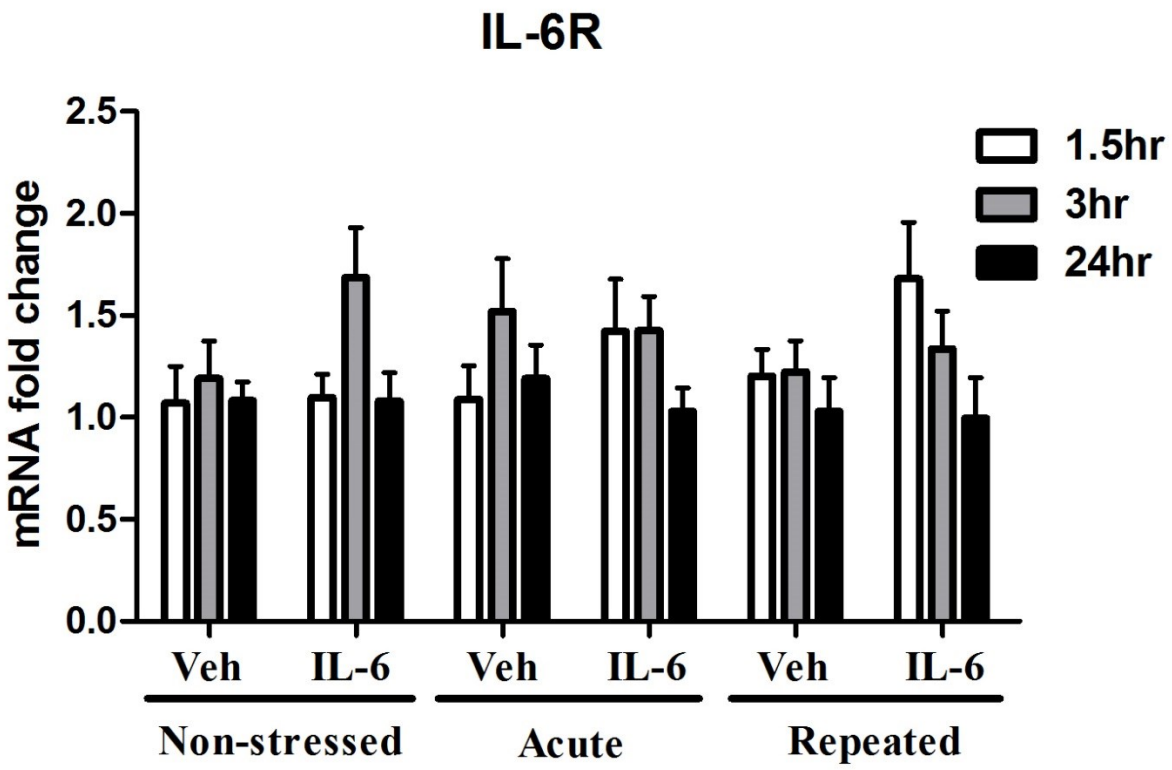

Figure 27. Mean (±SEM) mRNA expression of IL-6R within the hippocampus at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). N = 6/group.

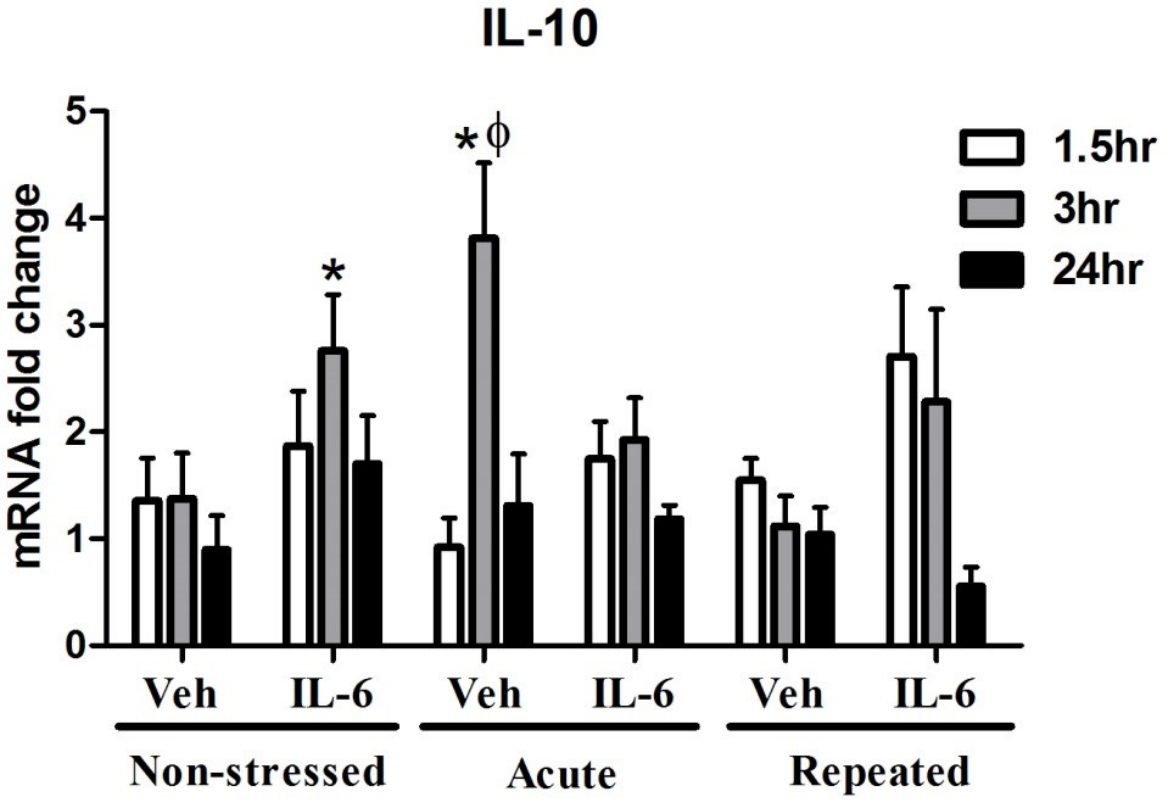

Figure 28. Mean (±SEM) mRNA expression of IL-10 within the hippocampus at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ relative to non-stressed, vehicle injected controls; Φ $p < 0.05$ enhanced response relative to mice in the 1.5 hr group and IL-6 injected counterparts . N = 6/group.

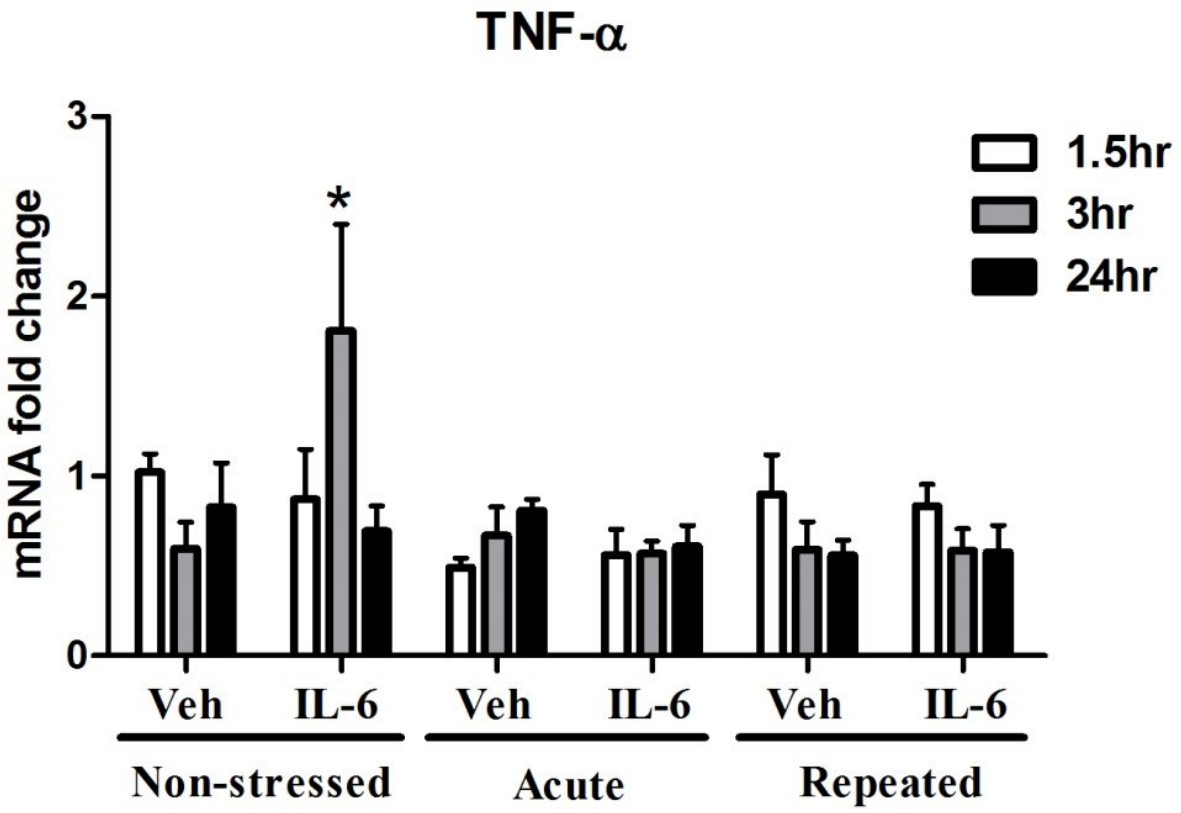

Figure 29. Mean (±SEM) mRNA expression of TNF-α within the hippocampus at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to vehicle injected counterparts, and mice in the 1.5 hr group. N = 6/group.

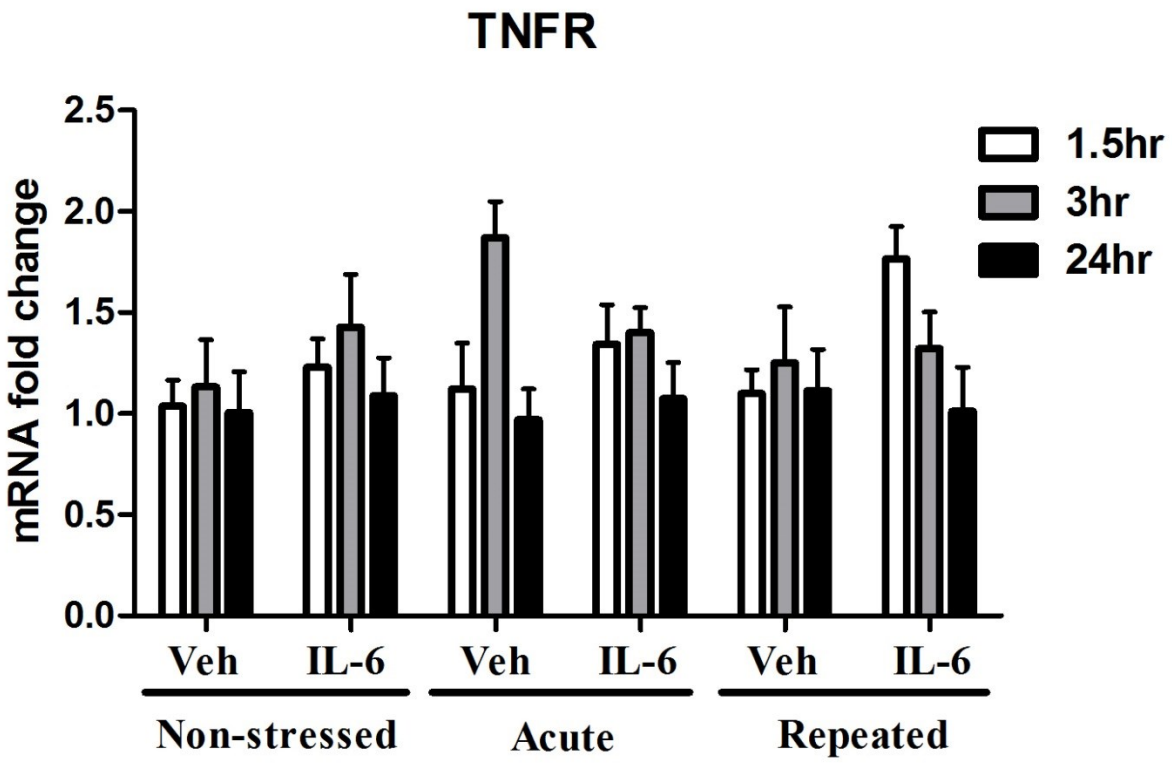

Figure 30. Mean (±SEM) mRNA expression of TNFR within the hippocampus at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had either not been stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). N = 6/group.

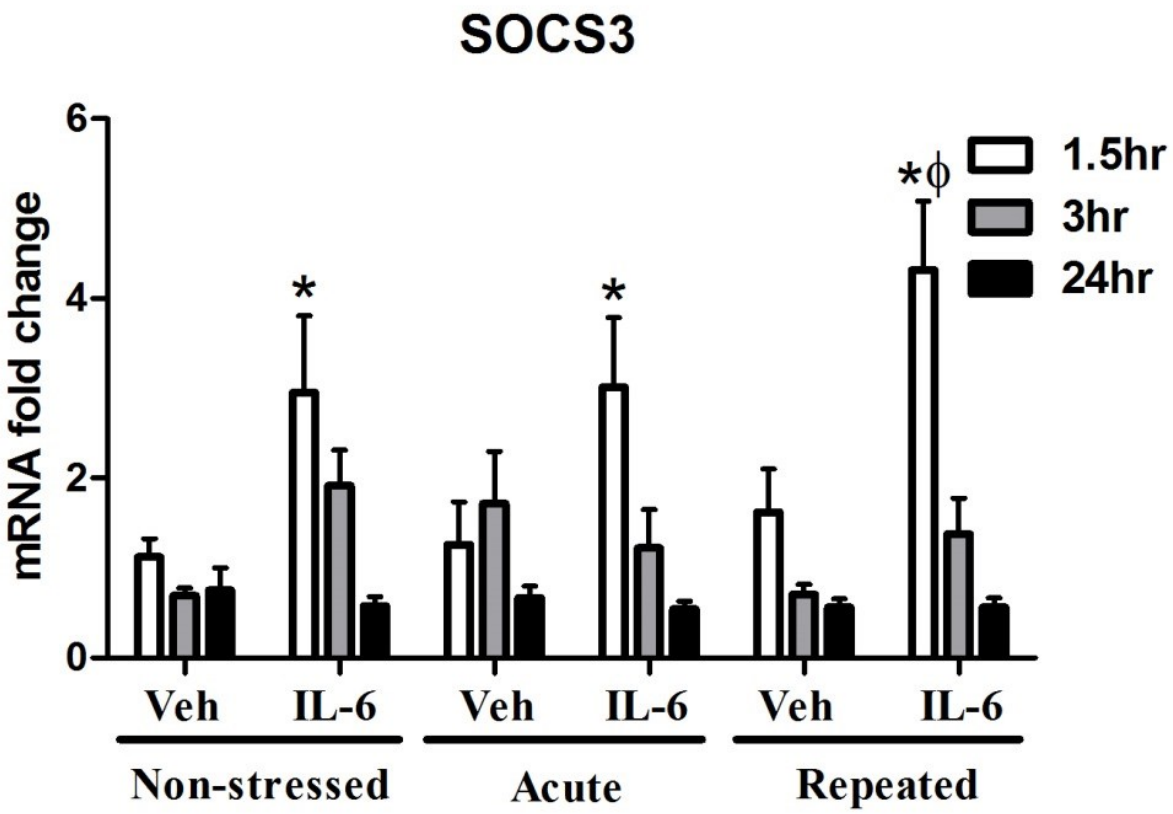

Figure 31. Mean (±SEM) mRNA expression of SOCS3 within the hippocampus at 1.5, 3 or 24 hr following IL-6 (4 μg) or vehicle (BSA) treatment. Mice had been not stressed previously, exposed to an acute social stressor (retired breeder) or exposed to a repeated stressor regimen (retired breeder for 7 days). * $p < 0.05$ enhanced response relative to vehicle injected counterparts and mice in the 3 hr group; Φ $p < 0.05$ relative to non-stressed, IL-6 injected counterparts. N = 6/group.

4. Discussion

Psychosocial stressors in rodents have been shown to influence circulating and brain cytokine as well as other facets of immune functioning (Bartolomucci et al., 2005; Audet et al., 2010). These outcomes can be exacerbated when an immune challenge is superimposed on a stressor backdrop (Gibb et al., 2008, 2013; Merlot et al., 2003). Indeed, studies with humans suffering from chronic inflammatory diseases revealed that stressors tend to aggravate arthritis and cardiovascular disease, often leading to a co-morbid depressive condition (Kemeny & Schedlowski, 2007; Nemeroff & Goldschmidt-Clermont, 2012). In the present study, as observed in the context of bacterial challenges (Brydon et al., 2009; Frank et al., 2010; Gibb et al., 2011, 2013), stressors and immune-related factors additively or synergistically influence cytokine processes. In this regard, combining IL-6 with an acute stressor activated transcription of pro-inflammatory cytokines as well as that of SOCS3, but did so selectively, in that each of the cytokines was not influenced in the same fashion, and the effects that were observed were specific to particular brain regions. As expected, the cytokine variations were time-dependent and varied as a function of the stressor being acute or repeated.

Cytokines are produced in the CNS by a variety of cells, including macrophages, microglia, and neurons (Sébire et al., 1993) and are released in response to infection as well as seizures, concussive injuries, and in neurodegenerative diseases (e.g., Alzheimer's; Rivest, 2009). Stressors have also been found to alter brain cytokine expression, along with changes of brain monoamines and that of plasma corticosterone (Anisman et al., 2008). As cytokines may elicit effects similar to those provoked by stressors, including brain region-specific neurochemical changes (Besedovsky et al., 1986; Lyson & McCann, 1991; Sharp et al., 1989), it was suggested that the brain interprets inflammation much as it does other stressors (Anisman et

al., 2008). In fact, in the present study the use of an acute social stressor accelerated the onset of some of the effects of IL-6 administration on expression of pro-inflammatory cytokines, and additively enhanced the activating effect of IL-6 on SOCS3 expression in the PFC.

One of the most ubiquitous features of depression is it's co-morbidity with other psychiatric disorders, most notably anxiety disorders (Gorman, 1996). In the present investigation anxiety was assessed in an open field test and on the elevated plus maze, where it was observed that stressed mice generally spent more time in the inner square of the open field and on the open arms of the plus maze. This behavioral profile, at first blush, appears counterintuitive as stressors might have been expected to promote increased anxiety characterized by decreased open-field activity and reduced entries onto the open arms of a plus maze. However, within the first few hours of stressor exposure a relatively persistent hyperarousal is evident in mice, often reflected by impulsive behavioral responding. With the passage of time, this behavioral excitation diminishes and is replaced by anxiety changes that might still be evident when mice are retested two weeks later in the same test situation (Jacobson-Pick et al., 2013).

Like stressor treatments, IL-6 injection increased the number of entries into the open arms, but did not influence the time spent there. Curiously, combining the cytokine with the stressor attenuated the increased time spent in the open arms ordinarily elicited by the stressor alone. It has been suggested that endogenous IL-6 might promote an anxiolytic effect (Armario et al., 1998, Butterweck et al., 2003), reflected by reduced entries and time spent in the open arms of the plus maze among IL-6 knockout mice, although other investigators did not observe these disturbances in IL-6 KO mice (Swiergiel & Dunn, 2006). The source for these different findings is uncertain, although they might be related to the timing of the behavioral assessment

following treatment. Indeed, time-based anxiolytic effects of IL-6 could explain some of the findings of Experiment 2, such as the decrease in the latency to enter the center of the open field in response to IL-6, and the ability of the IL-6 injection to rescue the increase in time spent in the open arms of the plus maze seen in response to stress alone. Alternatively, IL-6 injection might have had these actions secondary to decreased locomotion reflected by the decreased time spent in the open arms of the plus maze. However, it was reported that 1.5 hrs after intracerebroventricularly administered rrIL-6, social investigatory behaviour and immobility in rats were not altered (Lenczowski et al., 1999), and the only decrease in locomotion caused by IL-6 was observed 9 hrs after administration in rats (Schöbitz et al., 1995). In the present study, we similarly did not observe differences between groups with respect to closed arm entries and time spent in closed arms, thus suggesting that the observed findings were not a result of altered levels of motor activity.

Stressors are known to influence circulating levels of plasma cortisosterone, but in response to most stressors of moderate severity the hormone levels normalize within less than an hour (Anisman et al., 2008; McEwen et al., 2006). In this investigation, the use of the single acute social stressor in the form of being exposed to an aggressive retired breeder caused an increase of plasma corticosterone levels that were still elevated 1.5 hrs after the encounter. However, in mice that were repeatedly stressed over 7 days the elevated corticosterone was not evident. Such an outcome has been reported frequently following exposure to the same stressor on repeated occasions (Stam et al., 2000; Huether, 1996), and the present results suggest that this same outcome occurs even though the social stressor may be one that is particularly significant and tends to have relatively lasting effects. This said, it might not be productive to consider the limited corticosterone changes associated with a repeated stressor to represent an adaptation in

the form of down-regulated HPA functionality, as it was reported that when animals are exposed to a novel challenge following the repeated stressor regimen, a hyper-elevation of corticosterone may ensue (Aguilera, 1994; Anisman et al., 2008; Stam et al., 2000).

Although pro-inflammatory cytokines, such as IL-1β, produce marked corticosterone elevations, and to a lesser extent so does TNF-α (Anisman & Merali, 1999; Besedovsky et al., 1986; Dunn, 1988; Hayley et al., 1999), there have been few studies that examined the influence of IL-6. In general, however, those studies that did assess the influence of this cytokine, reported modest corticosterone variations. For instance, modest, but significant elevations of corticosterone were observed in response to systemic IL-6 administration (1 and 5 μg) (Wang & Dunn, 1998), and I.C.V treatment of rat with recombinant rat IL-6 caused an elevation of corticosterone 1.5 hrs afterward (Lenczowski et al., 1999). Thus it seems that IL-6 treatment may cause HPA axis activation, peaking at 1 hr, though this varies depending on the dosage used (Matta et al., 1992; Wang & Dunn, 1998; Lenczowski et al., 1999; Nevarra et al., 1991). In the present investigation, injection of 4μg of IL-6 alone did not influence corticosterone levels measured 1.5 hr later, but it is possible that the IL-6 injection caused a rise of plasma corticosterone that was not sufficiently great to persist 1.5 hrs after the injection.

Although it has been reported that central cytokine variations may occur in response to acute and/or chronic stressors (Bartolomucci et al., 2003; Goshen et al., 2008), in the present investigation central mRNA levels of IL-1β, TNF-α, and IL-6 in the PFC and the hippocampus were largely unaffected by the stressor, regardless of whether it was administered on a single or repeated occasions. We previously observed similar results in mice that had been exposed to a series of different stressors administered over 6 weeks (Gibb et al., 2013). The source for the inter-laboratory differences is uncertain given the numerous procedural differences involved in

the studies. This said, in the context of the present investigation, when mice were injected with IL-6, there was a noticeable increase of mRNA expression of IL-1β, IL1R1, and IL-6R in the PFC, and IL-6 and TNF-α in the hippocampus, peaking 3 hrs after injection. Interestingly, when IL-6 administration was superimposed upon the acute stressor, IL-1β expression in the PFC was increased when assessed 1.5 hrs later, as opposed to the increase that occurs at 3 hr in otherwise naïve mice, essentially indicating that the stressor provoked a leftward shift of the temporal changes engendered by IL-6 consequences. In effect, the acute social stressor caused IL-6 to exert some of its effects earlier, and also to normalize sooner. However, in other instances, as in the case of expression of IL-1R1, the expression of this receptor occurred earlier, but persisted throughout the 3 hr post treatment interval, being absent at 24 hr.

As previously mentioned, repeated stressors did not influence mRNA levels of pro-inflammatory cytokines in the PFC and hippocampus. When the last of the repeated social stressors was followed by an injection of IL-6, the expression of IL-1R1 and IL-6 mRNA in the PFC were significantly increased 3 hrs later, but not at 1.5 and 24 hrs. In effect, the relative increase of IL-6 elicited by a combination of the cytokine treatment and the acute stressor was still present in mice that had been repeatedly stressed, indicating that an adaptation did not develop as observed in relation to corticosterone.

Stressors reliably increase plasma corticosterone levels, and as this hormone can suppress cytokine production, it is often assumed that both the peripheral and central cytokine changes introduced by stressors may be a reflection of corticosterone changes or that of corticoid receptors. However, stressors activate many physiological systems, beyond that of the HPA axis, including the sympathetic nervous system response which is characterized by release of catecholamines, epinephrine/adrenaline and norepinephrine (NE). It was found that acute

immobilization stress increases the expression of PNMT, an enzyme that converts NE into epinephrine, and β2-adrenergic receptor mRNA in the spleen (Jelokova et al., 2002; Laukova et al., 2010). Interestingly, repeated stressors not only down-regulated β2-adrenergic receptors, but also increased IL-6 gene expression in the spleen (Laukova et al., 2010).

Acute stressors ordinarily increase the utilization of NE in several brain regions, and if sufficiently intense and uncontrollable, brain NE levels may decline. However, this reduction of NE was not observed following a chronic stressor, possibly owing to a compensatory of NE synthesis (Shanks et al., 1994; Irwin et al., 1986; Stone, 1979). In the context of the current investigation, the acute stressor quickened the appearance of the effects of IL-6 on IL-1β, IL-1R1, and IL-6R, possibly owing to the release of NE provoked by the stressor. Unlike the apparent adaptation of corticosterone levels that occurs with a repeated stressor, this was not seen with respect to each of the cytokines. Indeed, in the case of IL-6 and IL-10, the mRNA expression was at least as great after the repeated stressor as it had been after the acute stressor, although there was a shift in the time course of IL-10 variations. As the cytokine and corticosterone variations were not congruent with one another, it is unlikely that corticosterone was causally related to the changes of these cytokines elicited by the stressor treatments. Interestingly, the mRNA changes of IL-1β associated with the acute stressor was entirely absent following the repeated stressor treatment, attesting to the independence of this cytokine from that of IL-6 and IL-10, but leaves open the possibility that IL-1β variations were, in fact, linked to that of corticosterone.

Several other monoamines, such as 5-HT and DA in the central amygdala (Brebner et al., 2000), and the ratio of 5-hydroxyindoleacetic acid (5-HIAA) to 5-HT in the brain stem (Wang & Dunn, 1998), were increased in response to IL-6. As 5-HT contributes to the regulation of HPA

axis activity, and may even be able to stimulate adrenal cells directly (Dinan, 1996; Lefebvre et al., 1996), a role for this transmitter in mediating the synergy between stress and IL-6 challenge cannot be discounted. Thus, while we did not examine monoamine variations directly, it is important to consider the indirect effects of IL-6 and stressors on these processes.

Ordinarily, the levels of pro- and anti-inflammatory cytokines should be in balance with one another, and perturbations in one way or the other have been linked to pathological outcomes (Sredni-Kenigsbuch, 2002). However, some treatments may selectively influence one type of cytokine over another, or may cause earlier (or later) expression of a particular cytokine, and hence during this period of imbalance, animals might be more responsive or vulnerable to certain challenges. In this regard, it was demonstrated that the peripheral and central responses to challenge with an endotoxin (LPS) varied across brain regions and peripheral blood, but was also further modified by stressors so that altered balances between IL-10 and both IL-6 and TNF-α were further dysregulated (Gibb et al., 2013). In the present investigation, levels of IL-10 mRNA in the PFC and the hippocampus increased 1.5 hrs after the repeated stressor / IL-6 treatment, whereas an elevation of IL-6 transcription in the PFC occurred 3 hrs after injection.

The presence of IL-6 may be important in T lymphocyte development and differentiation associated with immunogenic challenges, as it inhibits Th1 proliferation and promotes Th2 differentiation (Diehl & Rincón, 2002). It is thought that the balance of circulating IL-6/IL-10 is important for system homeostasis and that disruption of this balance favors the development of pathologies (Sredni-Kenigsbuch, 2002). It is uncertain to what extent such imbalances within the brain are important in promoting psychopathology. However, in the current investigation, the temporal imbalance seen in IL-6/IL-10 levels in response to a repeated stressor may promote neurochemical disturbances that could influence behavioral disturbances.

The findings of Experiment 3 largely corroborated those seen in Experiment 1, although there were differences observed that are noteworthy. In Experiment 1 the acute social stressor seemed to decrease TNF-α expression in the PFC 1.5 hrs after injection irrespective of whether mice received IL-6 or vehicle treatment. In contrast, in Experiment 3, the increase was only observed 3 hrs after injection of IL-6, and only when the injection was applied on a backdrop of an acute social stressor. Furthermore, in the hippocampus of mice from Experiment 1, TNF-α expression was increased in response to IL-6 injection among mice that had not been stressed. In Experiment 3, hippocampal TNF-α was also increased in response to the IL-6 treatment, but this occurred 3 hrs rather than 1.5 hrs after the treatment. The source for these differences is not certain, particularly with regard to the time-dependent variations of TNF-α that were induced by the IL-6 treatment in Experiment 1 and 3 where the nonstressed animals essentially received identical treatments. The differential effects of the stressor treatments on TNF-α in the two experiments could, of course, be spurious, although there were differences between these treatments that could potentially account for the differences observed. In Experiment 1, the mice were returned to their home cage after the first physical altercation. This generally occurred within a minute of being introduced into the cage with the retired breeder, and never exceeded 5 minutes. The IL-6 injection, however, always occurred 15 minutes after the start of the stressor. In Experiment 3, the animals were again allowed to interact until a physical altercation occurred; however, once such an interaction occurred, a partition was placed in the cage, thereby separating the two animals for the remainder of the 15 minutes. Thus, during this time the mice could see, hear and smell one another, but physical contact was not possible. It is possible that the differential mRNA levels of TNF-α between the two experiments might have been due to the intense stress procedure of Experiment 3. However, this still begs the question as to why this

effect was restricted to the hippocampus and was evident only with respect to TNF-α and was not apparent with respect to other cytokine or receptor mRNA expression.

A consistent finding of the present study was that SOCS-3 was increased by both IL-6 and by the acute stressor, and that these treatments had additive effects in this regard. Interestingly, the enhanced effects of the IL-6 treatment were not provoked by the chronic stressor, and the analysis of the time course for these changes revealed that the additive effects of IL-6 and the stressor were restricted to the 1.5 hr assessment time, being entirely absent 3 hrs following the stressor treatment. In fact, at the latter time point, the stressor seemed to antagonize the enhancing effects of the IL-6 treatment. A different picture emerged within the hippocampus. Although, IL-6 treatment enhanced the expression of SOCS-3, the stressors did not affect hippocampal SOCS-3 mRNA expression. However, the chronic stressor modestly enhanced the influence of IL-6 on SOCS-3 expression, although this was restricted to the 1.5 hr time point.

Ordinarily, IL-6 activates the JAK/STAT signalling pathway after it binds to its receptor and, along with the gp-130 signal transducer protein, forms the IL-6 receptor complex. This activates a signalling cascade that eventually leads to transcription of target genes involved in immune and CNS regulation (Wang & Campbell, 2002; Akira, 1999). This signalling cascade also enhances expression of the suppressors of cytokine signalling (SOCS proteins, specifically SOCS-1 and -3 in the case of IL-6). SOCS operates through a negative feedback loop by inhibiting different components of the JAK/STAT pathway, thus regulating the intensity and duration of cytokine expression and preventing excessive activation (Auernhammer & Melmed, 2001).

It is currently uncertain how stressors come to exert their effects on IL-6 functioning, although it can occur through stimulation of the JAK/STAT pathway. In this regard, it has been shown that the synthetic glucocorticoid dexamethasone, which normally plays an immunosuppressive role, is able to increase IL-6 induced gene expression and protein in the liver via prolonged activation of STAT3 and down-regulation of SOCS3 (Dittrich et al., 2012). While glucocorticoids may play a role in stress-induced IL-6 gene expression, it is unlikely that they are the only players involved, as we observed cytokine variations in repeatedly stressed animals where an adaptation had developed regarding the corticosterone response. Cytokines are usually unable to readily pass the BBB without the use of saturable transport systems (Banks et al., 1995), as they tend to be hydrophilic and have large molecular weights. Thus, cytokine entry might be restricted to sites where the BBB is compromised, such as the circumventricular organs (CVOs) and the choroid plexus (Vallières & Rivest, 1997). In addition, however, stressors could disturb the integrity of the blood brain barrier (BBB). In this regard, acute immobilization stressors have been found to increase the permeability of the BBB via CRH activation of mast cells in the brain (Esposito et al., 2001; 2002), allowing more IL-6 entry into the brain. As well, administration of IL-6 itself can increase BBB permeability in the rat brain (Saija et al., 1995). The two manipulations used in this investigation may both influence the BBB, and hence it is possible that the combined treatments significantly compromised BBB integrity, allowing for greater entry of cytokines into the parenchyma.

Although depression has consistently been associated with an increase of circulating IL-6 (Basterzi et al., 2005), less is known about SOCS-3 levels. Elevated levels of SOCS-3 are seen after cerebral ischemia, traumatic brain injury and focal strokes (Carmichael, 2003; Raghavendra Rao et al., 2003), just as cytokines are. Despite the limited knowledge concerning SOCS-3 levels

in depressed humans, it was found that animals that displayed learned helplessness behavior in response to an uncontrollable stressor also displayed lower SOCS-3 gene expression in the prefrontal cortex and hippocampus compared with mice that did not show learned helplessness (Mingmalairak et al., 2010). Thus, it was proposed in the current investigation that the combination of the IL-6 injection and a social stressor would cause elevated SOCS-3 expression.

The increase of circulating IL-6 seen in depression is not exclusive to this illness, as patients diagnosed with PTSD also display increased IL-6 (Maes et al., 1999). This said, depression is also associated with an increase in the membrane soluble form of the IL-6 receptor (sIL-6R) (Maes et al., 1995). Interestingly, patients with concurrent PTSD and depression have significantly higher levels of serum sIL-6R compared to those with PTSD alone (Maes et al., 1999). Thus, the two disorders may share some common pathophysiological mechanisms, although how they translate into one or the other disorder is uncertain. The importance of the soluble form of the receptor lies in it having several functions not shared with its membrane bound counterpart, such as the ability to trigger osteoclast formation in vitro (Tamura et al., 1993), which might be relevant to the frequent reductions of bone density elicited by stressors and has commonly been seen in depression (Rizzoli et al., 2012; Yirmiya et al., 2006), as well as inducing expression of certain neurotrophic factors in astrocytes that play a role in neuronal survival (März et al., 1999). It is unclear what role sIL-6R plays in the etiology of depression, however, it could be that the increase seen serves in a neuroprotective capacity meant to regulate the neuro-immune response to disease, although at relatively high levels it may become neurodestructive (Maes et al., 1995).

The present investigation did not attempt to assess anti-inflammatory properties associated with sIL-6R, but might still have had implications for novel treatments for affective

disorders that take the inflammatory component of the disease into consideration. Traditional treatments, such as SSRIs and SNRIs, are reported to only be effective in depression in 60% of individuals and only partially attenuate depressive symptoms (Trivedi et al., 2006). Furthermore, SSRI/SNRI resistant patients displayed significantly elevated levels of plasma IL-6 compared to those that responded positively to the SSRI/SNRI treatment (Yoshimura et al., 2009). In the patients that do respond to treatment, antidepressants decreased plasma IL-6 (Basterzi et al., 2005; Yoshimura et al., 2009) and attenuated depressive symptoms that result from cytokine treatment (IFN-α in Hepatitis C patients, Schramm et al., 2000). These data suggests that IL-6 could be used as a biomarker for depressive illness, and perhaps as a marker of potential treatment efficacy.

In addition to the elevation of circulating IL-6 and sIL-6R, depression is also associated with time-dependent suppression of certain immune molecules as well as variations of other markers of the immune system response, such as C-reactive proteins (Howren et al., 2009; Maes, 1995). Indeed, before a role of pro-inflammatory cytokines was suggested, depression was initially thought to only be associated with decreased immunity, pertaining to observations that individuals with major depression displayed a decrease in lymphocyte proliferative response in response to non-specific mitogens (Zorrilla et al., 2001), as well as a decrease in natural killer (NK) cell activity (Irwin et al., 1987; Zorrilla et al., 2001). These effects were also ameliorated upon antidepressant treatment (Frank et al., 1999; Ravindran et al., 1995). Moreover, people under chronic stress have reduced antibody responses to immunization against the influenza virus (Vedhara et al., 1999). Taken together, it is clear that depression causes an alteration in many aspects of immune functioning, including but not limited to cytokine expression, that may contribute to the pathophysiology of the disease.

Recently, there has been a call for an anti-inflammatory therapeutic approach to depression (Hayley, 2011). Combining the antidepressant fluoxetine with the tetracycline antibiotic minocycline, which blocks the activation of microglial cells, synergistically attenuated depressive-like behaviour (Molina-Hernández et al., 2008; Joks & Durkin, 2011). Several studies likewise found that the non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, and indomethacin had antidepressant effects (Müller and Schwarz, 2008, Teeling et al., 2010). NSAIDs work by inhibiting cyclooxygenase, an inflammatory enzyme. They non-selectively inhibit isoenzymes of COX (COX-1 and COX-2). COX-2 is induced in response to inflammatory factors, including cytokines (Cao et al., 1999; Goppelt-Struebe, 1995). Additionally, COX-1 and COX-2 are rate-limiting enzymes in the production of prostaglandins (PGs), and PGE_2 is a mediator of inflammation that is increased in depression (Milton, 1982; Müller and Schwarz, 2008). Moreover, PGE_2 release is stimulated by IL-6 (Fukunaga et al., 1991). Thus it follows that blocking COX-2 using NSAIDs led to anti-depressive effects. Nonetheless, questions remain as to whether PGE_2 is merely a correlate of depression or if it plays a causal role in the disease. Indeed, it was recently found that PGE_2 levels are increased in mice exposed to a repeated social defeat procedure, and mice that had the PGE receptor blocked did not display the social avoidance associated with repeated defeat (Tanaka et al., 2012). Moreover, this effect appeared to be at least partially facilitated by attenuation of the mesocortical dopaminergic pathway (Tanaka et al., 2012). Thus, it seems that by stimulating PGE_2 release, IL-6 is able to promote some social disturbances that may be indicative of depressive illness. However it is unlikely that this is the only link between IL-6 and depression.

The current investigation suffered from several limitations. First, the sample size in Experiment 3 was low (~6 animals/group). As previously mentioned with regard to the

behavioural tests, the results were fogged by the hyperarousal displayed by stressed mice. It would have been beneficial to add an extra group to the experiment that were injected then tested two weeks after the stressor, thereby allowing anxiety-related effects to appear. Also, as discussed previously, our investigation primarily examined mRNA levels in the brain, which might not necessarily represent protein synthesis and circulating cytokine levels.

The cytokine manipulation in these experiments comprised the injection of IL-6, and while this served as a challenge to the mice, it is important to consider that cytokines are expressed at very low levels under basal conditions and serve many normal functions. It has been suggested that elevated cytokine levels can be neuroprotective, neurodestructive, or both, depending on several factors such as region, chronicity, and nature of the inciting event (Pascoe et al., 2011; Stoll et al., 2002). Moreover, affective disorders are chronic conditions that develop over time, thus a more appropriate approach would be to assess the effects of chronic treatments, preferably through continuous infusion that would be relevant to changes of daily IL-6 variations. Finally, while the observed cytokine variations were taken at face value, it is important to consider that cytokine levels have been found to vary between stressor-reactive and stressor-resilient strains of mice (Gibb et al., 2011), thus making the generalization of the current results to human populations difficult.

4.1 Conclusion

Inflammatory immune activation may promote neurochemical changes that ultimately increase susceptibility to affective disorders (Anisman et al., 2008). Combining an immune challenge, such as LPS, with a psychosocial stressor has been shown to exacerbate the effects of stressors on circulating and brain cytokines and to induce behavioural and immune disturbances (Gibb et al., 2008, 2013; Merlot et al., 2003). In the present investigation, it was demonstrated

that an acute social stressor seemed to augment the influence of IL-6 administration on certain pro-inflammatory cytokines, as well as additively enhancing the effects of IL-6 on SOCS-3 gene expression. The combined treatment also induced a temporal imbalance in the IL-6/IL-10 ratio, which might also contribute to pathology. As expected, these results were dependent on the chronicity of the stressor, the brain region examined, and the timing of outcome measurement. Stress may be affecting the IL-6 activated JAK/STAT pathway via actions on glucocorticoids and their receptors, as well as possibly compromising the integrity of the BBB and allowing more IL-6 access to the brain parenchyma. It is unclear whether the role IL-6 plays in the development of depression and other affective disorders is a causal one, or if it is a mediator in this process, as there are many other factors involved in the immune response to depression. Nevertheless, IL-6 may serve as a bio-marker for depressive illness and potential treatment efficacy.

5. References

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