

**STRESSOR AND CYTOKINE INTERACTIONS: IMPLICATIONS IN
DEPRESSIVE-LIKE PATHOLOGY**

by

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Abstract

Stressful events have been implicated in the development of affective disorders through their effects on neuronal functioning. In addition to their independent effects, it has been shown that stressor exposure can interact with, and even potentiate inflammatory immune system activation, to produce exaggerated neurochemical and behavioural responses. As both stressors and cytokines have been implicated in the development of behavioural disturbances, the present series of studies were undertaken to further investigate the independent and combined effects of stressors and immunogenic agents on behavioural, neuroendocrine and inflammatory processes. In this regard, an initial study examined whether acute and chronic stressors differentially influenced sickness behaviour, circulating corticosterone and central cytokine mRNA expression alone, or in combination with the bacterial endotoxin lipopolysaccharide (LPS). It was found that both acute and chronic stressor exposure synergistically increased sickness and circulating corticosterone when combined with LPS. Central cytokine mRNA expression was also markedly increased following the combined treatments, but this depended on the chronicity of the stressor and timing of the outcome measures. As there is considerable inter-individual variability in the impact of stressors, another investigation assessed whether the synergistic effects of stressors and an immune challenge (either LPS or the viral mimic poly I:C) would differentially influence strains of mice that were either stressor reactive (BALB/cByJ) or stressor resilient (C57BL/6ByJ). It was found that the combined treatments markedly increased sickness and corticosterone, and this was more prominent in the BALB/cByJ strain, whereas circulating and central cytokine elevations were more prominent in the C57BL/6ByJ strain. Thus, synergistic effects of stressors and

immune challenges not only depend on the chronicity of the stressor, but also on the emotionality of the strain. Finally, as immune system activation appears to be related to behavioural disturbances, a last series of studies demonstrated that pretreatment with the antibiotic minocycline could attenuate endotoxin-induced anxiety-like behaviour in an openfield and central cytokine expression; although its stressor like effects augmented corticosterone and sickness behaviour. These data indicate that the effects of immunogenic treatments, including those that are meant to attenuate immunologically based illnesses, ought to consider the stressor background upon which these are superimposed.

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List of Abbreviations

5-HT, serotonin; 5-HTT, serotonin transporter; ACTH, adrenocorticotrophic hormone; ANOVA, analysis of variance; BBB, blood brain barrier; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; COX, cyclooxygenase; CRH, corticotropin-releasing hormone; CSF, cerebrospinal fluid; GC, glucocorticoid; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenal axis; IDO, 2,3-indoleamine-dioxygenase; IFN, interferon; I κ B, inhibitor of kappa B; IL, interleukin; IP, intra-peritoneal; LPS, lipopolysaccharide; LTP, long-term potentiation; MHC, major histocompatibility; MDD, major depressive disorder; mPFC, medial prefrontal cortex; NE, norepinephrine; NF κ B, nuclear factor kappa B; poly I:C, polyinosinic:polycytidylic acid; PVN, paraventricular nucleus of the hypothalamus; RA, receptor antagonist; RIA radioimmunoassay; RT-QPCR, reverse-transcription quantitative polymerase chain reaction analysis; SEM, standard error of the mean; SOCS, suppressors of cytokine signaling; SSRI, selective serotonin reuptake inhibitors; TH, T helper type; TLR, toll-like receptor; TNF, tumor necrosis factor.

Introduction

The etiology of major depressive disorder (MDD) is complex, likely involving the interaction between multiple factors, including genetic and biological vulnerability as well as individual life experiences. Although the pathophysiology of depression remains to be elucidated, it is thought that MDD stems from disruptions of several biological processes, including monoaminergic neurotransmission, growth factors (e.g. brain-derived neurotrophic factor; BDNF), corticotropin-releasing hormone (CRH), and inflammatory factors (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Duman & Monteggia, 2006; Krishnan & Nestler, 2008; Maes, et al., 2009; Miller, Maletic, & Raison, 2009). Although any number of factors may lead to the neurobiological disturbances that accompany MDD, there is considerable evidence that stressful experiences might act in this capacity (Anisman, 2009; Bartolomucci & Leopardi, 2009; Chrousos, 2009; Leonard & Myint, 2009; McEwen, 2008). In fact, acute and chronic stressor experiences have been found to provoke the dysregulation of neuroendocrine systems, disruptions of monoamine neurotransmission, and altered synaptic plasticity, and these effects are typically accompanied by depressive-like behaviour and can be reversed with antidepressant treatment (Anisman, 2009; Bartolomucci & Leopardi, 2009; Leonard & Myint, 2009; McEwen, 2008).

Beyond the involvement of external stressors, there has been increasing attention devoted to the view that activation of the inflammatory immune system may act as a systemic stressor, and may play a provocative role in the evolution of MDD (Anisman, Merali, & Hayley, 2008; Dantzer, et al., 2008; Miller, et al., 2009). Although the effects of traditional stressors can be dissociated from those in response to a systemic stressor, it

has been shown that activation of the inflammatory immune system, and subsequent release of proinflammatory cytokines (signaling molecules of the immune system), can elicit neurochemical effects reminiscent of those provoked by traditional stressors (Anisman, Gibb, & Hayley, 2008; Brebner, Hayley, Zacharko, Merali, & Anisman, 2000; Linthorst & Reul, 1998). Paralleling these neurochemical effects, cytokines can also produce behavioural disturbances similar to those observed in MDD (Dantzer, 2009; Gibb, Audet, Hayley, & Anisman, 2009). Furthermore, in addition to their unique effects, stressors and immune challenges may additively or synergistically influence behaviour, neurochemical and cytokine functioning (Anisman, Poulter, Gandhi, Merali, & Hayley, 2007; Gandhi, Hayley, Gibb, Merali, & Anisman, 2007; Gibb, Hayley, Gandhi, Poulter, & Anisman, 2008).

Given the potential contribution of stressor-related and inflammatory processes in MDD, the aim of the current thesis was to further elucidate behavioural and central alterations that occur in response to cytokines and stressors. To this end, in the present investigation we examined the independent and combined effects of acute or chronic stressors and lipopolysaccharide (LPS) on sickness behaviour and circulating glucocorticoids (GCs), as well as the expression of central cytokine mRNA in an outbred strain of mice. Furthermore, considering the intricate relationship between genetic vulnerability and environmental factors, we then examined the combined effects of an acute social stressor and two types of immune challenges (LPS and the viral mimic polyinosinic:polycytidylic acid [Poly I:C]) in inbred strains that are known to be either stressor-reactive or stressor-resilient. Finally, we assessed whether minocycline, an antibiotic that inhibits the activation of microglial cells and thus decreases cytokine

expression in the brain (Kim & Suh, 2009), can act as an antidepressant and dampen the neurochemical and behavioural effects of an immune challenge and stressful events.

Stressful Events and the Development of MDD

Major depressive disorder is a debilitating condition that affects a large percentage of the population worldwide, and is predicted to become one of the three leading causes of burden of disease in the world by 2030 (Mathers, Ezzati, & Lopez, 2007). Part of the difficulty in diagnosing and treating MDD is the fact that it is a heterogeneous disorder, comprising of several subtypes (e.g. dysthymia, typical and atypical depression, unipolar and bipolar disorders), each with their own, but not mutually exclusive, set of symptoms and diverse underlying processes (Antonijevic, 2006). A further complication is the fact that MDD tends to be co-morbid with a broad spectrum of other inflammatory conditions and diseases, making a sole diagnosis or treatment considerably more difficult (Anisman, Merali, & Hayley, 2008; Evans, et al., 2005; Uzun, Kozumplik, Topic, & Jakovljevic, 2009; Yirmiya, et al., 1999). Therefore, despite recent advances in depression research, the mechanisms behind its development are still relatively ambiguous.

There have been substantial advancements in depression research in the past few decades, combining data from both clinical patients and animal models. Amongst the extensive literature, there is little dispute over the involvement of stressors in the pathophysiology of MDD (Anisman, 2009; Chrousos, 2009; Leonard & Myint, 2009; McEwen, 2008). The term 'stressor' can be defined as a negatively appraised situation or event that elicits a biological response (stress response) in an effort to adapt or

accommodate environmental or psychological challenges (McEwen, 2008). There is considerable individual variability in the impact of stressors, likely owing to factors relating to individual characteristics, such as age, gender, genetic and biological vulnerability, as well as characteristics of the stressor itself; like severity, chronicity and controllability (Anisman & Matheson, 2005).

The stress response is ordinarily thought to have adaptive value, preparing the organism to deal with environmental insults. However, prolonged stress can become maladaptive as individual resources may become overly taxed. This situation, termed allostatic overload, can lead to the development of physiological and psychological disorders (McEwen, 2008). In this regard, it is thought that depression may be associated with the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis functioning provoked by stressors, leading to disruption of monoaminergic neurotransmitter systems, particularly in hippocampal and prefrontal cortical regions, and hence the evolution of depression (Bartolomucci & Leopardi, 2009; Chrousos, 2009; McEwen, 2008). This view was recently elaborated upon with the proposal that stressors can alter neuronal plasticity by decreasing the expression of growth factors (such as BDNF and nerve growth factor), which has been associated with MDD (Alleva & Francia, 2009; Duman & Monteggia, 2006). Regardless of the mechanisms involved, stressors seem to consistently provoke and exacerbate depressive symptoms.

Behavioural Effects of Stressors

Stressors can be characterized as being either 'processive' (those involving higher-order sensory processing) or 'systemic' (those involving physical insults resulting

in circulatory, respiratory, hemodynamic or immune alterations). The former stressors can be further characterized as being either psychogenic or neurogenic; psychogenic stressors are those that are purely psychological in nature (e.g. restraint or predator exposure), whereas, neurogenic stressors are those that involve a physical stimulus (e.g. foot shock; Herman & Cullinan, 1997). Although the effects of a wide variety of psychogenic and neurogenic stressors have been investigated, increasing attention has been devoted to assessing the impact of ethologically relevant stressors, notably social stressors (for a review see Bartolomucci, 2005). These stressors focus on the natural tendency of rodents to develop social hierarchies, and function to either disrupt pre-existing hierarchies by including a dominant intruder (Bailey, Kinsey, Padgett, Sheridan, & Leblebicioglu, 2009; Bartolomucci, 2005; Bartolomucci, et al., 2001; Quan, et al., 2001; Sheridan, Stark, Avitsur, & Padgett, 2000), or by forcing rodents to create new social hierarchies by regrouping previously individually housed animals in a novel cage (Anisman, Poulter, et al., 2007; Gandhi, et al., 2007; Gibb, et al., 2008). Other paradigms focus on the fighting behaviour of rodents, and investigate the behavioural and neurochemical effects associated with being dominant or submissive (Audet & Anisman, 2009; Merlot, Moze, Dantzer, & Neveu, 2003; Pizarro, et al., 2004; Powell, et al., 2009; Razzoli, Carboni, & Arban, 2009). This recent surge of ethologically relevant paradigms has permitted the analysis of social stressors on behaviour, neurochemistry, neuroplasticity and inflammation in relation to depressive-like states.

Stressors consistently elicit behavioural changes in rats and mice, which to some extent mimic the depressive symptoms seen in humans (Bartolomucci, 2005). For instance, both acute and chronic social stressors can lead to the development of

anhedonia, the inability to experience pleasure, which is a core symptom of depression in humans (Razzoli, et al., 2009; Schweizer, Henniger, & Sillaber, 2009; Wann, Audet, Gibb, & Anisman, 2010). Behavioural despair, the endpoint of learned helplessness, is another behavioural characteristic observed in response to stressor exposure in animals (Rygula, et al., 2005), which can be comparable to depressive affect in humans. Other depressive-like behaviour provoked by social stressors include decreased locomotor, grooming, and exploratory activities, reduced food and water intake, as well as increased anxiety-like behaviour (Bartolomucci, et al., 2005). As expected, these behaviours can be attenuated with antidepressant treatment (Rygula, et al., 2006; Von Frijtag, Van den Bos, & Spruijt, 2002).

Stressors and HPA Dysregulation

Dysregulation of the HPA axis is one of the most consistent biomarkers of depression, and has been hypothesized to play a major role in its pathophysiology (Chrousos, 2009; Gillespie & Nemeroff, 2005; Holsboer, 2001; Marques, Silverman, & Sternberg, 2009). As such, depression has often been associated with HPA hyperactivity (as measured by levels of cortisol and adrenocorticotropic hormone [ACTH] within plasma, urine and cerebral spinal fluid [CSF]; Gillespie & Nemeroff, 2005; Marques, et al., 2009; Parker, Schatzberg, & Lyons, 2003). Consistent with this view, social stressors, like neurogenic and psychogenic insults, prompt neuroendocrinological changes, and particularly increased secretion of corticosterone (Audet & Anisman, 2009; R. J. Blanchard, McKittrick, & Blanchard, 2001; Keeney, et al., 2006; Keeney, Hogg, & Marsden, 2001; Razzoli, et al., 2009). Specifically, these stressors activate select

neurons in the paraventricular nucleus of the hypothalamus (PVN), which secretes CRH. The CRH triggers the release of ACTH from the pituitary gland, which stimulates the adrenal cortex to release GCs, such as corticosterone (cortisol, in humans; Sapolsky, 2000). Chronic stimulation of HPA functioning, through repeated exposure to social stressors, has also been found to result in relative hypertrophy of the adrenal gland (Bartolomucci, 2005; Engler, Engler, Bailey, & Sheridan, 2005), an effect typically associated with MDD (Nemeroff, et al., 1992; Rubin, Phillips, Sadow, & McCracken, 1995).

Although transient increases in HPA activity are expected following stressor exposure, chronic social stressors have been found to provoke more protracted and exaggerated increases, which have been attributed, in part, to a dampened functioning of the negative HPA feedback loop (Bartolomucci, et al., 2005; Bartolomucci, et al., 2004). Once the HPA axis is activated and GCs are secreted, they bind to GC receptors on the hippocampus and PVN, which inhibit the further release of CRH, deactivating the HPA axis (Chrousos, 2009). The proper functioning of the negative feedback loop is typically investigated using the dexamethasone suppression test, or more recently, the dexamethasone/CRH test, which assesses the ability of the central nervous system (CNS) to terminate the HPA axis (Schule, et al., 2009). Using the dexamethasone suppression test, it was found that social stress impairs the ability of the CNS to terminate the HPA response (Bartolomucci, et al., 2005; Bartolomucci, et al., 2004), leading to prolonged hyperactivity of the HPA axis, which may overtax available resources and lead to the development of stressor-related pathologies.

Effects of Stressors on Neurotransmission

Stressors have long been known to influence neurotransmitter synthesis and release (Linthorst & Reul, 2008). Such effects have been observed in response to a variety of different stressors, although it is acknowledged that not all stressors engage the same neural circuits (Herman & Cullinan, 1997). In this regard, it seems that innate stressors, learned stressors (cues associated with a previously encountered aversive event) and systemic stressors may activate different processes and might be moderated by different variables (e.g., Anisman, Hayley, Kelly, Borowski, & Merali, 2001). As the focus of the present series of studies entails mainly psychosocial stressors, a considerable degree of the literature review will deal with challenges of this sort.

Like neurogenic and other psychogenic challenges, psychosocial stressors increase the release of serotonin (5-HT) in the prefrontal cortex (PFC), amygdala, and hippocampus in rodents (Audet & Anisman, 2009; Beekman, Flachskamm, & Linthorst, 2005; Blanchard, et al., 2001; Fuchs & Flugge, 2003; Keeney, et al., 2006). Furthermore, stressor-induced release of norepinephrine (NE) was found in the PFC, locus coeruleus, hippocampus, and hypothalamus (Audet & Anisman, 2009; R. J. Blanchard, et al., 2001). Interestingly, NE utilization differed between mice that were dominant and those that were submissive, as submissive animals had higher elevations of NE within the PFC and hippocampus (Audet & Anisman, 2009), revealing differences in neurotransmission in response to an animal's social status. Finally, dopamine also appears to be influenced by social stressors, with increased levels being evident in the PFC and nucleus accumbens (Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993; Blanchard, et al., 2001). It will

be appreciated that some of these same neurochemical disturbances have been implicated in human depressive disorder.

In addition to altering monoamine secretion, it has been reported that both acute and chronic social stressors can provoke the downregulation or desensitization of NE and 5-HT receptor subtypes (Bambico, Nguyen, & Gobbi, 2009; Berton, Durand, Aguerre, Mormede, & Chaouloff, 1999; Flugge, 1995; Korte, Buwalda, Meijer, De Kloet, & Bohus, 1995; McKittrick, Blanchard, Blanchard, McEwen, & Sakai, 1995). For instance, chronic stress was found to provoke a desensitization of 5-HT_{1A} autoreceptors in the dorsal raphe, which was paralleled by reduced 5-HT firing activity and the development of anhedonia (Bambico, et al., 2009). Furthermore, acute social defeat resulted in downregulated 5-HT_{1A} receptor binding, as measured using the administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (Korte, et al., 1995), and decreased binding to the 5-HT transporter in the hippocampus (Berton, et al., 1999). Alterations of NE receptor subtypes, namely β ₁- and β ₂-adrenoceptors, have also been reported; however, these occur mainly in response to chronic social stress (Flugge, Ahrens, & Fuchs, 1997). In addition to 5-HT and NE receptor subtype variations, a hyposensitivity of the D₂receptor sub-type was found along with decreased binding capacity in subordinate female Cynomolgous monkeys (Shively, Grant, Ehrenkauf, Mach, & Nader, 1997).

The monoamine view of depression has, until recently, been the reigning theory of depression. The reason for this is two-fold. First, antidepressant treatments specifically target and enhance monoamine neurotransmission. Second, stressful experiences can elicit reductions of these neurochemicals in humans and animals, and depressive affect (in animal models) has been associated with these reductions (Beekman,

et al., 2005; Fuchs & Flugge, 2003; Keeney, et al., 2006). Although this theory has not been entirely abandoned, the fact remains that only about 65% of patients treated with 5-HT antidepressants display alleviation of depression, and even when the drug is “effective”, symptom remission is not always complete (Moncrieff & Kirsch, 2005). In view of the limitations of the monoamine-based hypotheses, several alternative substrates have been proposed as potential mediators of major depressive illness.

Stress and Neuroimmunomodulation

It had been previously thought that stressors solely had immunosuppressant actions, downregulating immune competence. For instance, social stressors were shown to reduce lymphocyte proliferation, natural killer cell cytotoxicity and central cytokine production in animals (Bartolomucci, 2005; Bartolomucci, et al., 2001; Bartolomucci, et al., 2003; Stefanski & Engler, 1999). Recent evidence, however, suggests that the influence of stress on the immune system is not clear-cut, and under certain conditions may trigger enhanced inflammatory output, which persists despite the anti-inflammatory effects of GCs (Avitsur, Stark, & Sheridan, 2001; Bailey, Avitsur, Engler, Padgett, & Sheridan, 2004; Engler, et al., 2008; Quan, et al., 2003; Quan, et al., 2001; Sheridan, et al., 2000; Stark, et al., 2001).

Acute social stressor exposure in humans has been shown to provoke increased circulating cytokines (namely Interleukin- [IL-]1 β and IL-6) which were correlated with depressive affect (Brydon, et al., 2009; Steptoe, Hamer, & Chida, 2007; Wright, Strike, Brydon, & Steptoe, 2005). Similarly, exposure to acute stressors in animals provoked increased circulating proinflammatory cytokines (Johnson, et al., 2005; Nguyen, et al.,

2000; K. A. O'Connor, et al., 2003; Shintani, et al., 1995). For instance, stressors such as inescapable shock (Johnson, et al., 2005; Nguyen, et al., 2000; K. A. O'Connor, et al., 2003) and immobilization (Shintani, et al., 1995) enhanced plasma levels of IL-1 β and IL-6. Social stressors, such as social isolation and social defeat were also found to increase plasma IL-1 β and IL-6 (Engler, et al., 2008; Merlot, et al., 2003; Merlot, Moze, Dantzer, & Neveu, 2004). It has been suggested that the observed alterations of circulating cytokines may be mediated by the increased secretion of catecholamines (namely epinephrine and NE) in response to the stressor (Blandino, Barnum, & Deak, 2006; Connor, Brewer, Kelly, & Harkin, 2005; Johnson, et al., 2005). For instance, it was found that blocking α -adrenergic receptors attenuated the increased IL-1 β and IL-6 ordinarily provoked by inescapable shock, while stimulation of the β -adrenergic receptors increased these cytokines (Johnson, et al., 2005). Furthermore, it was reported that the enhanced IL-10 following forced swim stress and LPS challenge were mediated by β -adrenergic receptors (Connor, et al., 2005). Although catecholamines may be involved in mediating peripheral cytokine secretion in response to stressors, central mechanisms have also been implicated, such as Substance P and CRH (Johnson, et al., 2005; Rosenkranz, 2007). Thus, it is premature to ascribe the cytokine variations solely to catecholamine release.

In addition to peripheral cytokine induction, both social and non-social stressors have been found to provoke alterations of central cytokine expression in several brain regions, including the hypothalamus, hippocampus and pituitary (Bartolomucci, et al., 2003; Bartolomucci, et al., 2005; Goshen, et al., 2008; O'Connor, et al., 2003).

Interestingly, although acute stressor exposure (i.e. footshock and restraint) and chronic

mild stressor regimes were found to increase central expression of IL-1 β , IL-6 and tumor-necrosis factor (TNF)- α (Goshen, et al., 2008; Nguyen, et al., 2000; O'Connor, et al., 2003), chronic social stressor exposure downregulated central expression of these cytokines (Bartolomucci, et al., 2003). Central inflammatory responses may therefore be stressor-specific.

Glucocorticoids that are readily released in response to stressors have been implicated as major immunomodulatory factors. However, under some stressor conditions, the sensitivity of the corticoid system may be altered, thus affecting immune and cytokine activity. GCs are typically immunosuppressant, reducing the proliferation, circulation and activation of lymphocytes, suppressing the activity of natural killer cells, and inhibiting the secretion of IL-1 β , IL-6 and TNF- α (Calcagni & Elenkov, 2006; O'Connor, O'Halloran, & Shanahan, 2000). The anti-inflammatory and immunosuppressant effects of GCs have been attributed in part to their upregulation of inhibitor of κ B (I κ B), which prevents the translocation of nuclear factor κ B (NF κ B), a transcription factor that is highly involved in transcribing inflammatory genes (Vermeulen, Vanden Berghe, & Haegeman, 2006) It has been shown that chronic social stressors, but not physical stressors, increase systemic GC levels that can lead to changes in the responsiveness of the immune cells to the anti-inflammatory properties of this hormone (Quan, et al., 2003; Stefanski, 2000). After exposure to a social stressor, the GC receptor is no longer able to translocate to the nucleus of mononuclear cells where it suppresses NF κ B (Almawi & Melemedjian, 2002). This causes the cell to be resistant to the suppressive effects of GCs, which can result in increased cell viability, and continued production of proinflammatory mediators (Almawi & Melemedjian, 2002; Quan, et al.,

2003). This effect appears to be mediated by IL-1 β , as IL-1R1 knockout mice fail to develop GC resistance (Engler, et al., 2008). In effect, social stressors may provoke GC resistance in immune cells (Avitsur, Kavelaars, Heijnen, & Sheridan, 2005; Avitsur, et al., 2001; Engler, et al., 2005; Sheridan, et al., 2000; Stark, et al., 2001; Stefanski, 2000), and the relatively unrestricted cytokine production can lead to inflammatory conditions, as well as immunopathology (Maes, et al., 2009).

Similar observations were made in response to chronic stress in humans. For instance, the chronic stress associated with caregiving led to GC resistance and increased NF κ B signaling in monocytes, resulting in elevated levels of inflammatory mediators (Miller, et al., 2008). Furthermore, altered immune sensitivity to GCs has been found in patients with treatment resistant major depression (Bauer, et al., 2003). Taken together, these findings suggest that stressors can interrupt the regulatory functions of the immune system, which can lead to increased production and secretion of inflammatory mediators, which favor the development of depression.

Stress, depression and synaptic plasticity

The link between growth factors and depression emerged when it became apparent that the monoamine theory of depression alone couldn't account for all cases of depression. As mentioned, antidepressant treatment which targets the monoaminergic system (i.e. SSRIs) is only effective in approximately 60-70% of the population (Moncrieff & Kirsch, 2005). Furthermore, simply increasing the availability of serotonin is not sufficient to abate the symptoms of the disorder, as the therapeutic effects of antidepressants are only observed after 2-3 weeks (Blier, 2001). Subsequently, new

theories of depression emerged, which incorporate different processes, such as growth factors, in the development and exacerbation of the disorder (Alleva & Francia, 2009; Duman & Monteggia, 2006).

It has long been noted, through functional imaging and post-mortem analyses, that depression is associated with reduced volume within several brain regions, especially in areas thought to be associated with depression, such as the PFC and most consistently the hippocampus (MacQueen, et al., 2003; Sheline, 2000; Sheline, Gado, & Kraemer, 2003; Sheline, Wang, Gado, Csernansky, & Vannier, 1996). Animal studies also suggest that exposure to long periods of chronic stress can result in dendritic retraction, apoptosis and reduced neuro and gliogenesis (Brown, Henning, & Wellman, 2005; Magarinos, McEwen, Flugge, & Fuchs, 1996; Y. Watanabe, Gould, & McEwen, 1992). Furthermore, cognitive deficits, including impaired learning and memory are often observed as a symptom of the disorder (Ravnkilde, et al., 2002). Importantly, these morphological and cognitive alterations can often be reversed with antidepressant therapy (Sheline, et al., 2003).

Brain-derived neurotrophic factor is a growth factor, part of the neurotrophin family, that is widely expressed in the mammalian brain and in particular the hippocampus, as well as in peripheral tissues like the heart and the lungs (Hofer, Pagliusi, Hohn, Leibrock, & Barde, 1990). BDNF is highly involved in synaptic plasticity and long term potentiation (LTP), and has been implicated in the pathophysiology of depression (Alleva & Francia, 2009; Duman & Monteggia, 2006; Khairova, Machado-Vieira, Du, & Manji, 2009). There are several lines of evidence, derived from clinical and animal research, linking BDNF to depression. Reduced serum BDNF levels were reported in

depressed patients, and levels of this growth factor were correlated with symptom severity (Cunha, et al., 2006; Karege, et al., 2002; Sen, Duman, & Sanacora, 2008; Shimizu, et al., 2003). This reduction of BDNF could also be normalized with antidepressant treatment (Aydemir, Deveci, & Taneli, 2005; Gervasoni, et al., 2005; Gonul, et al., 2005; Lee, Kim, Park, & Kim, 2007; Sen, et al., 2008; Shimizu, et al., 2003), or electroshock therapy (Marano, et al., 2007), which often corresponded to a decrease in depressive symptoms. Post-mortem samples from patients on antidepressant therapy had higher BDNF protein levels within the hippocampus compared to non-medicated controls (B. Chen, Dowlatshahi, MacQueen, Wang, & Young, 2001). It was further shown that suicide attempters had significantly reduced serum BDNF levels (Deveci, Aydemir, Taskin, Taneli, & Esen-Danaci, 2007; Y. K. Kim, et al., 2007; Lee, et al., 2007), and that suicide brains had reduced BDNF mRNA expression in several areas of the forebrain (Karege, Vaudan, Schwald, Perroud, & La Harpe, 2005).

In animal models, acute and chronic stressor exposure were found to reduce BDNF expression in the brain, especially within the hippocampus (Haenisch, Bilkei-Gorzo, Caron, & Bonisch, 2009; Jacobsen & Mork, 2006; Murakami, Imbe, Morikawa, Kubo, & Senba, 2005; Pizarro, et al., 2004), an outcome that could be reversed with the administration of antidepressants (Haenisch, et al., 2009; Molteni, et al., 2009). Furthermore, acute or chronic infusion of BDNF directly into the hippocampus or midbrain had long lasting antidepressant effects when animals were subsequently exposed to a chronic stressor regimen (Hoshaw, Malberg, & Lucki, 2005; Shirayama, Chen, Nakagawa, Russell, & Duman, 2002; Siuciak, Lewis, Wiegand, & Lindsay, 1997). Moreover, animals with enhanced BDNF signaling (in transgenic mice with an over

expression of *trkB* receptors), appeared to be more resistant to behavioural despair than their wild-type counterparts (Koponen, et al., 2005).

The work using BDNF overexpressing mice supported the involvement of this growth factor in depression, and was, indeed, impressive. Data from studies assessing knockouts, however, were less encouraging. Research using homozygote knockouts has been limited (due to lethality), but studies using heterozygote knockouts revealed that although these mice display hyperphagia, hyperactivity, and some cognitive impairments, they did not differ from wild-type mice in anxiety- and depressive-like behaviours (Chourbaji, et al., 2004; Ibarguen-Vargas, et al., 2009). In fact, it seems that reduced BDNF levels did not even leave them more vulnerable to develop depressive-like behaviours than wild-type when exposed to a chronic stressor regimen (Ibarguen-Vargas, et al., 2009). To further explore the BDNF-depression relation, inducible knockout mice with BDNF deletion within the forebrain were assessed. As with the heterozygote knockouts, conditional knockouts did not display increased basal anxiety- or depressive-like behaviour (Autry, Adachi, Cheng, & Monteggia, 2009; Monteggia, et al., 2004; Monteggia, et al., 2007). However, there appeared to be a sexual dimorphism in the role of BDNF expression in response to stress in these knockout mice (Autry, et al., 2009; Monteggia, et al., 2007). Specifically, female knockout mice were more prone to develop stressor-induced anxiety and depressive-like behaviour (as measured by exaggerated corticosterone release, decreased duration in the centre of the open field and number of entries, and decreases of grooming, locomotor activity, and sucrose consumption), relative to male knockouts. These findings not only support BDNF involvement in

depression, but are in line with the well known sex differences that exist in the development of major depressive disorder.

Despite the lack of clarity concerning the precise role BDNF plays in the development of depression, there has been one consistent finding: BDNF appears to be essential in the effectiveness of antidepressant therapy. Several studies have shown a blunted antidepressant effect in BDNF or *trkB* deficient mice (Ibarguen-Vargas, et al., 2009; Monteggia, et al., 2007; Saarelainen, et al., 2003), suggesting that BDNF mediates the therapeutic action of antidepressants, and it was shown that BDNF specifically within the dentate gyrus was necessary for the effectiveness of antidepressants (Adachi, Barrot, Autry, Theobald, & Monteggia, 2008). Furthermore, antidepressant therapy and electroshock therapy can increase BDNF mRNA expression as well as BDNF protein in the brain, and is protective against stress-induced reductions of BDNF (Jacobsen & Mork, 2004; Nibuya, Morinobu, & Duman, 1995; Piccinni, et al., 2009). In a like fashion, it was found that BDNF increases tryptophan metabolism, which is responsible for an increase of 5-HT synthesis (Siuciak, Clark, Rind, Whitemore, & Russo, 1998). As such, there might be a reciprocal relationship between BDNF and 5-HT functioning. It was shown that 5-HT receptor activation induces BDNF expression in the brain, and BDNF infusion stimulated 5-HT turnover and synthesis, and promoted the development of serotonergic neurons in the dorsal raphe (Koponen, et al., 2005; Mamounas, Blue, Siuciak, & Altar, 1995; Siuciak, Boylan, Fritsche, Altar, & Lindsay, 1996; Siuciak, et al., 1998). In effect, 5-HT – BDNF functioning could be an important mechanism through which BDNF might be related to depression. It should be noted, however, that NE transporter knock-out mice were found to be resistant to stressor-induced depressive-like

behaviour as well as alterations of BDNF, suggesting that NE may also play a role in the altered synaptic plasticity associated with depressive-affect (Haenisch, et al., 2009).

Inflammatory Processes and Depression

It had long been thought that the brain was an immuno-privileged organ, isolated from the inflammatory immune system. It is now widely accepted that there exists bi-directional communication between these two systems, which is largely mediated by cytokines (Dantzer, et al., 2008). Cytokines are the signaling molecules of the immune system, and are secreted by immunocompetent cells (e.g. lymphocytes and macrophages in the periphery, and microglia in the CNS), in order to regulate the immune response. Cytokines serve a wide variety of biological functions, such as stimulating the proliferation and differentiation of immune cells, trafficking cells to infection sites, as well as activating or inhibiting cellular functions (Elenkov, 2008). These chemical messengers can act in an autocrine (self-stimulating), paracrine (stimulating nearby target cells) or endocrine (secreted into the bloodstream to stimulate distant cells) fashion, and thus their effects can be far ranging.

Cytokines have been classified according their principle actions (pro- vs anti-inflammatory). Proinflammatory cytokines, including IL-1, IL-6, TNF- α , interferon [IFN]- α and INF- γ , are responsible for the activation of the inflammatory immune response, synthesis of acute phase proteins, and activation of neuroendocrine systems (Turrin & Plata-Salaman, 2000). In contrast, anti-inflammatory cytokines, such as IL-4, IL-10, and IL-1 receptor antagonist (ra), are responsible for dampening the immune response by inhibiting the actions of their proinflammatory counterparts (Turrin & Plata-

Salaman, 2000). Cytokines have been further divided based on their target pathogens (e.g. T-helper [Th]1 vs Th2). Th1 cytokines, such as IL-1 β , IL-2, TNF- α and IFN- γ , respond primarily to intra-cellular pathogens and regulate cell-mediated immunity, largely through their influence on cytotoxic T cells and natural killer cells (Chizzolini, Chicheportiche, Burger, & Dayer, 1997; Elenkov, 2008). In contrast, Th2 cytokines, such as IL-4, IL-5, IL-6, IL-10 and IL-13, respond to extra-cellular pathogens, and regulate humoral immunity through their influence on B cells (Elenkov, 2008). Interestingly, maintaining homeostasis depends on the delicate balance of these two types of cytokines. For instance, a shift towards Th2 immunity can lead to inflammatory conditions such as allergies and asthma, through the increase of circulating IgE antibodies, whereas a shift towards Th1 immunity can lead to autoimmune diseases such as rheumatoid arthritis, due to excess levels of TNF- α , and a deficiency of IL-10 (Murali, Hanson, & Chen, 2007). It is therefore apparent that healthy functioning depends on the proper orchestration of cytokines, and that disruption of this harmony may have pathological outcomes.

There are ample clinical data supporting the role of inflammatory processes in the development of depression. For instance, circulating levels of proinflammatory cytokines were found to be elevated in patients with chronic inflammatory conditions, such as rheumatoid arthritis, cancer, and cardiovascular disease, and these levels were positively correlated with severity of depression (see (Yirmiya, et al., 1999). Furthermore, patients with depression were found to have increased levels of circulating inflammatory mediators, such as lymphocytes and monocytes, complement proteins, acute phase proteins, as well as proinflammatory cytokines including IL-1 β , soluble IL-2 receptors,

IL-1ra, IL-6, soluble IL-6 receptors and INF- γ (Huang & Lee, 2007; Maes, 1995; Maes, et al., 2009; Piletz, et al., 2009). In fact, meta-analyses revealed that the most consistent biomarker in depression is elevated levels of circulating IL-6 (Dowlati, Herrmann, Swardfager, Liu, Sham, Reim, et al. 2010; Mossner, et al., 2007; Zorrilla, et al., 2001). Post-mortem analyses have also demonstrated an increase in pro-inflammatory gene expression in the PFC of depressed patients compared to controls (Shelton, et al., 2010). Consistent with a relationship between cytokines and depression, it was reported that immune disturbances associated with depressive illness can be often be attenuated with antidepressant treatment (Basterzi, et al., 2005; Janssen, Caniato, Verster, & Baune; Piletz, et al., 2009).

Further evidence for the association between cytokines and depression comes from studies of patients administered immunotherapy in treatment of hepatitis C or cancer. Chronic treatment with the proinflammatory cytokine IFN- α over several months is associated with adverse effects, including depressive affect, anhedonia, anorexia, fatigue, lethargy, disrupted sleep, social and cognitive impairment (for a review see Myint, Schwarz, Steinbusch, & Leonard, 2009). Additionally, treatment with IFN- α is associated with high rates of attempted suicide, which necessitate the discontinuation of the treatment (Yokoyama, Kimura, & Shigemura, 1996). These symptoms are thought to be a reflection of the cytokine actions on monoaminergic systems, and they can, in part, be attenuated with pretreatment with antidepressants (Capuron & Miller, 2004; Musselman, et al., 2001). Although these data are more correlational in nature, animal studies have supported a causal role of cytokines in depression, as administering cytokines directly, or by increasing their expression using a bacterial endotoxin or viral

mimic, not only provokes depressive-like symptoms, but also provokes alterations of HPA functioning, neurotransmission and synaptic plasticity that are thought to be associated with depression (Anisman, Merali, & Hayley, 2008; Khairova, et al., 2009; A. H. Miller, et al., 2009; Pace & Miller, 2009).

Behavioural Effects of Cytokines

Activation of inflammatory processes, through the administration of LPS or the Salmonella Typhi vaccine, provokes general malaise as well as depressive affect in otherwise healthy individuals (Brydon, et al., 2009; Reichenberg, et al., 2001). Likewise, exposure of animals to proinflammatory cytokines (e.g. IL-1 β , TNF- α , IFN- α and to a lesser degree IL-6) or cytokine inducers (e.g. LPS or Poly I:C) can elicit a wide spectrum of behavioural alterations, including lethargy, decreased social and exploratory behaviour, anorexia, anxiety, and altered sleep patterns (for a review see Gibb, et al., 2009). These constellations of symptoms have been collectively referred to as sickness behaviour (Dantzer, et al., 2008). Sickness behaviour can be considered an adaptive process, whereby an organism reorganizes its priorities to recuperate from illness or infection (Hart, 1988). For instance, some symptoms (e.g. lethargy, reduction of social behaviour) are thought to reflect a reallocation of energy resources, as considerable energy is required to mount a febrile response (Dantzer, et al., 2008). Furthermore, altered sleep patterns and increased anxiety may be reflecting a state of hyper-vigilance, in order to protect from further injury. Taken together, it appears that cytokines can trigger behavioural disturbances that are reminiscent of the neurovegetative symptoms seen in depression.

Although there is considerable overlap between sickness behaviour and depressive-like behaviour, these two conditions can be readily dissociated (Capuron, et al., 2009). In fact, they appear to have different temporal dynamics, with sickness symptoms emerging quickly following the administration of an acute treatment, and depressive symptoms emerging later, and persisting for several days following exposure (Frenois, et al., 2007). Similar findings were demonstrated in mice receiving chronic inoculation with *Bacillus Calmette-Guerin*, or clinical patients receiving chronic IFN- α , where the onset of general malaise was relatively quick, but the depressive symptoms were only apparent with prolonged treatment (Capuron & Miller, 2004; Moreau, et al., 2008). This is likely an indication that different processes may be responsible for the emergence of the two conditions.

Even though sickness behaviour is not fully congruent with depression, the two share many overlapping features, and it has been suggested that sickness behaviour may be mimicking the neurovegetative symptoms of depression (Castanon et al., 2002, Dantzer et al., 2008, Loftis et al., 2010). It has further been postulated that depression may be a maladaptive form of sickness behaviour, resulting from intense and/or prolonged inflammation and the subsequent neurochemical disturbances, especially in vulnerable populations such as the elderly or those with preexisting medical conditions and/or a predisposition toward depression (Dantzer et al., 2008; Henry et al., 2008).

Beyond the general malaise or sickness, cytokines (or cytokine inducers), also provoke hallmark features of depression in laboratory animals, including anhedonia and behavioural despair. For instance, the administration of LPS and IL-1 β were found to decrease responding for rewarding brain stimulation, and decrease consumption of a

palatable snack (Anisman, Kokkinidis, Borowski, & Merali, 1998; Borowski, Kokkinidis, Merali, & Anisman, 1998; Brebner, et al., 2000; Merali, Brennan, Brau, & Anisman, 2003). Furthermore, endotoxin treatment or viral inoculation were found to increase learned helplessness in mice (Frenois, et al., 2007; Moreau, et al., 2008), as measured by the forced swim and tail suspension tests. In sum, these findings suggest that cytokines can cause depressive-like symptoms in laboratory animals. Interestingly, these symptoms can often be attenuated with antidepressant therapy (Yirmiya, 1996), or with anti-inflammatory agents such IL-1ra (Bluthe, Dantzer, & Kelley, 1992; Fortier, et al., 2004) and IL-10 (Bluthe, et al., 1999).

Communication between cytokines and the CNS

Cytokines are relatively large molecules that are not readily able to penetrate the blood-brain barrier (BBB). However, there are several routes through which cytokine signals may access the CNS: a) passive diffusion through brain regions where the BBB is naturally less restrictive (e.g. circumventricular organs; (Goehler, Erisir, & Gaykema, 2006) b) active transport by saturable carrier molecules (Banks, Farr, & Morley, 2002) c) diffusion through areas where the BBB has been compromised (for instance by illness, or through the destructive influence of cytokines; (Quan & Banks, 2007) d) binding to receptors on vagal afferent fibers, originating in the abdominal cavity, which rapidly transmits the signals to the nucleus solitary tract in the brain stem, which then serves as a hub, relaying the signals to the necessary brain regions, often using secondary messengers, such as prostaglandins (Wieczorek, Swiergiel, Pournajafi-Nazarloo, & Dunn, 2005).

In addition to infiltration from the periphery, cytokines (including IFN- α , IFN- γ , IL-1, IL-2, IL-6 and TNF- α) are also produced in the CNS itself, mainly by astrocytes and microglia (McGeer & McGeer, 1995). Cytokine production has been found to occur in several brain sites, including the hypothalamus, hippocampus, cerebellum, forebrain regions, basal ganglia, and brainstem nuclei (Kronfol & Remick, 2000). It was previously thought that cytokines were solely secreted in the brain following trauma, infections and disease, however, it is now known that cytokines (such as IL-1 β , IL-6 and TNF- α) are naturally expressed in low levels within the brains of healthy individuals, and are important in synaptic plasticity and LTP (for a review see Khairova, et al., 2009). It will be recalled that increased central cytokine expression has been shown to be provoked by psychosocial stressors (Bartolomucci, et al., 2003; Goshen, et al., 2008; K. A. O'Connor, et al., 2003). Similarly, exposure to IL-1 β (Anisman, Gibb, et al., 2008; Goshen, et al., 2008), Poly I:C (Cunningham, Campion, Teeling, Felton, & Perry, 2007) and endotoxin (Andre, et al., 2008; Deak, Bellamy, & Bordner, 2005; Gibb, et al., 2008; Turrin, et al., 2001) treatments, have also been shown to act in this capacity.

The transcription factor NF κ B has been proposed to be one of the mediators in the communication of immune system molecules between the peripheral and central nervous systems at the level of the BBB (Dantzer, et al., 2008). In response to inflammation, I κ B kinase, an NF κ B inhibitor, is activated and phosphorylated, which causes it to dissociate from NF κ B. This subsequently results in the translocation of NF κ B into the cell nucleus where it promotes the transcription of cytokines and their receptors (Miyamoto & Verma, 1995). Social stressors are also capable of initiating this cascade through their influence on GC receptors (Quan, et al., 2003). Interestingly, the central administration of a NF κ B

antagonist abolished the behavioural effects of a peripheral IL-1 β challenge, and attenuated c-Fos expression in several brain regions, including the hypothalamus, supraoptic nucleus and amygdala (Nadjar, Bluthe, May, Dantzer, & Parnet, 2005). Similar results were found using the central administration of a NF κ B decoy, which significantly reduced LPS-induced behavioural alterations (Godbout, Berg, Krzyszton, & Johnson, 2005). These findings support the premise that NF κ B plays a role in the activation of neural substrates necessary for the development of cytokine induced behavioural alterations, and may explain how peripheral signals are relayed to the brain.

In addition to NF κ B, other mechanisms have been proposed to explain the central effects of peripheral cytokines, such as cyclooxygenases (COX). Considered to be secondary mediators, COX enzymes act by converting arachidonic acid into prostaglandin endoperoxide H₂, leading to the synthesis of prostaglandins (Aid, Langenbach, & Bosetti, 2008). Two COX subtypes have been identified, COX-1 and COX-2, which share 60% homology (Aid, et al., 2008). However, whereas COX-1 is ubiquitously expressed in most cell types and is responsible for maintaining normal physiological functioning, COX-2 is only expressed in certain cell types (e.g. endothelial cells), and is induced primarily by inflammatory stimuli (Morita, 2002). Considering its role in inflammation, most studies have focused on COX-2 receptors, and both LPS and IL-1 β have been shown to increase its expression in brain endothelial cells, which corresponded with depressive-like symptoms (Dunn, Swiergiel, Zhang, & Quan, 2006). Clinical studies have shown that antidepressant therapy is more efficient when combined with non-steroidal anti-inflammatory drugs (NSAIDs), such as celecoxib and acetylsalicylic acid, which are non-selective and target both COX-1 and COX-2 enzymes

(Mendlewicz, et al., 2006; Muller, et al., 2006). Likewise, animal studies have demonstrated that selective COX-2 inhibitors, such as indomethacin and ibuprofen (de Paiva, Lima, Fernandes, Soncini, Andrade, & Giusti-Paiva, 2010; Teeling, Cunningham, Newman, & Perry, 2009; Teeling, et al., 2007) as well as nonselective inhibitors such as celecoxib (Swiergiel & Dunn, 2002), reduced the behavioural effects associated with an acute immune challenge. These findings are supported by studies using COX knockout mice, where the behavioural effects of inflammation were reduced or absent following a challenge (Aid, et al., 2008; Hayley, Mangano, Strickland, & Anisman, 2008). Taken together, it seems that although high levels of circulating cytokines provoke behavioural and neurochemical disturbances, their actions are at least partly dependent on the action of COX enzymes.

Neurochemical effects of cytokines

Cytokines, like stressors, can provoke a wide variety of neuroendocrine alterations. For instance, the systemic administration of proinflammatory cytokines, or cytokine inducers, can result in HPA hyperactivity, as indicated by increased circulating levels of CRH, ACTH and corticosterone (Brebner, et al., 2000). It will be recalled that GCs are important modulators of cytokine activity, stimulating or limiting the transcription of cytokines and their receptors through several pathways, including NF κ B (Pace, Hu, & Miller, 2007). Interestingly, like the effects observed in response to social stressors (Quan, et al., 2003), cytokines can also provoke the development of GC resistance in immune cells by reducing glucocorticoid receptor (GR) functioning (Pace & Miller, 2009). These effects have been attributed, in part, to the reduced translocation of

the GR into the nucleus where it can inhibit the actions of NF κ B (Pace, et al., 2007). This can lead to exacerbated and protracted increases of proinflammatory cytokines, which can lead to the development of inflammatory conditions and neurodegenerative conditions.

In addition to neuroendocrine effects, the administration of proinflammatory cytokines can provoke profound alterations of monoaminergic neurotransmission in stressor-related brain regions. Reminiscent of stressor effects, increased utilization of 5-HT and NE were found within the PFC, amygdala and hypothalamus following treatment with cytokines or a bacterial endotoxin (Brebner, et al., 2000; Day, Curran, Watson, & Akil, 1999; Hayley, Brebner, Lacosta, Merali, & Anisman, 1999; Zalcman, et al., 1994). Furthermore, centrally administered IL-1 β , and systemically administered LPS and IL-1 β were found to increase in vivo extracellular concentrations of 5-HT, NE and dopamine within several hypothalamic and extra-hypothalamic sites (Linthorst, Flachskamm, Muller-Preuss, Holsboer, & Reul, 1995; Linthorst & Reul, 1998; Merali, Lacosta, & Anisman, 1997; Song, Merali, & Anisman, 1999; J. Zhang, Terreni, De Simoni, & Dunn, 2001).

Treatment with IFN- α can also provoke neurochemical disturbances. For instance, clinical studies show that INF- α can suppress tryptophan availability, which in turn decreases 5-HT synthesis (Capuron, et al., 2009; Raison, Borisov, et al., 2009). In fact, it was found that the occurrence of depressive symptoms induced by cytokine treatment was negatively correlated with dose-dependent decreases in serum tryptophan levels (Capuron, et al., 2002), which could be normalized with pretreatment with antidepressants (Capuron, et al., 2003). In addition to directly affecting monoamine

secretion, cytokines can also influence monoamine re-uptake by increasing the activity of transporters. For instance, it was found that IL-1 β and TNF- α increased 5-HT, NE and dopamine transporters, mainly through their effects on the p38 mitogen-activated protein kinase (MAPk) pathway (Zhu, Blakely, & Hewlett, 2006; Zhu, Carneiro, Dostmann, Hewlett, & Blakely, 2005).

The effects of cytokines on 5-HT are thought to be mediated, in part, by 2,3-dioxygenase (IDO). IDO is considered to be a rate-limiting enzyme in the kynurenine pathway. This enzyme converts tryptophan, the precursor of serotonin, into kynurenine and quinolinic acid. Increased production of kynurenine has been shown to degrade tryptophan, which subsequently decreases the rate of synthesis of serotonin (Raison, Dantzer, et al., 2009). In fact, administering kynurenine directly has been associated with depressive-like symptoms (O'Connor, et al., 2009). Proinflammatory cytokines, such as IL-1 β and TNF- α , as well as LPS have been shown to independently and synergistically increase IDO activity (Babcock & Carlin, 2000; Hu, Hissong, & Carlin, 1995), whereby decreasing tryptophan metabolism. This effect has been attributed to the synergistic activation of both the p38 MAPK and NF κ B pathways (Fujigaki, et al., 2006). In contrast, anti-inflammatory cytokines have been shown to decrease activity of this enzyme, thus increasing tryptophan metabolism and serotonin synthesis (Muller & Schwarz, 2007).

Inhibiting IDO activity directly has been found to have an antidepressant-like effect. Specifically, when administered centrally, the IDO antagonist 1-methyltryptophan, was shown to attenuate the neurochemical and behavioural disturbances associated with the peripheral administration of LPS (O'Connor, et al.,

2009). Similarly, the peripheral administration of minocycline, a potent anti-inflammatory agent that works by inhibiting the activation of microglial cells and by decreasing the expression of IDO, has been found to attenuate depressive-like behaviours and altered monoamine neurotransmission within the CNS following an immune challenge (Henry, et al., 2008; O'Connor, et al., 2009). In this regard, it seems that behavioural disturbances as well as alterations of 5-HT neurotransmission may be significantly influenced by IDO activity.

Cytokines and Synaptic Plasticity

Emerging evidence suggests a role of cytokines in the regulation of neurogenesis, synaptic plasticity and learning and memory, through their actions in the hippocampus (Khairova, et al., 2009). For instance, basal levels of IL-1 β and TNF- α within the hippocampus appear to be required for synaptic plasticity and LTP, whereas elevated levels can impair these functions (Khairova, et al., 2009). Further emphasizing this association, IL-1 receptor 1 (R1) knockout mice were found to display severe cognitive deficits, and impairments in LTP (Avital, et al., 2003). Likewise, in response to chronic stressors, mice lacking IL-1R1 failed to display reduced hippocampal neurogenesis compared to their wild-type counterparts (Goshen, et al., 2008), emphasizing that IL-1 β may mediate stressor-induced disruptions of neuroplasticity. In support of this view, it was found that the blockade of IL-1 β , through the administration of IL-1ra, abrogated the downregulation of BDNF mRNA expression induced by a social isolation stressor (Barrientos, et al., 2003).

Cytokines may directly influence the activity of growth factors, such as BDNF. In this regard, the administration of LPS, like stressors, was found to decrease BDNF protein levels (Guan & Fang, 2006), and mRNA expression (Richwine, Sparkman, Dilger, Buchanan, & Johnson, 2009) in the frontal cortex and hippocampus, alterations that were accompanied by sickness behaviour and cognitive impairments (Richwine, et al., 2009). Moreover, both IL-1 β and TNF- α were found to dose-dependently influence BDNF mRNA expression in vitro in hypothalamic and primary cortical cells (Taishi, Churchill, De, Obal, & Krueger, 2008).

The mechanisms by which cytokines influence the activity of growth factors remain to be fully elucidated. However, activated microglial cells and astrocytes have been shown to release BDNF (Juric, Loncar, & Carman-Krzan, 2008). Moreover, BDNF signaling may be triggered through the activation of prostaglandin receptors (EP2 and EP3), which as mentioned are secondary mediators of inflammation (Hutchinson, Chou, Israel, Xu, & Regan, 2009). Regardless of the mechanisms involved, it is thought that cytokines, like stressors, may influence synaptic plasticity by reducing the availability of growth factors, which has been associated with the development of depression.

Genetic vulnerability and stressful events

The impact of stressors vary considerably among individuals, and although stressful life experiences are thought to play a causal role in the development of MDD, not everyone faced with chronic adversity or a traumatic event will develop an affective disorder. In this regard, individual characteristics such as genetic predisposition have been receiving considerable attention (Anisman, Merali, & Stead, 2008). Although there

may be no such thing as a 'depression gene', certain polymorphisms of genes thought to be involved in depression have been elucidated (e.g. the 5-HT transporter [5HTT] and BDNF). It is important to note, however, that these polymorphisms are only significantly related to depression when combined with stressful experiences (Caspi, et al., 2003; Gatt, et al., 2009; Kaufman, et al., 2006; Shalev, et al., 2009; Wilhelm, et al., 2006), highlighting the importance of studying the interaction between genes and the environment.

Considering the key role of 5-HT neurotransmission in MDD, polymorphisms of 5-HT receptor subtypes and transporters were examined, in the hope of finding genetic markers for depression (for a review see Anguelova, Benkelfat, & Turecki, 2003). Among these polymorphisms, the most robust finding was the 5HTTLPR, which was shown to decrease 5-HTT expression (Lesch, et al., 1996). It will be recalled that the 5HTT is intricately involved in serotonergic neurotransmission by promoting the reuptake of 5HT from the synapse (Zhu, et al., 2005). Associations between this polymorphism and the development of depression have been inconsistent, and a meta-analysis failed to reveal a direct relationship (Anguelova, et al., 2003). In fact, a significant relationship was only revealed when the polymorphism was combined with stressful experiences, such as early life trauma (Kaufman, et al., 2006; Kaufman, et al., 2004), and chronic life stressors (Caspi, et al., 2003; Kim, et al., 2007; Wilhelm, et al., 2006). It is possible that 5HTTLPR may be moderating the serotonergic response to stressors, which can lead to increased vulnerability to stressor-related pathologies.

Similar evidence was found with the polymorphism of the gene encoding for BDNF expression. The Val66Met polymorphism was associated with a decrease in

activity-dependent secretion of BDNF (Egan, et al., 2003). Individuals with this polymorphism had smaller hippocampal, PFC and amygdala volumes (Bueller, et al., 2006; Montag, Weber, Fliessbach, Elger, & Reuter, 2009; Pezawas, et al., 2004; Szeszko, et al., 2005), increased cognitive impairments (Hariri, et al., 2003; Ho, et al., 2006) and emotional instability (Montag, et al., 2009). As with 5HTT, just having this polymorphism alone was not sufficient for the development of depression (Verhagen, et al., 2008). However, the gene x environment interaction was significant, as the combination of early life trauma and the BDNF polymorphism was significantly related to depression (Gatt, et al., 2009; Shalev, et al., 2009). Inasmuch as genetic vulnerability is an important determinant in the susceptibility to MDD, its interaction with environmental triggers need to be taken into consideration.

The gene x environment interaction has also been investigated in animal models using inbred strains of rodents that differ in emotional responsiveness. For instance, the behavioural and neurochemical effects of a wide range of stressors were investigated using mice that were genetically predisposed to being either stressor-reactive or stressor-resilient (BALB/cByJ or C57Bl/6ByJ, respectively), and it has been posited that the BALB/cByJ strain could be a good model to explore stressor-induced anxiety – and depressive-like behaviour, compared to the more stressor-resilient C57Bl/6ByJ (Anisman, Prakash, Merali, & Poulter, 2007).

BALB/cByJ mice consistently demonstrate exaggerated anxiety- and depressive-like behavioural responses across several paradigms compared to C57Bl/6ByJ mice (Dulawa, Holick, Gundersen, & Hen, 2004; Kim, Lee, Ryu, Suk, & Park, 2002), and these behavioural abnormalities have been found to normalize with the chronic

administration of fluoxetine (Dulawa, et al., 2004). Aside from their basal differences, BALB/cByJ mice also displayed heightened behavioural responses to psychogenic and neurogenic stressors, and increased sickness behaviour in response to a peripheral injection of IL-1 β (Anisman, et al., 2001). Paralleling these behavioural disturbances, BALB/cByJ mice have also displayed stressor-induced HPA hyperactivity, as evidenced by elevated levels of circulating ACTH and corticosterone, and displayed more profound changes of CRH in the orbital frontal cortex, hypothalamus and central amygdala compared to the more hardy strain (Anisman, Prakash, et al., 2007; Anisman, et al., 2001; Brinks, van der Mark, de Kloet, & Oitzl, 2007; Shanks, Griffiths, & Anisman, 1994).

Beyond the behavioural and neuroendocrine alterations, C57Bl/6ByJ and BALB/cByJ mice also differed in their neurochemical responses to stressors, as the stressor-reactive mice displayed greater monoamine alterations in stressor-related brain regions (Hayley, Borowski, Merali, & Anisman, 2001; Norcross, et al., 2008; Prakash, Merali, Kolajova, Tannenbaum, & Anisman, 2006; Shanks, et al., 1994). Interestingly, a polymorphism for the gene encoding the 5-HT synthesizing enzyme, tryptophan hydroxylase2, was recently discovered within the BALB/cByJ strain (X. Zhang, Beaulieu, Sotnikova, Gainetdinov, & Caron, 2004). This polymorphism was associated with a reduction of 5-HT synthesis and 5-HT cellular content in the CNS (X. Zhang, et al., 2004). In this regard, the behavioural and neurochemical discrepancies between the two strains have, in part, been attributed to presence of this polymorphism (Norcross, et al., 2008; X. Zhang, et al., 2004). To conclude, studying inter-strain variability in stressor responsiveness is a valuable tool for examining gene x environment interactions. In this model, it appears as though a genetic predisposition toward stressor vulnerability

can lead to behavioural and neurochemical disruptions that are reminiscent of those seen in MDD.

The Proposed Research

Major depressive disorder is multifaceted, and its pathophysiology likely involves the interaction between several biological processes. Both stressful events and cytokines have been found to independently and synergistically influence depressive-like behaviour through their influence on neuroendocrine functioning, monoamine neurotransmission, neuroimmunomodulation, as well as synaptic plasticity (Anisman, Merali, & Hayley, 2008; Duman & Monteggia, 2006; Gandhi, et al., 2007; Gibb, et al., 2008; Miller, et al., 2009; Pace & Miller, 2009). Given the contribution of stressor-related and inflammatory processes in MDD, the aim of the current thesis was to further investigate the independent and combined effects of stressors and cytokines on behavioural, neurochemical, and inflammatory processes. In this regard, in Chapter 1, we investigated the independent and interactive effects of acute or chronic stressors and LPS on sickness behaviour and circulating corticosterone, as well as the expression of central cytokine mRNA in an outbred strain of mice. Furthermore, as these effects may emerge at different timepoints, the behavioural, immune and neurochemical outcomes were assessed at 1.5, 3 and 24 hrs following LPS administration.

Considering the great inter-individual variability that exists in relation to stressor-reactivity and vulnerability to developing an affective disorder (Anisman et al., 2008), in Chapter 2, we assessed the behavioural, neuroendocrine and neuroinflammatory effects of an immunogenic agent administered following a social stressor in mice from inbred

strains known to be either stressor-reactive or stressor-resilient (BALB/cByJ and C57Bl/6ByJ mice, respectively). Furthermore, as these strains also demonstrate different immune characteristics, we examined two different types of immune challenges, namely LPS that primarily elicits a Th2 response, and the viral mimic poly I:C which predominantly provokes a Th1 response, to determine whether they differentially influence these strains.

Lastly, as immune activation has been linked to the development of depression, in Chapter 3, we assessed whether minocycline, a synthetic antibiotic that inhibits the activation of microglial cells and thus decreases cytokine expression in the brain (Kim et al., 2009), can attenuate the behavioural, neuroendocrine and central cytokine alterations produced by an acute endotoxin challenge.

CHAPTER 1: Synergistic actions of chronic or acute social stressors and an endotoxin challenge on central stress systems

Abstract

Acute stressor exposure and immunogenic challenges have been shown to independently influence neuronal activity within brain regions involved in affective disorders, and can elicit depressive-like behaviour in animal models. When combined, these treatments can produce synergistic effects on behavioural, endocrine and neuroinflammatory outcomes. Chronic stressors have been associated with the diminution of several neurochemical and hormonal effects ordinarily elicited by acute stressors, but little is known about the relative effects of acute and chronic stressors coupled with immune challenges on behavioural and biological outcomes. In the present investigation we assessed whether these stressor treatments, independently and combined with a bacterial endotoxin, influenced the development of sickness behaviour, circulating corticosterone levels and central cytokine mRNA expression in the pre-frontal cortex (PFC) and hippocampus, at 1.5, 3 and 24 hrs following LPS administration. Sickness behaviour and corticosterone were strongly influenced by the combined treatments, with exaggerated effects peaking early at 1.5 hrs post-LPS. In contrast, central cytokine mRNA expression (especially in the case of IL-1 β and TNF- α) were markedly diminished at 1.5 hrs following stressor and LPS administration, but were then synergistically enhanced at 3 hrs compared to non-stressed controls. Although acute and chronic stressors provoked similar behavioural and neuroendocrine responses when combined with LPS, the effects of chronic stressors and LPS on brain cytokines were generally reduced, an effect more pronounced in the PFC, perhaps indicating an

adaptation due to the repeated stressors. Although the effects of cytokine activity and stressors may involve different neurological circuits, their combined actions may render an individual vulnerable to depression.

Introduction

Activation of the inflammatory immune system elicits a wide variety of behavioural and neurochemical alterations that have been implicated in the development of psychological illnesses, such as major depressive disorder (MDD; Anisman et al., 2008; Dantzer, et al., 2008; Miller et al., 2009). When an immunogenic challenge is applied on the backdrop of an acute stressor, circulating cortisol and cytokines are synergistically enhanced, and this is typically accompanied by increased depressive affect (Brydon et al., 2009; Yeager et al., 2009). Corroborating these findings in humans, animals exposed to a stressor comprising social disruption combined with an immunogenic agent (namely a bacterial endotoxin, interferon- α or poly I:C) displayed enhanced sickness behaviour, circulating cytokines and neurochemical disturbances (Anisman et al., 2007; Gandhi et al., 2007; Gibb et al., 2008; Quan, et al., 2001). Stressor exposure has also been shown to influence endotoxin-induced expression of pro-inflammatory cytokine mRNA and protein levels in stressor-related brain regions, although the nature and direction of the changes were not always consistent (cf. Johnson et al., 2002; Munhoz et al., 2006 vs. Gibb et al., 2008, Goujon et al., 1995).

Typically, acute stressors of moderate to high severity profoundly influence plasma corticosterone levels, various monoamine levels and turnover, expression of brain-derived neurotrophic factor (BDNF), as well as other growth factors (Anisman et al., 2008; Duman, & Monteggia, 2006; Leonard & Myint, 2009). However, several of the effects of acute stressors were diminished when animals were exposed to the same stressor repeatedly (homotypic stressors), whereas the magnitude of this “adaptation” was less notable if the chronic stressor regimen varied from day to day (heterotypic stress;

Armario, Vallès, Dal-Zotto, Márquez & Belda, 2004; Sabban & Serova, 2007). In fact, when animals were exposed to heterotypic stressors, the magnitude of some biological changes was greater than those associated with an acute stressor (Armario et al., 2004; Sabban & Serova, 2007).

There is little information, however, as to whether acute and chronic stressors differentially affect brain cytokine expression, and whether these changes vary across brain regions (e.g. prefrontal cortex [PFC] and hippocampus) that have been associated with psychopathology, such as depression. It was reported that chronic stressor exposure potentiated central pro-inflammatory cytokine release as well as glial and neuronal cell loss when lipopolysaccharide (LPS) was directly administered into the PFC (de Pablos et al., 2006). Similarly, chronic stressor exposure combined with intrahippocampal administration of LPS synergistically enhanced BDNF mRNA, neuronal cell loss, and neuroinflammation (Espinosa-Oliva, de Pablos, Villaran, Arguelles, Venero, Machado et al., 2011). Yet, scant data are available concerning the influence of a chronic stressor regimen in modifying the impact of systemic LPS treatment despite the fact that bacterial insults typically involve systemic processes. The present investigation was conducted to determine whether exposure to an acute stressor would enhance endotoxin-induced sickness behaviour, endocrine and inflammatory output, and whether an “adaptation” would be evident following repeated stressors leading to diminished biological responses. Furthermore, given that we previously observed that stressors and immune challenges differentially influenced IL-6 vs both IL-1 β and TNF- α , it was expected that the effects of the stressor treatments would be cytokine-specific. To this end, the present investigation assessed the independent and combined effects of acute or chronic stressors and LPS, on

sickness behaviour and circulating corticosterone, as well as the expression of pro-inflammatory cytokine mRNA in the PFC and hippocampus. Furthermore, as the emergence of these effects may have different temporal dynamics, behavioural and central measures were taken at three different time points following treatment exposure (1.5, 3 and 24 hrs).

Materials and Methods

Subjects

Male CD-1 mice were obtained from Charles River Canada (St. Constant, Quebec) at approximately 6-8 weeks of age. Upon arrival, mice were individually housed in standard polypropylene cages (27 x 21 x 14 cm) and permitted 2 weeks to acclimatize to the vivarium before being used as experimental subjects. Mice were maintained on a 12-h light-dark cycle (light phase: 0800-2000 h), with temperature (22°C) and humidity (63%) kept constant, and were permitted free access to food (Ralston Purina, St. Louis, MO) and water. The studies met the guidelines set out by the Canadian Council on Animal Care and were approved by the Carleton University Animal Care Committee.

Procedures

Experiment 1: Combined effects of chronic or acute stressors and bacterial endotoxin on sickness behaviour, circulating corticosterone, and central mRNA expression of pro-inflammatory cytokines

Mice were randomly assigned to either a chronic, acute or non-stressed condition. Following the stressor treatments, mice were further divided by drug treatment; LPS (10 µg in a volume of 0.3 ml; Sigma L-3755 from *Escherichia coli* serotype O26:B6) or saline, and time of sacrifice (1.5, 3 and 24 hrs) (n = 6/group).

Chronically stressed mice were exposed to a series of different stressors each day over the duration of 6 weeks. During this time, stressors were applied twice per day, with the exception of the 24hr stressors, and were administered on a variable and unpredictable schedule. Animals were returned to their home cages between the two stressor sessions each day. The chronic stressor regimen included the following stressors: 15 min restraint in a semicircular Plexiglas tubes (4 x 12cm), 5 min forced swim in tepid water (19-21°C), 15 min restraint in a tight-fitting triangular plastic bag (with a hole cut out for unrestricted breathing), 60 min wet bedding in home cage, 60 min exposure to dirty bedding taken from breeding animals, 15 min of regrouping mice separated by a partition, and 24 hrs of light. Mice in the acute and non-stressed conditions remained in their home cages and were not disturbed.

On the test day, chronically stressed mice were submitted to social disruption stress as their final stressor. This consisted of grouping 4 previously individually housed mice in a novel cage for 1 hr. This stressor was previously shown to elicit potent behavioural and neurochemical alterations (Gandhi et al., 2007; Gibb et al., 2008). Mice in the acute group were also exposed to the social stressor, whereas the non-stressed controls remained undisturbed. Immediately following stressor treatment, or an equivalent time for controls, mice were administered 10 µg of LPS or saline intraperitoneally (i.p.), and sickness behaviour (See General Methods) was monitored.

Mice were sacrificed at 1.5, 3 or 24 hrs following drug treatment. Trunk blood was collected, centrifuged and the plasma stored at -80°C for subsequent corticosterone determinations (see General Methods). Brains were removed, sliced, and tissue was punched to extract brain tissue for qPCR analysis. The mRNA expression of pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) were quantified within the mPFC and hippocampus. These regions were assessed as immune challenges markedly affect both these stressor-sensitive brain regions (Anisman, Poulter et al., 2007; Gandhi et al., 2007; Gibb et al., 2008).

General Methods

Sickness behaviour

Sickness behaviour was scored within the home cage for 10 sec epochs at 1.5, 3 or 24 hrs following LPS administration. To this end, the presence or absence of the following symptoms was recorded: lethargy (demonstrated by diminished locomotion and exploratory activities; curled body posture), ptosis (drooping eyelids), and piloerection (ruffled and greasy fur, typically at the neck). Animals were rated on a 4-point scale with respect to the number of symptoms present (0 = no sickness symptoms, 1 = 1 symptom, 2 = 2 symptoms, 3 = three symptoms). This procedure was found to provide less than 10% variability between raters blind to the treatment mice received and was highly correlated with other methods of scoring sickness (e.g., assessing severity of each symptom independently; see Gandhi et al., 2007).

Plasma Corticosterone Analysis

Following decapitation, trunk blood was collected in tubes containing 10 µg of EDTA, centrifuged for 15 min at 3600 RPM, and the plasma was stored at -80°C for subsequent corticosterone determination using a commercial radioimmunoassay RIA kit (ICN Biomedicals, CA). For each study, corticosterone levels were determined, in duplicate, in a single run to avoid inter-assay variability, and intra-assay variability was less than 10%.

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

mRNA from the mPFC and hippocampus was isolated and purified by standard methodologies employing Trizol according to the manufacturer's protocol (Invitrogen; Burlington, ON, Canada). The mRNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen; Burlington, Ontario), and aliquots of this reaction were used in simultaneous QPCR reactions.

BioRad MyIQ real time thermocycler was used to collect the data. For QPCR, SYBR green detection was used according to the manufacturer's protocol (Bio-Rad). 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 software. All primer pairs had a minimum of 90% percent efficiency.

Primers that amplify Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and Beta-Actin mRNA were used as a control to normalize the data. Primer sequences were

as follows: GAPDH, Forward: GGTCGGTGTGAACGGATTTG, Reverse: TGCCGTTGGAGTCATACTG, Beta-Actin, Forward: GAACCCTAAGGCCAACCGTG, Reverse: GGTACGACCAGAGGCATACAGG; Mus IL-1 β , Forward: TGTCTGAAGCAGCTATGGCAAC: Reverse: CTGCCTGAAGCTCTTGTTGATG; Mus IL-6. Forward: TCTTGGGACTGATGCTGGTG, Reverse: CAGAATTGCCATTGCACAACCTC; Mus TNF- α , Forward: CTC AGCCTCTTCTCATTCTGC Reverse: GCCATAGAACTGATGAGAGGG. To compensate for inter-individual variability that ordinarily exists within the assay, the expression of each species was normalized by subtracting its Ct from the housekeeping Ct. Following the procedure described by (Livak & Schmittgen, 2001), normalized brain mRNA expression values were converted to fold changes relative to controls.

Statistical Analyses

In each experiment, the outcome measures were analyzed through between-subjects analyses of variance (ANOVA). As such, sickness behaviour, plasma corticosterone, as well as the mRNA expression of cytokines were all analyzed independently. 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.

Results

Sickness Behaviour

Endotoxin-induced sickness behaviour was influenced by the Stressor x Time interaction, $F(4, 45) = 3.30$, $p < .05$. As shown in Figure 1.1, sickness among non-stressed mice varied over time following treatment, peaking 3 hrs after LPS administration. Furthermore, the extent of the sickness was elevated by the acute and chronic stressors at the 1.5 hr time point, but was less pronounced at the 3 hr interval. In the control and acutely stressed mice, sickness 24 hr after LPS had returned to basal levels; however, LPS-treated mice that also received the chronic stressors displayed increased sickness symptoms at 24 hrs, compared to LPS-treated mice that received the acute stressor, and non-stressed controls.

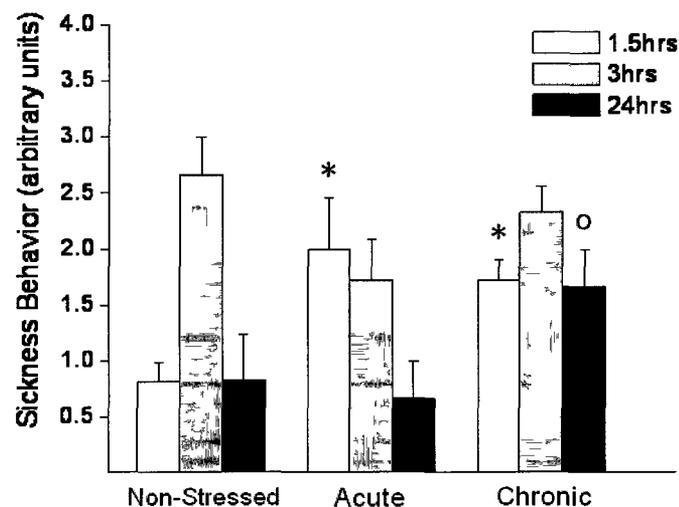


Figure 1.1. Mean (\pm SEM) sickness scores among mice that were exposed to acute or chronic stressors, and non-stressed controls, at 1.5, 3 and 24hrs following the administration of LPS. * $p < .05$ relative to non-stressed mice at 1.5hrs post-LPS exposure. ° $p < .05$ relative to acutely stressed and non-stressed controls at 24hrs post-LPS exposure.

Plasma Corticosterone

Circulating corticosterone levels varied as a function of the Stressor x Time and LPS x Time interactions, $F_s(4, 88) = 10.97$ and $(2, 88) = 9.20$, $p < .001$, respectively, as shown in Figure 1.2. As well, the Stressor x LPS x Time interaction was just shy of significance, $F(4, 88) = 2.32$, $p > .06$, $\eta^2 = 0.11$. As an *a priori* prediction had been made concerning the Stressor x LPS x Time interaction, and this interaction accounted for a fairly large portion of the variance (11%), follow-up tests were conducted of the simple effects comprising this interaction. These comparisons revealed that the LPS treatment increased plasma corticosterone at the 1.5 hr interval, peaking at 3 hrs, and returning to control levels at the 24 hr time point. The acute stressor alone provoked an elevation of corticosterone at 1.5 hrs, was still elevated at 3 hrs, although the corticosterone rise was diminished, and was entirely absent by 24 hrs. Among acutely stressed mice, the effects of LPS on corticosterone at the 1.5 hr time point exceeded that of mice that received only a single treatment, but the combined stressor + LPS treatment effect was no longer apparent at the 3 hr time point. Finally, the effects of the chronic stressor on plasma corticosterone were similar to that elicited by the acute stressor, being elevated relative to that of mice that had received LPS treatment in the absence of a stressor. Of particular note, however, was the corticosterone rise elicited by LPS among chronically stressed mice was markedly diminished at the 3 hr time interval relative to that evident in the nonstressed mice that had received LPS. Thus, it seems that the combined treatments diminished the ongoing synthesis of corticosterone or increased the clearance rates of the hormone relative to non-stressed mice.

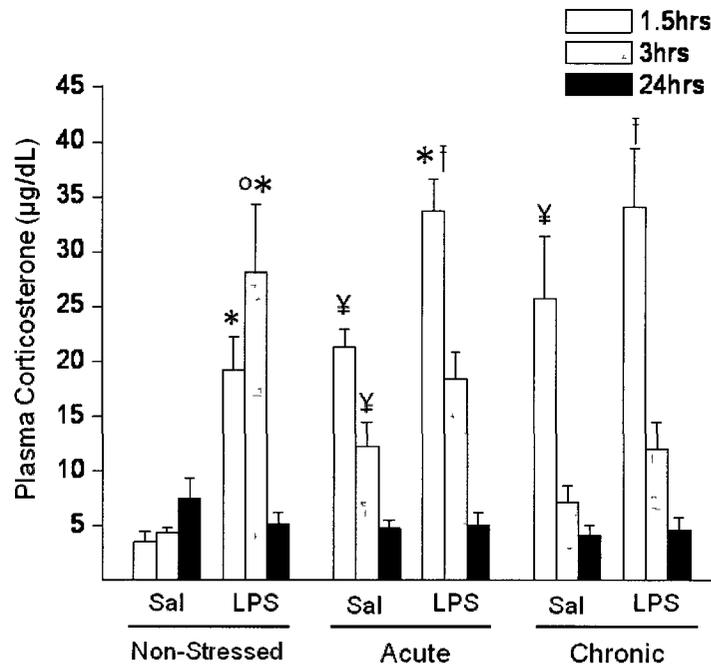


Figure 1.2. Mean (\pm SEM) plasma corticosterone among mice that were exposed to acute or chronic stressors, and non-stressed controls, at 1.5, 3 and 24hrs following the administration of LPS or saline. * $p < .05$ relative to saline treated animals at the equivalent time post-LPS. ‡ $p < .05$ relative to saline treated non-stressed mice at the equivalent time post-LPS. † $p < .05$ relative to LPS-treated acute and chronically stressed mice at 3hrs post-LPS. ¶ $p < .05$ relative to similarly treated non-stressed mice at 1.5hrs post-LPS.

Central Cytokine mRNA Expression

Prefrontal Cortex

Within the mPFC, IL-1 β mRNA expression varied as a function of the Stressor x LPS x Time interaction, $F(4, 83) = 4.09$, $p < .005$; Figure 1.3, top panel. The follow-up comparisons indicated that in non-stressed mice LPS increased mRNA expression at the 1.5 and 3 hr time points, but had normalized at 24 hrs. The acute stressor alone did not influence expression of this cytokine, but when LPS was combined with an acute stressor, the effects on IL-1 β 1.5 hrs later were markedly diminished relative to that elicited by LPS alone, but were significantly enhanced at 3 hrs. Like the acute stressor,

the chronic stressor alone did not affect mPFC expression of IL-1 β . When the LPS was administered to chronically stressed mice, the expression of the cytokine at the 1.5 and 3 hr time points were slightly lower than that elicited by LPS alone,. At the latter time, the LPS-stressor synergy was entirely absent.

Paralleling the effects of IL-1 β , TNF- α mRNA expression was also influenced by the Stressor x Drug x Time interaction $F(4, 82) = 3.49, p < .01$; Figure 1.3 middle panel. The follow-up comparisons revealed that in mice that LPS increased the mRNA expression of this cytokine at the 1.5 and 3 hr intervals, whereas the acute stressor had no effect. Among mice that received the combined treatment of endotoxin and acute stressor, the rise of TNF- α ordinarily elicited by LPS was entirely suppressed at the 1.5 hr time point, but was strikingly enhanced at 3 hrs. In the chronically stressed mice the expression of TNF-a was moderately increased 3 hrs following LPS treatment, but the expression of the cytokine was markedly lower than that evident in mice that received the LPS alone, and the synergy between LPS and the acute stressor was entirely absent.

The effects associated with the stressor-LPS treatments on IL-6 could be readily dissociated from those of IL-1 β and TNF- α . In this regard, IL-6 mRNA expression within the mPFC was influenced by the LPS x Time interaction, $F(2, 83) = 4.83 p < .01$; Figure 1.3 lower panel. Follow-up comparisons revealed that LPS provoked a significant increase of IL-6 mRNA at 1.5 hrs, and to a more modest degree at 3 hrs. Due to the small sample size, and high variability, alterations of this cytokine due to Stressor conditions were not significant.

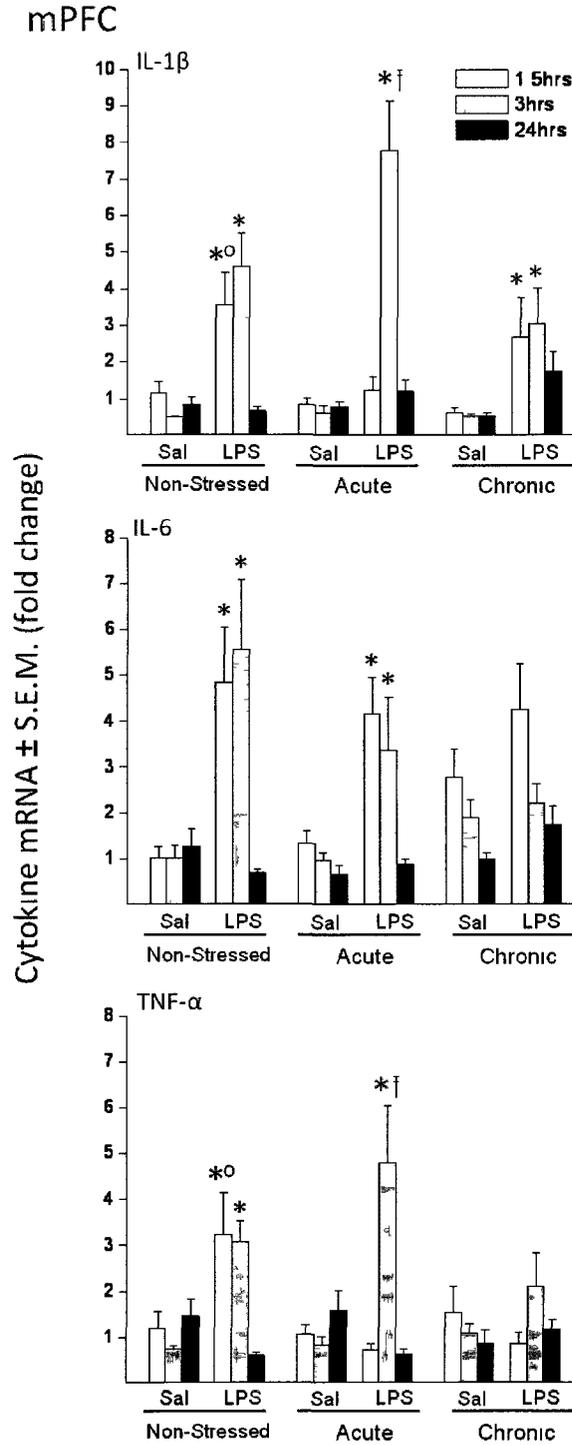


Figure 1.3. Mean (\pm SEM) IL-1 β , IL-6 and TNF- α mRNA expression in the prefrontal cortex among mice that were exposed to acute or chronic stressors and non-stressed controls, at 1, 5, 3 and 24hrs following the administration of LPS or saline. Data are presented as fold changes. *p < 0.05 relative to saline treated mice at the equivalent time post-LPS. †p < 0.05 relative to acutely stressed mice 1 5hrs following LPS administration. ‡p < 0.05 relative to LPS-treated chronically stressed and non-stressed mice at 3hrs post-LPS.

Hippocampus

Analysis of IL-1 β mRNA expression within the hippocampus revealed a significant LPS x Time interaction, $F(2, 84) = 9.36$, $p < .001$, although the Stressor x LPS x Time interaction was just shy of significance $F(4, 84) = 2.31$, $p = .07$, $\eta^2 = .10$. Once again, based on a priori predictions, follow-up comparisons were conducted of the means comprising the simple effects for this interaction. These analyses, as depicted in Figure 1.4 upper panel, indicated LPS produced a significant increase of IL-1 β mRNA at 1.5 hrs, which was absent by 24 hrs. Neither the acute nor the chronic stressor itself influenced the expression of this cytokine, but when combined with LPS the IL-1 β expression was greatly elevated at the 3 hr time point relative to mice that received LPS.

Within the hippocampus, TNF- α mRNA expression varied as a function of the Stressor x Time and Drug x Time interactions, $F_s(4, 85) = 4.16$ and $(2, 85) = 5.39$, p 's $< .01$, respectively. The follow-up comparisons indicated that overall, the LPS treatment increased TNF- α expression at the 3 hr time point. These trends were consistent with those observed in the mPFC where the effects of the endotoxin and stressor were diminished at 1.5 hrs, yet enhanced at 3 hrs. However, the suppressive effect of the chronic stressor that evident within the mPFC was not apparent in the hippocampus.

The IL-6 mRNA expression within the hippocampus was significantly influenced by the LPS x Time interaction $F(2, 85) = 4.85$ $p < .01$. As shown in Figure 1.4 lower panel, and confirmed by multiple comparisons, LPS provoked a marked increase of IL-6 mRNA at 1.5 hrs, an effect that was no longer statistically significant at 24 hrs. Overall, as was the case in the mPFC, the effect of Stressor condition on IL-6 expression was non-significant.

Hippocampus

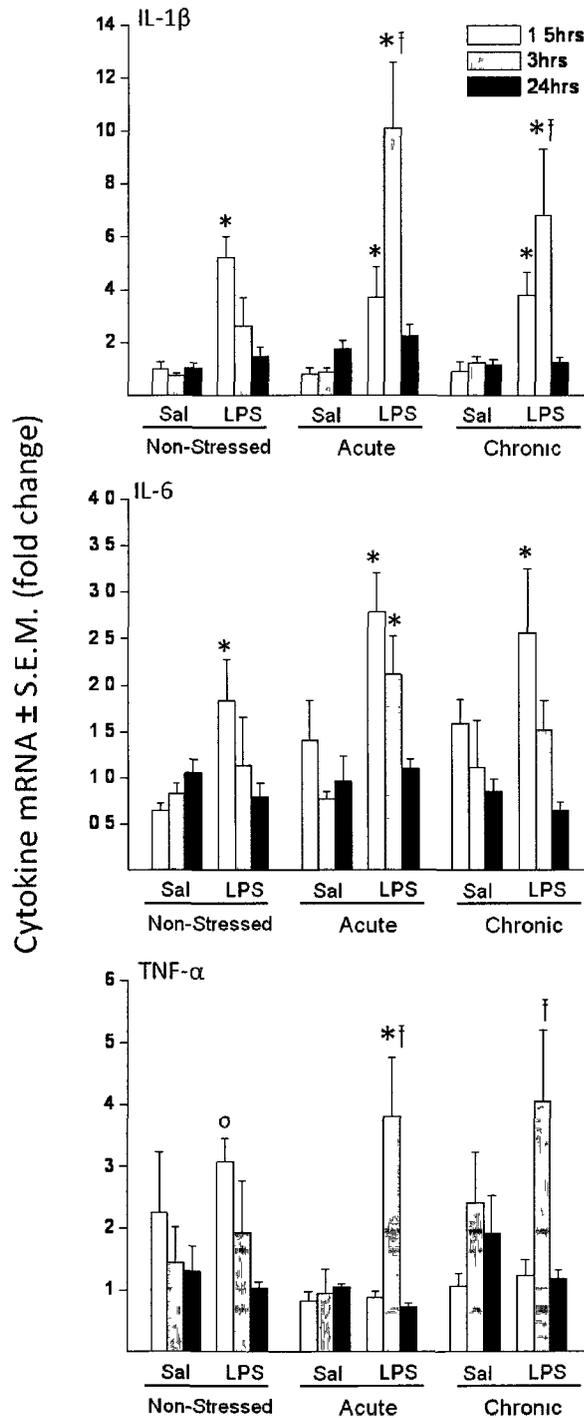


Figure 1.4. Mean (± SEM) IL-1β, IL-6 and TNF-α mRNA expression in the hippocampus among mice that were exposed to acute or chronic stressors, and non-stressed controls, at 1.5, 3 and 24hrs following the administration of LPS or saline. Data are presented as fold changes. *p < .05 relative to saline treated mice at the equivalent time post LPS. †p < .05 relative to acute and chronically stressed mice 1.5hrs following LPS administration. ‡p < .05 relative to LPS-treated non-stressed mice at 3hrs post-LPS.

Discussion

Psychosocial stressors have been shown to influence immune functioning and circulating cytokines in animal studies (Bartolomucci, et al., 2003; Bartolomucci, et al., 2005; Goshen, et al., 2008; O'Connor, et al., 2003). Likewise, in humans, stressors are known to exacerbate chronic inflammatory conditions, such as asthma, arthritis and cardiovascular disease, conditions that are often co-morbid with major depression (Black & Garbutt, 2002; Kemeny & Schedlowski, 2007). This said, it appears that immune challenges and stressors may have additive or synergistic effects on inflammatory immune processes as well as cytokine variations within the brain. However, as acute and chronic stressors may have very different effects on several biological processes, in the present investigation we assessed whether these stressor regimens would differentially influence LPS-elicited sickness, plasma corticosterone and brain cytokine variations. It was observed that when stressor exposure was combined with an LPS challenge, sickness behaviour and plasma corticosterone were additively enhanced at 1.5 hrs, but this effect became less pronounced thereafter, even though the nature of the stressor (social disruption or social defeat) is one that has relatively long lasting effects on corticosterone levels (Koolhaas, Meerlo, De Boer, Strubbe, & Bohus, 1997).

In contrast to sickness behaviour and corticosterone levels, which peaked relatively early, the temporal dynamics of central cytokine expression were very different, particularly among mice that received both the LPS and the stressor challenge. Moreover, these effects varied with the specific cytokine being considered. Specifically, whereas the stressor did not influence the rise of IL-6 elicited by LPS, the increase of IL-1 β and TNF- α within the mPFC and hippocampus was very much attenuated 1.5 hrs after

endotoxin administration in mice that had experienced a stressor comprising social disruption. In contrast, 3 hrs after the LPS challenge, the expression of these cytokines was strikingly increased. These effects were not apparent in the mPFC in response to LPS among chronically stressed mice assessed 3 hrs after the LPS challenge. In fact, some of the actions of LPS were diminished in the chronically stressed mice. Within the hippocampus, in contrast, the effects of the chronic stressor were very much like those evident in mice that had been exposed to the acute stressor and treated with LPS.

As alluded to earlier, it is unclear how acute and chronic stressors might come to differentially influence behaviour and central cytokine expression, especially when combined with an immunogenic agent. In the present study, neither acute nor chronic stressor exposure independently elicited elevations of central cytokine expression, despite provoking significant increases of circulating corticosterone. This contrasts with previous findings showing that stressor exposure provoked central cytokine mRNA and protein alterations (Bartolomucci, et al., 2003; Goshen, et al., 2008; O'Connor, et al., 2003).

When combined with the endotoxin, chronic and acute stressor exposure had similar effects on sickness behaviour and plasma corticosterone levels, namely, they both additively enhanced these outcomes measured 1.5 hrs after the LPS challenge. However, 3 hrs after LPS treatment corticosterone levels had declined to a greater extent in stressed mice. In effect, it seemed that the stressor resulted in the time curve for the corticosterone variations shifting to the left so that peak effects occurred earlier and were greater than that seen in non-stressed mice. Alternatively, the biphasic effects observed with respect to the stressor-LPS outcomes may reflect two independent processes at play at the different

time points; one that increases corticosterone release, and a second that either inhibits release or increases the clearance of the hormone.

The enhanced effects of stressor exposure and an immunogenic challenge on behavioural, neuroendocrine and inflammatory markers has consistently been observed in human studies (Brydon et al., 2009; Frank et al., 2010; Yeager et al., 2009), as well as studies involving animal models (Anisman et al., 2008; Gandhi et al., 2007; Gibb et al., 2008). For instance, similar to the present report, animals exposed to an acute social stressor were found to display exaggerated sickness behaviour and neurochemical alterations following the administration of an immunogenic agent, namely interferon- α , the double-stranded viral mimic poly I:C or a bacterial endotoxin (Anisman et al., 2008, Gandhi et al., 2007; Gibb et al., 2008). It was also reported that socially stressed animals were more susceptible to developing LPS-induced endotoxic shock (Carobrez et al., 2002; Quan et al., 2001), further highlighting the potentially harmful effects of stressor and cytokine exposure. The present findings, however, make the point that the combined effects of these treatments are time-dependent and vary with the nature of the stressor treatment (acute vs chronic stressor), and that the time course for the effects of the treatment on corticosterone levels and on sickness may be very different from one another, the latter still being evident long after corticosterone levels had normalized.

The mechanisms through which the synergy between stressors and cytokines occurs is currently unknown, however, it has been postulated that glucocorticoids (GCs) are the most important mediators of this interaction (Sorrels & Sapolsky, 2007). GCs are normally immunosuppressant, decreasing the expression and release of inflammatory mediators (Calcagni & Elenkov, 2006; O'Connor et al., 2000). Contrary to the typical

anti-inflammatory effects, reports have shown that GCs can also have pro-inflammatory effects, especially when combined with an immune challenge, which may vary according to the timing and nature of stressors, the dose of the immune challenge, as well as the area of interest (e.g. peripheral vs central; Sorrels & Sapolsky, 2007).

The profile of the corticosterone changes in the present study was, in the main, a reverse image of the IL-1 β and TNF- α variations seen in the PFC and hippocampus. When corticosterone levels were highest (1.5 hrs following LPS treatment in stressed mice), central mRNA expression of IL-1 β and TNF- α was relatively diminished, possibly reflecting the immunosuppressant actions that are characteristic of this stress hormone. This is consistent with previous reports (Frank et al., 2010; Gibb et al., 2008; Goujon et al., 1995) demonstrating a suppression of LPS-induced cytokine expression before or immediately following stressor exposure, when GC levels were highest. With the decline of corticosterone levels at 3 hrs following LPS administration, cytokine expression was exaggerated in response to the combined stressor and endotoxin treatments, possibly reflecting the progressively diminished anti-inflammatory effect exerted by corticosterone. Interestingly, it has been reported that the GC receptor antagonist RU486 was effective at reversing the inflammatory and damaging effects of stressors and LPS, further demonstrating the involvement of GCs in this interaction (de Pablos et al., 2006; Espinosa-Oliva et al., 2011; Munhoz et al. 2006).

It is curious that although the effects of the acute stressor and LPS on IL-1 β and TNF- α on mPFC were diminished in animals that had been chronically stressed, this outcome was not as evident in the hippocampus. Why this differentiation occurred between the mPFC and hippocampus is uncertain especially as the hippocampus is

particularly rich in GC receptors (Tasker & Herman, 2011). Whatever the case, the present findings demonstrate that the impact of the concomitant application of stressor exposure and an immune challenge varies not only with time of endotoxin administration, but also with the nature of the stressor and the brain region examined.

It has been demonstrated through *In vitro* studies in animals that stressors can prime immune cells so that subsequent exposure to an immune challenge provokes exaggerated effects (Bailey et al., 2009; Dong-Newsom, Powell, Bailey, Padgett, & Sheridan, 2009). For instance, splenic cells stimulated with LPS from *Porphyromonas Gingivalis* displayed increased cytokine production (IL-1 β and TNF- α) in animals that had experienced a social disruption stress, compared to non-stressed controls (Bailey et al., 2009). These cells also showed decreased GC sensitivity, with the cells remaining viable even with high doses of glucocorticoids. Similar results were found using herpes simplex virus type 1 infection within the cornea and trigeminal ganglia (Dong-Newsom et al., 2009). These results have, in part, been attributed to direct effect of stressors on microglial cells. For instance, GCs, either exogenously administered or released in response to an acute stressor, have been shown to directly activate microglial cells, as evidenced by increased major histocompatibility (MHC)-II mRNA expression and protein levels (de Pablos et al., 2006; Frank et al., 2007; Nair and Bonneau, 2006). When microglia are subsequently exposed to an immune challenge, the effects are then synergistically enhanced (de Pablos et al., 2006; Frank et al., 2007). In fact, it has been shown that stressor exposure is essential in provoking LPS-induced microglial activation and degeneration in the hippocampus (Espinosa-Oliva et al., 2011). In addition to microglial activation, stressors have also been shown to provoke microglia proliferation,

where the increased number of activated immunocompetent cells could be responsible for the enhanced effects of the immune challenge (de Pablos et al., 2006; Nair and Bonneau, 2006). It should be noted, however, that the order in which stressor exposure and an immune challenge is presented may affect the synergistic outcomes of the combined treatments. For instance, although the administration of exogenous GCs potentiated the inflammatory effects of a peripheral endotoxin challenge, these effects were not apparent when LPS preceded GC exposure (Frank et al., 2010). Evidently, whatever mechanisms are responsible for the synergy, priming essential cells by stressors is fundamental in eliciting augmented responses to immunogenic challenges.

In addition to directly activating microglial cells, GCs may also be mediating the stressor and LPS interaction by priming immunocompetent cells by increasing toll-like receptor (TLR) expression, so that subsequent stimulation with an immune agent will result in heightened reactivity (Bailey et al., 2007; Frank et al., 2010). TLRs are pattern recognition molecules, an integral part of the innate immune system, which detect and respond to antigens by promoting an inflammatory response and phagocytosis (Rivest, 2003). LPS effects are primarily mediated through TLR-4, and to a lesser degree TLR-2 (Rivest, 2003). Interestingly, exposure to social disruption stress was found to upregulate TLR-2 and TLR-4 expression on splenic macrophages (Bailey et al., 2007), as well as hippocampal microglia (Frank et al., 2010), making cells more reactive to LPS-induced activation (Frank et al., 2010). Thus, stressors in the present investigation might likewise have had their augmenting actions by influencing TLR-2 and TLR-4 expression in brain microglia, just as such effects can occur peripherally on macrophages.

Stressors might also potentiate LPS inflammation through their effects on nuclear factor κ B (NF κ B), a transcription factor that is involved in transcribing inflammatory genes (Vermeulen, Vanden Berghe, & Haegeman, 2006). In a typical response to inflammation, Inhibitor κ B (I κ B) kinase, an NF κ B inhibitor, is activated and phosphorylated, which causes it to dissociate from NF κ B. This results in the translocation of NF κ B into the cell nucleus where it promotes the transcription of cytokines and their receptors (Miyamoto & Verma, 1995; Quan et al., 2000). As indicated earlier, GCs can exert their anti-inflammatory effects in two complimentary ways. First, they induce the expression of I κ B, which prevents the translocation of NF κ B into the nucleus (Quan et al., 2000). Second, they antagonize the NF κ B receptor, the p65 subunit, within the nucleus, thereby blocking NF κ B binding and limiting its effects on DNA transcription (McKay & Cidlowski, 2000; Unlap & Jope, 1997). NF κ B has recently become a target of interest in the study of depression, as severe stressors in both humans (Miller et al., 2008; Pace, Mletzko, Alagbe, Musselman, Nemeroff, Miller, et al., 2006) and animals (Bierhaus et al., 2003, Munhoz et al., 2006), have been associated with an increase in its expression, and severity of depressive symptoms were positively correlated with NF κ B binding (Pace, et al., 2006). Interestingly, despite the normal anti-inflammatory properties of GCs, it was found that when LPS was combined with a chronic unpredictable stressor, the treatments synergistically enhanced NF κ B expression in the PFC and hippocampus, resulting in heightened inflammation, an effect which was inhibited with pre-treatment with the GC receptor antagonist, RU486 (Munholtz et al., 2006). It should also be noted that IL-1 β and TNF- α are both mediated by NF κ B activation (Baldwin, 1996), so it's interesting that the elevations of these cytokines

resulting from the combined treatments in the present study were only observed in these cytokines, whereas IL-6 (which is influenced by different pathways, namely JAK/STAT and MAPK; Heinrich, Behrmann, Haan, Hermanns, Müller-Newen, & Schaper, 2003) was differentially affected.

Conclusion

Activation of the inflammatory immune system by an immunogenic agent produces elevated levels of pro-inflammatory cytokines effects in the CNS, and can provoke the development of behavioural disturbances. Interestingly, these effects appear to be moderated, at least in part, by prior stressor exposure, as the administration of an immune challenge on the backdrop of acute or chronic stress can provoke exaggerated effects. In the present study, sickness behaviour and corticosterone were markedly enhanced by the combined LPS and stressor treatments. Furthermore, when LPS was administered following an acute stressor, central cytokine mRNA expression was markedly enhanced, though this outcome was not as apparent when the endotoxin was administered following chronic stressor exposure. These data have implications for the therapeutic use of cytokines when administered on a backdrop of stressors, such as those ordinarily associated with severe illness, as their combined actions may render an individual vulnerable to depression.

CHAPTER 2: Effects of stressors and immune activating agents on peripheral and central cytokines in mouse strains that differ in stressor responsivity

As seen in Chapter 1, stressor exposure can potentiate the behavioural, endocrine and neuroinflammatory effects of a bacterial endotoxin, an effect dependent on the chronicity of the stressor, the brain region examined and the timing of the outcome measures. However, as considerable inter-individual variability exists regarding the impact of stressors, in Chapter 2, we explored whether inter-strain differences of stressor reactivity altered sickness behaviour, elevations of circulating corticosterone and cytokine levels and changes of central cytokine mRNA expression normally provoked by an immune challenge administered on the backdrop of a social stressor. Furthermore, as different immune profiles are evident in each strain, we investigated two different types of immune challenges, namely lipopolysaccharide which mainly elicits a Th2 response and Poly I:C which primarily elicits a Th1 responses, to assess whether these would differentially influence outcomes in these strains.

Abstract

The impact of inflammatory immune activation on behavioural and physiological processes varies with antecedent stressor experiences. We assessed whether immune activation would differentially influence such outcomes as a function of stressor reactivity related to genetic differences. To this end, we assessed the influence of a social stressor (exposure to a dominant mouse) in combination with an acute immune challenge on behaviour and on peripheral and central cytokines in stressor-reactive BALB/cByJ mice and the less reactive C57BL/6ByJ strain. As C57BL/6ByJ and BALB/cByJ mice are highly Th1 and Th2 responsive, respectively, the stressor effects were assessed in response to different challenges, namely the viral analogue poly I:C and the bacterial endotoxin LPS. The stressor enhanced the effects of LPS on sickness behaviours and plasma corticosterone particularly in the BALB/cByJ mice, whereas the effects of poly I:C, which primarily affects Th1 processes, were not augmented by the stressor. As well, the stressor increased circulating cytokines in LPS treated C57BL/6ByJ mice, whereas the effects of poly I:C were diminished. Finally, like circulating cytokines, mRNA expression of pro-inflammatory cytokines within the PFC and hippocampus varied with the mouse strain and with the stressor experience, and with the specific cytokine considered. Together, the experiments indicated that the impact of stressors vary with the nature of the immune challenge to which animals had been exposed. Moreover, given the diversity of the stressor effects on central and peripheral processes, it seems likely that the cytokine changes, HPA activity and sickness operate through independent mechanisms.

Introduction

The effects of systemic insults, such as bacterial endotoxins, on neuroendocrine and brain neurochemical changes are, in several respects, reminiscent of those elicited by psychological and physical stressors (Anisman, et al., 2008a, b). For instance, the actions of LPS, like those of IL-1 β , include increased HPA functioning (Anisman, et al., 2008c) and monoamine variations in hypothalamic and limbic regions (Merali, et al., 1997; Schiepers, Wichers, & Maes, 2005). As well, some of the effects elicited by immune challenges may be exaggerated when administered on the backdrop of an acute social stressor (Anisman, et al., 2007; Gibb, et al., 2008; Quan et al., 2001). Moreover, features reminiscent of depression (e.g. anhedonia and lethargy/sickness; Dantzer, 2001; Konsman, Parnet, & Dantzer, 2002; Merali, et al., 2003), can be attenuated by antidepressant treatment (Merali et al., 2003; Yirmiya, 1996).

One of the most prominent characteristics of the stress response is the pronounced inter-individual and inter-strain variability that occurs with respect to both behavioural and neurochemical outcomes (Anisman, et al., 2008b). Relative to C57BL/6 mice, BALB/c mice are highly stress reactive, exhibiting pronounced signs of anxiety and depression (e.g., anhedonia) in response to stressors (Anisman, et al., 2001; Belzung, El Hage, Moindrot, & Griebel, 2001; Crawley, et al., 1997; Griebel, Belzung, Perrault, & Sanger, 2000; Shanks & Anisman, 1993; Zacharko, Gilmore, MacNeil, Kasian, & Anisman, 1990). In line with neurochemical perspectives of depression and anxiety (Nestler, et al., 2002; Nutt, 2002; Reul & Holsboer, 2002), in response to stressors BALB/cByJ mice exhibit particularly marked elevations of corticosterone, altered monoamine variations in stress-sensitive brain regions (Prakash, et al., 2006), as well as

increased expression of CRH content and altered CRH1 receptor mRNA expression in the frontal cortex (Anisman, et al., 2007b).

It has been proposed that activation of the inflammatory immune system may contribute to the evolution of depression (Dantzer, et al., 2008), through its stressor-like actions on central neurotransmitter and peptide processes, or by cytokine changes (Anisman, et al., 2008a,b). If immune activation acts like a stressor, it would be expected that immune activation might differentially influence behavioural and neuroendocrine functioning in the C57BL/6ByJ and BALB/cByJ strains of mice, just as traditional stressors have such effects. Among other things, it would be expected that LPS challenge would provoke greater sickness behaviour and plasma corticosterone in stressor-reactive BALB/cByJ than in the more resilient C57BL/6ByJ mice, and that the previously reported synergistic or additive effects of LPS and stressors (Gibb, et al., 2008) would also be strain dependent. As well, it might be expected that stressors and immune activation would differentially influence brain cytokine mRNA expression in these strains.

The differences between the two strains are not, however, limited to stress responses as they differ from one another in numerous other respects, including but not limited to, prostaglandin 2 production by macrophages (Kuroda, Noguchi, Doi, Uematsu, Akira, & Yamashita, 2007), mast cell production by splenocytes (Hu, Zhao, & Shimamura, 2006), and prolactin production (Meerlo, Easton, Bergmann, & Turek, 2001). Any of these or other factors could potentially influence the stress response, in general, and may contribute to the differential effects of immune challenges that have been reported. This said, BALB/cByJ and C57BL/6ByJ also display unique immune

characteristics that are particularly germane to the present investigation. Specifically, whereas BALB/cByJ mice are highly Th2 responsive, the C57BL/6ByJ mice exhibit greater Th1 responses (Watanabe, Numata, Ito, Takagi, & Matsukawa, 2004), leading to differential resistance to infection (Brenner, Cohen, & Moynihan, 1994; Kruszezwska, Felten, & Moynihan, 1995) and different immune responses to stressors (Brenner & Moynihan, 1997). Thus, beyond assessing the effects of LPS, which primarily influences macrophages and B cells, we also evaluated the effects of a viral analogue poly I:C, which primarily elicits a Th1 immune response (Manetti, et al., 1995). It was expected that stressors would enhance the effects of LPS in both strains, but more so in BALB/cByJ mice, whereas the impact of the stressor on poly I:C effects would be more prominent in C57BL/6ByJ than in BALB/cByJ mice.

Materials and Methods

Subjects

BALB/cByJ and C57BL/6ByJ male mice, approximately 70 days of age, were used to assess the effects of an immune challenge and a social stressor. Mice were bred at Carleton University from parent stock obtained from the Jackson Laboratory (Bar Harbor, Maine). As well, male CD-1 mice (Charles River Canada, St. Constant, Quebec) were used as a social stressor for the inbred strains. Male inbred mice were housed in pairs, in standard (27 x 21 x 14 cm) polypropylene cages, and maintained on a 12-h light-dark cycle (light phase: 0700-1900 h), with temperature (22°C) and humidity (63%) kept constant, and were permitted free access to food and water. The studies met the

guidelines set out by the Canadian Council on Animal Care and were approved by the Carleton University Animal Care Committee.

Procedures

Experiment 1: effects of LPS and a social stressor on sickness behaviours, plasma corticosterone and cytokine levels

Mice (48 of each strain) were tested between 08:30 and 13:00h. On the day of the experiment, half of the mice were submitted to the social stressor procedure, whereas the remaining mice were not exposed to any stressor. During a stressor session, a pre-selected CD-1 mouse was introduced into the home-cage of an individual naïve BALB/cByJ or C57BL/6ByJ mouse, and direct interactions were permitted for 30min, after which the CD-1 mouse was returned to its home-cage. As the CD-1 mouse was generally much larger and more aggressive, the inbred mice (BALB/cByJ or C57BL/6ByJ) were almost invariably submissive in these encounters. Aggressive behaviours (e.g., incessant fighting) were reduced by having the CD-1 introduced to the submissive animals' home cage (rather than the other way around), but if the aggression was excessive, it was interrupted by knocking gently on the side walls of the cage.

Following the 30min session (or an equivalent time for mice that did not experience the stressful interaction), mice were individually housed and injected intraperitoneally (i.p.) with either saline or the bacterial endotoxin, LPS (2 or 10 μ g in a volume of 0.3ml). Sickness behavior, as described shortly, was monitored every 15min over the 1.5h post-injection period, commencing 30min after injection. Mice were then decapitated and trunk blood was collected for later plasma corticosterone determinations.

Experiment 2: effects of LPS and social disturbance on cytokine mRNA expression in brain

To evaluate cytokine mRNA expression as a function of the strain, LPS and stressor treatments, a study was conducted that was identical to that of Experiment 1, except that brain samples from BALB/cByJ and C57BL/6ByJ mice (N=20 and 16, respectively) were used for qPCR analyses. For these analyses both the mPFC and hippocampus were assessed given that immune challenges markedly affect both these stressor-sensitive brain regions (Anisman et al., 2007a; Gibb et al., 2008).

Experiment 3: effects of poly I:C and social disturbance on sickness behaviours, plasma corticosterone and cytokine levels, and cytokine mRNA expression in brain

Experiment 3 was conducted to assess the influence of poly I:C when administered on the background of a social stressor. Thus, mice of each strain (N=24/strain) received either no treatment or exposed to the social stressor. These groups were further subdivided and treated with either poly I:C (1.0mg/kg; Sigma P-1038) or vehicle, and then decapitated 90min afterward (N=6/group). Trunk blood was collected for the determination of plasma corticosterone and cytokines, and brain samples were collected for determination of cytokine mRNA expression as described in Experiment 2.

General Methods

Sickness behaviour

Sickness behaviour was scored for 10s epochs at 15min intervals over the 1.5h period following injection. To this end, the presence or absence of the following symptoms were recorded: lethargy (demonstrated by diminished locomotion and exploratory activities; curled body posture), ptosis (drooping eyelids), and piloerection (ruffled and greasy fur, typically at the neck). Animals were rated on a 4-point scale with respect to the number of symptoms present (0=no sickness symptoms, 1=1 symptom, 2=2 symptoms, 3=three symptoms). This procedure was found to provide less than 10% variability between raters blind to the treatment mice received and was highly correlated with other methods of scoring sickness (e.g., assessing severity of each symptom independently; see Gandhi et al., 2007).

Plasma corticosterone analysis

Following decapitation, trunk blood was collected in tubes containing 10 μ g of EDTA, centrifuged for 15min at 3600 RPM, and the plasma was stored at -80°C for subsequent corticosterone determination using a commercial radioimmunoassay RIA kit (ICN Biomedicals, CA). For each study, corticosterone levels were determined, in duplicate, in a single run to avoid inter-assay variability, and intra-assay variability was less than 10%.

Plasma cytokine analyses

The analysis of plasma cytokines (pro-inflammatory, including tumor necrosis factor [TNF]- α , IL-6, and the anti-inflammatory, IL-10) was conducted using a Beadlyte Mouse Multi-Cytokine Detection kit (Upstate, Cell Signaling Solutions, Cat # 48-004), in combination with a Luminex 100 system. This assay is a suspension based bead array system wherein sets of microspheres (5.6 μ m beads) are internally dyed with different ratios of fluorophores, each conjugated to a different capture probe (cytokine specific antibody). Following incubation, a classification laser identifies the particular cytokine bound and a second reporter laser quantifies the signal. A mouse diluent kit (Cat # 43-007) was used to dilute plasma supernatants and a serial dilution series performed to cover a range of standards (from 0 to 5000pg/ml). Then, 25 μ l of Beadlyte Cytokine Assay Buffer was placed in each well followed by addition of 25 μ l of diluent and 25 μ l of plasma. The filter plate was then incubated on a shaker for 20min and vortexed. To finalize the initial reaction, 25 μ l of bead solution was added to each well, the plate was covered and vortexed. Following overnight incubation, samples were washed twice and 25 μ l of Biotin a conjugated Beadlyte Anti-Mouse Cytokine cocktail, that included antibodies against mouse IL-1, IL-6, TNF- α , interferon- γ , IL-4 and IL-10, was added to each well and incubated for 1.5h at room temperature. This was followed by 25 μ l of diluted beadlyte streptavidin–phycoerythrin being added and incubated for 30min on a plate shaker. Finally, 25 μ l of Beadlyte stop solution was added, samples were then resuspended in sheath fluid, and cytokine levels quantified using the Luminex 100. The lower level of detection using this procedure ranged from 0.1pg/ml (TNF- α) to 3.2pg/ml

(IL-10). Assays were performed in duplicate and yielded less than 10% intra- and inter-assay variability.

Reverse transcription-quantitative polymerase chain reaction analysis (RT-QPCR)

Total brain RNA was isolated and purified by standard methodologies employing Trizol according to the manufacturers 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.

Bio-Rad MyIQ real time thermocycler was used to collect the data. For QPCR, SYBR green detection was used according to the manufacturer's protocol (Bio-Rad). 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 Bio-Rad software. All primer pairs had a minimum of 90% percent efficiency.

Primers that amplify cyclophilin and synaptophysin mRNA were used as a control to normalize the data. Although there was inter-subject variability in the cycle threshold (Ct) for synaptophysin, there were no significant differences in the (Ct) across the treatment groups ($F's < 1$) within any of the brain regions examined. Moreover, whether the data were analyzed using cyclophilin or synaptophysin as the reference gene, the results were the same, and hence the analyses presented are based on the basis of using cyclophilin in a reference capacity. To compensate for inter-individual variability

that ordinarily exists within the assay, the expression of each species was normalized by subtracting its Ct from the housekeeping Ct. Following the procedure described by Livak and Schmittgen (2001), normalized brain mRNA expression values were converted to fold changes relative to non-stressed BALB/cByJ mice that had been treated with vehicle.

Primer sequences were as follows: Mus Ppia (Cyclophilin A), Forward: ATTCAT GTGCCAGGGTGGTG, Reverse: CCGTTTGTGTTTGGTCCAGC, Synaptophysin, Forward: GGACGTGGTGAATCAGCT GG, Reverse: GGCGAAGATGGCAAAGACC; Mus IL-1 β , Forward: TGTCTGAAGCAGCTATGGCAAC: Reverse: CTGCCTGAAGCTCTTGTTGATG; Mus IL-1R1, Forward: ATGAGTTACCCGAGGTCCAGTG; Reverse: TACTCGTG TGACCGGATATTGC1; Mus IL-6. Forward: TCTTGGGACTGATGCTGGTG, Reverse: CAGAATTGCCATTGCACAACCTC; Mus IL-6R, Forward: CTCTCCAACCAC GAAGGCTG, Reverse: TGCAACGCACAGTGACACTATG; Mus TNF- α , Forward: CTC AGCCTCTTCTCATTCTGC Reverse: GCCATAGAACTGATGAGAGGG; Mus IL-10, Forward: AATTCCTGGGTGAGAAGCTG; Reverse: TCATGGCCTTG TAGACACCTTG.

Statistical analyses

Each of the outcome measures was analyzed through a between-subjects analysis of variance (ANOVA) in which strain, LPS or poly I:C treatments, and stressor condition were used as independent variables. Elevated sickness behaviour scores over time were highly correlated, thus scores from 75 to 90min were averaged to create a composite sickness score which was used in the ANOVA. The plasma corticosterone and cytokine

levels, as well as the mRNA expression for each of the cytokines were analyzed independently. 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.

Results

Experiment 1: influence of strain and social stress on LPS-induced peripheral actions

Sickness Behaviour

The sickness scores and corticosterone levels for each group are shown in Figure 2.1 (left panel). Sickness (averaged over 75–90min after LPS treatment) was greater in BALB/cByJ than in C57BL/6ByJ mice, $F(1,84)=15.75$, $p<.01$, and this outcome was further increased by the social stressor, $F(1,84)=32.86$, $p<.001$, and by the LPS treatment, $F(2,84)=106.80$, $p<.001$. As well, the stressor \times LPS treatment interaction was significant, $F(2,84)=12.59$, $p<.001$, indicating that sickness behaviour was more pronounced in LPS-treated mice (at both doses) exposed to the stressor than in mice that had not been stressed.

Plasma corticosterone

The concentration of corticosterone (Figure 2.1, right panel) was higher in the BALB/cByJ than in the C57BL/6ByJ mice, $F(1,83)=18.73$, $p<.001$ and in mice that had been exposed to the social stressor relative to non-stressed mice, $F(1,83)=20.69$, $p<.0001$. As well, corticosterone was dose-dependently increased by the LPS treatment, $F(2,83)=22.07$, $p<.001$. In addition, the effects of the stressor in elevating plasma

corticosterone levels in BALB/cByJ significantly exceeded that evident in C57BL/6ByJ mice (stress×strain interaction, $F(1,83)=4.81$, $p<.05$). The effects of the LPS treatment were also somewhat greater in BALB/cByJ than in C57BL/6ByJ mice, but the interaction between these treatments was shy of significance, $F(1,83)=2.44$, $p=0.09$.

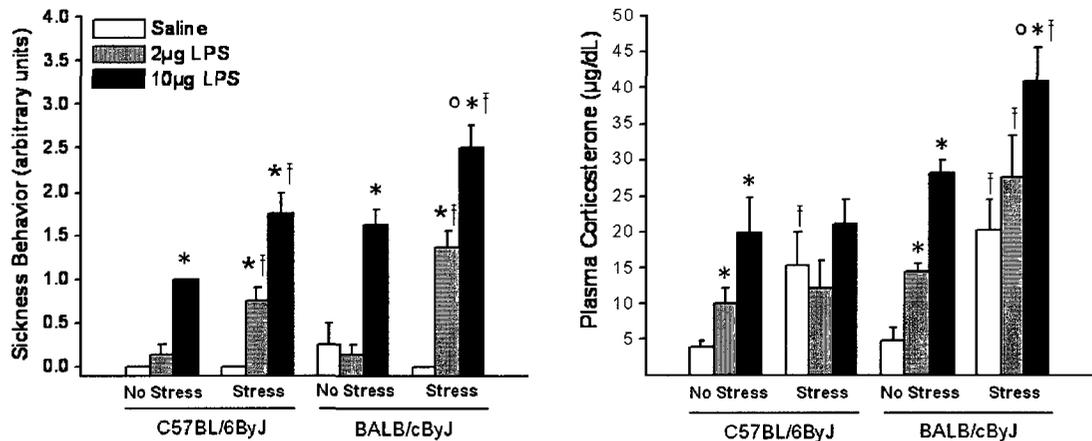


Figure 2.1. Mean (\pm SEM) sickness score (left panel) and plasma corticosterone concentrations (right panel) among non-stressed and stressed C57BL/6ByJ and BALB/cByJ mice that had been treated with LPS (2 or 10µg) or saline. * $p < .05$ relative to saline treated mice. † $p < .05$ relative to similarly treated C57BL/6ByJ mice. ‡ $p < .05$ relative to similarly treated non-stressed mice of the same strain.

Plasma cytokines

The levels of IL-6 varied as a function of the LPS×strain and the LPS×stressor interactions, F 's (4,81 and 2,81)=4.22 and 5.22, p 's=.01 and .05, respectively. The follow-up tests for the simple effects comprising these interactions indicated that the higher dose of LPS markedly increased IL-6 levels, whereas this was not evident following the lower dose. The effect of the higher dose of LPS on circulating IL-6 levels was dramatically enhanced in mice of both strains that had been exposed to the stressor ($p<.001$). Moreover, the effects of LPS were significantly greater in C57BL/6ByJ mice relative to their BALB/cByJ counterparts ($p<.001$) (see Figure 2.2, top panel).

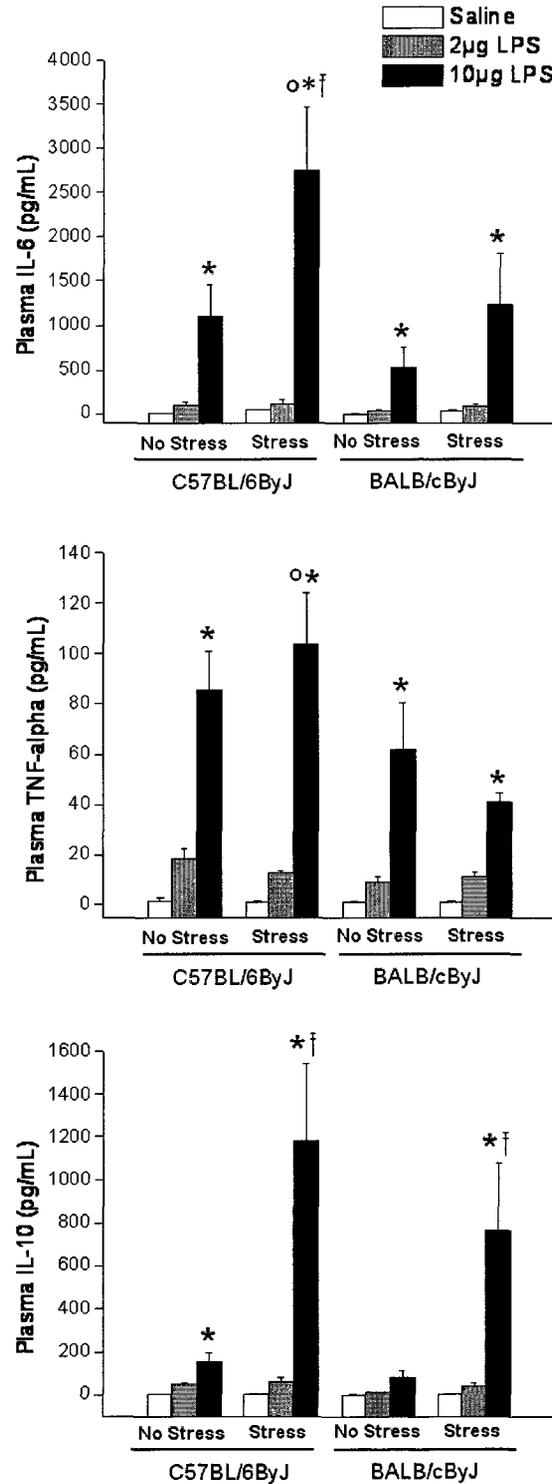


Figure 2.2. Mean (\pm SEM) plasma IL-6 (top panel), TNF- α (middle panel) and IL-10 (bottom panel) among C57BL/6ByJ and BALB/cByJ mice as a function of the stressor condition and LPS treatment (saline, 2 or 10µg), * $p < .05$ relative to saline treated mice, and mice treated with 2µg of LPS. $^o p < .05$ relative to similarly treated BALB/cByJ mice. $^\dagger p < .05$ relative to similarly treated non-stressed mice of the same strain.

As in the case of IL-6, the circulating levels of TNF- α were greater in C57BL/6ByJ than in BALB/cByJ mice, $F(1,81)=9.41$, $p<.01$, and this difference was moderated by the LPS treatment, $F(2,81)=6.26$, $p<.01$. The follow-up tests of the simple effects of this interaction indicated that in the absence of the LPS treatment the levels of TNF- α were very low, often not being detectable in either strain. Following LPS treatment the levels increased markedly, significantly more so in the C57BL/6ByJ than in the BALB/cByJ mice ($p<.01$; Figure 2.2, middle panel). The stressor, however, did not significantly influence TNF- α levels.

The concentrations of the anti-inflammatory cytokine, IL-10, did not differ between the two strains of mice, but varied as a function of the Stress \times LPS treatment interaction, $F(2,81)=12.78$, $p<.001$. As seen in Figure 2.2 (bottom panel), and confirmed by the follow-up tests, in the absence of the stressor, the levels of IL-10 increased moderately in response to the higher dose of LPS. However, in mice that received the LPS treatment following the social stressor a very marked increase of IL-10 levels was apparent, which significantly exceeded that of mice that had been treated with LPS but had not been stressed.

Experiment 2: influence of strain and a social stressor on LPS-induced brain cytokine mRNA expression

Prefrontal cortex

The profile of cytokine mRNA variations in brain elicited by the LPS and stressors could be distinguished from those evident in the blood, although in some respects the treatments yielded similar outcomes. Figure 2.3 presents the fold changes of

mRNA expression in the mPFC for IL-1 β , IL-6 and TNF- α (left hand panels) and that of their receptors (right hand panels) as a function of the strain, the social stressor condition, and the LPS treatment. The mRNA expression of IL-10 was very low and was thus not deemed to be meaningful, and hence these data are not reported.

The analysis of IL-1 β mRNA expression within the prefrontal cortex revealed a significant strain \times LPS treatment interaction, $F(1,42)=5.33$, $p<.01$. Follow-up comparisons indicated that the 2 μ g dose of LPS increased IL-1 β mRNA expression in C57BL/6ByJ ($p<.01$), but not in BALB/cByJ mice. At the 10 μ g dose this strain difference was no longer evident. Furthermore, the analysis revealed that relative to non-stressed mice, the cytokine's expression was diminished among mice that had been exposed to the social stressor, $F(1,42)=3.77$, $p<.05$. Although the interaction with strain was not significant, based on a priori hypotheses regarding the strain \times stress \times LPS interaction, follow-up comparisons were conducted. It appeared that in C57BL/6ByJ mice the effects of the stressor were relatively limited (Figure 2.3, top, left), whereas in BALB/cByJ mice the effects of the higher dose of LPS were diminished in stressed animals relative to similarly treated mice that had not been stressed ($p<.05$).

Overall, IL-6 mRNA expression (calculated as fold changes) was considerably higher in C57BL/6ByJ than in BALB/cByJ mice, $F(1,42)=94.81$, $p<.001$, and the LPS treatment provoked a significant dose dependent increase of IL-6 mRNA expression, $F(2,42)=10.14$, $p<.001$. The effect of LPS on IL-6 expression interacted with the strain and the stressor treatments, $F(2,42)=2.58$, $p<.05$. The follow-up tests for the simple effects of this 3-way interaction confirmed that the greater IL-6 expression in C57BL/6ByJ was further increased by LPS (10 μ g) in the C57BL/6ByJ mice, and this outcome was

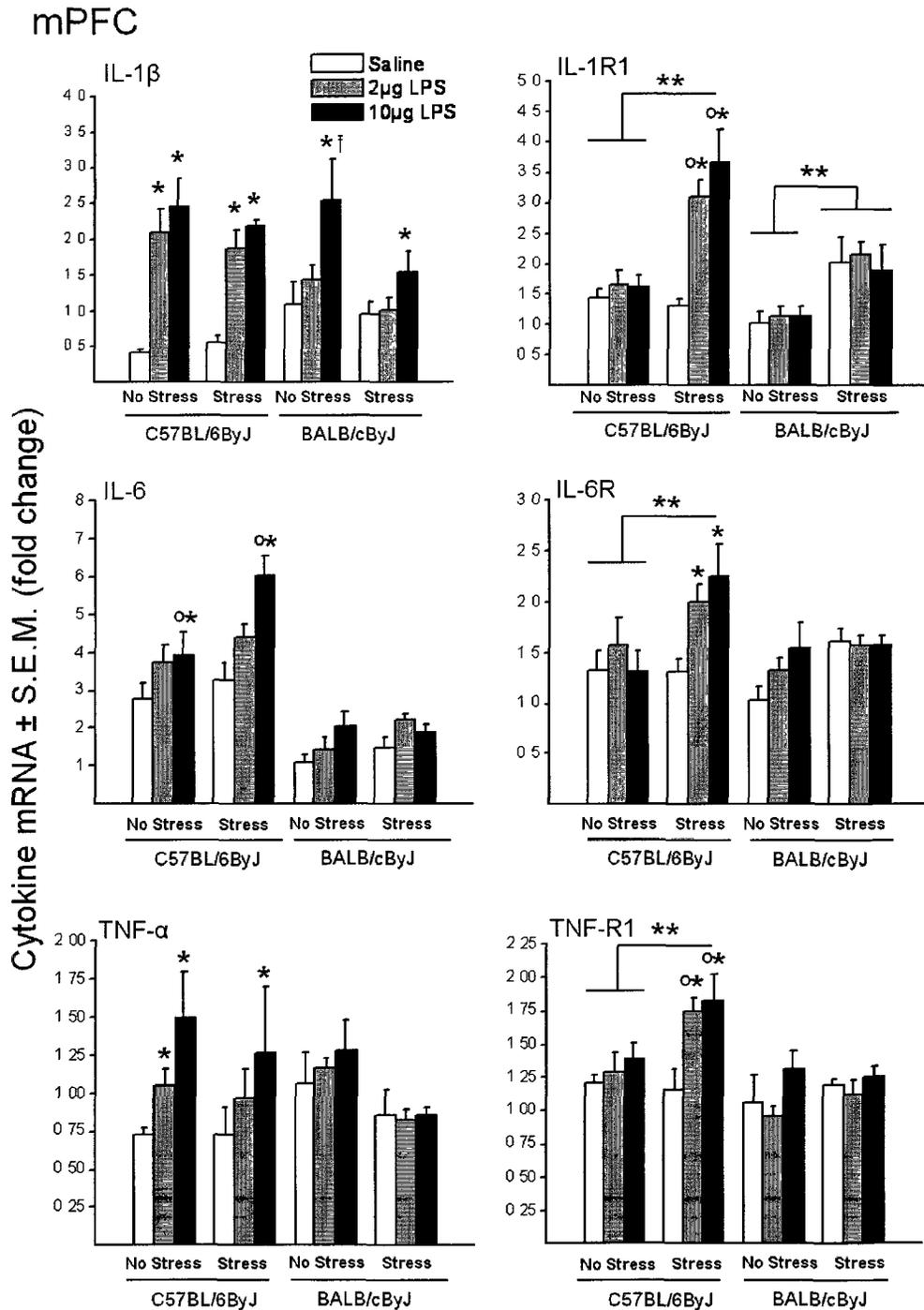


Figure 2.3. Mean (\pm SEM) IL-1 β , IL-6 and TNF- α (left hand panels) and their respective receptors (right hand panels) within the prefrontal cortex of stressed and non-stressed C57BL/6ByJ and BALB/cByJ mice that were treated with either LPS (2 or 10 μ g) or saline. Data are presented as fold changes. * $p < .05$ relative to saline treated mice. ^o $p < .05$ relative to similarly treated BALB/cByJ mice. [†] $p < .05$ relative to similarly treated mice of the same strain. ** $p < .05$ relative to non-stressed mice.

augmented to a still greater extent if these animals had also been stressed. Similarly augmented IL-6 expression did not occur in BALB/cByJ mice in response to either the LPS, the stressor treatment, or their combination (see Figure 2.3, middle, left panel).

The expression of TNF- α was dose-dependently increased by LPS, $F(2,42)=3.20$, $p<.05$. The effects of the LPS treatment did not vary with the strain or stressor treatment, nor did these variables moderate the effects of LPS. Nevertheless, as depicted in Figure 2.3 (lower, left panel), although the increased TNF- α expression was marked in the C57BL/6ByJ, this was definitely not the case in their BALB/cByJ counterparts. However, the low power due to the small N, coupled with the relatively high variability precluded the stress \times strain \times LPS interaction being significant.

Expression of the IL-1R1 transcripts (fold changes are shown at the top, right of Figure 2.3) varied as a function of the strain \times stressor \times LPS treatment interaction, $F(2,42)=4.32$, $p<.05$. The follow-up tests indicated the stressor itself provoked a significant rise of IL-1R1 expression, but only in BALB/cByJ mice. However, in the BALB/cByJ mice LPS did not affect IL-1R1 irrespective of whether a stressor was or was not presented. In the absence of the stressor, LPS had negligible effects on IL-1R1 expression in either strain. However, as depicted in Fig. 3 (right hand panels), in C57BL/6ByJ mice the LPS plus the stressor significantly increased the IL-1R1 mRNA expression, whereas a comparable change of the receptor expression was not apparent in BALB/cByJ mice.

The IL-6R fold changes also varied as a function of the strain \times stressor \times LPS treatment interaction, $F(2,42)=3.37$, $p<.05$. Comparable receptor expression was apparent in the two strains in the absence of any treatment, and neither the stressor nor the LPS treatments alone affected expression. However, as depicted in Figure 2.3, a

significant rise of IL-6R was evident in C57BL/6ByJ mice that had been treated with LPS after exposure to the social stressor, relative to either vehicle treated mice, non-stressed mice that had been treated with LPS, as well as BALB/cByJ mice that had been treated with LPS following the stressor treatment.

Finally, the expression of TNF- α receptors were elevated in C57BL/6ByJ relative to BALB/cByJ mice, $F(1,42)=13.67$, $p<.05$, were increased by LPS, $F(2,42)=4.89$, $p<.05$, as well as being increased by the stressor, $F(1,42)=4.98$, $p<.05$. Although the LPS and cytokine effects were significant, as depicted in Figure 2.3, the magnitude of the effects of these manipulations was actually quite small. In fact, the additive effects of the treatments were only notable in C57BL/6ByJ mice ($p<.05$) and the effects of the stress and LPS treatments and their combination in the BALB/cByJ mice were less than 0.25 cycles.

Hippocampus

Cytokine expression within the hippocampus, as in the PFC, was generally affected by factors such as strain, stress and LPS treatment; however, in several respects these effects were very different from those apparent in the PFC. The mRNA fold changes for IL-1 β within the hippocampus varied as a function of the strain \times stress \times LPS interaction, $F(2,41)=3.29$, $p<.05$. The follow-up comparisons indicated that LPS treatment increased mRNA expression of IL-1 β in both strains. Although expression of this cytokine was lower in C57BL/6ByJ than in BALB/cByJ mice in the absence of LPS treatment, following the endotoxin treatment the levels of the cytokine's expression was higher in the C57BL/6ByJ strain. As in the PFC, the increased expression of the cytokine

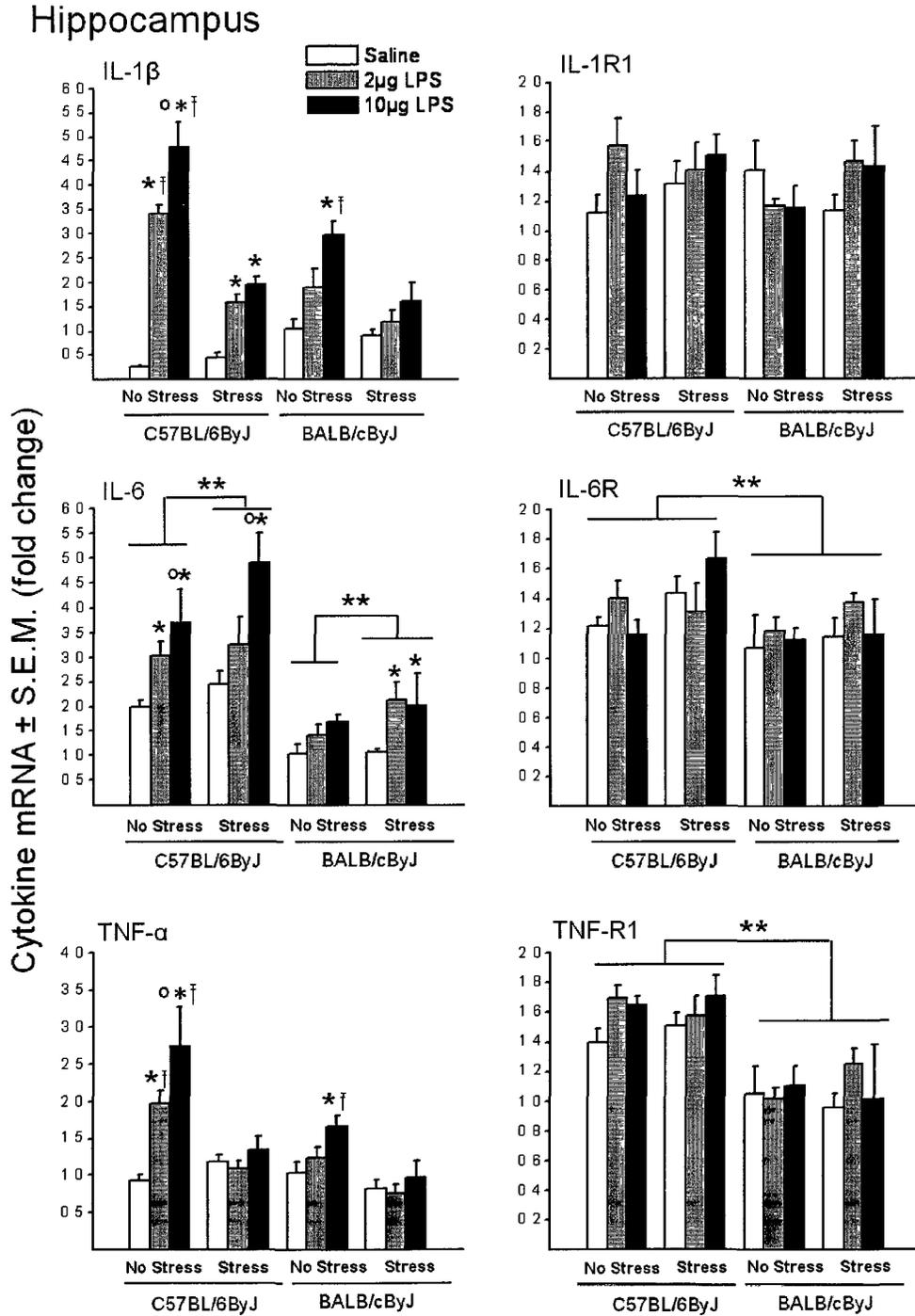


Figure 2.4. Mean (± SEM) IL-1β, IL-6 and TNF-α (left hand panels) and their respective receptors (right hand panels) within the hippocampus of stressed and non-stressed C57BL/6ByJ and BALB/cByJ mice that were treated with either LPS (2 or 10µg) or saline. Data are presented as fold changes. *p < .05 relative to saline treated mice. ^op < .05 relative to similarly treated BALB/cByJ mice. †p < .05 relative to similarly treated stressed mice of the same strain. **p < .05 relative to non-stressed mice.

elicited by LPS was reduced by the stressor, and the extent of the reduction was greater in C57BL/6ByJ than in BALB/cByJ mice (Figure 2.4, top).

The expression of TNF- α was elevated in C57BL/6ByJ relative to BALB/cByJ mice, $F(1,42)=12.53$, $p<.001$. Moreover, the expression of TNF- α varied as a function of the LPS \times stressor condition, $F(1,42)=5.79$, $p<.01$. The follow-up comparisons indicated that LPS alone increased the expression of TNF- α within the hippocampus, but this outcome was eliminated by the stressor treatment.

The hippocampal expression of IL-6 was greater in C57BL/6ByJ than in BALB/cByJ mice, $F(1,42)=46.93$, $p<.001$, but unlike the effects evident with respect to IL-1 β and TNF- α , the expression of IL-6 was increased by both the stressor, $F(1,42)=4.11$, $p<.05$, and by the LPS treatment, $F(1,42)=11.60$, $p<.001$. These data, as depicted in Figure 2.4 (middle, left panel), show that the various conditions additively influenced IL-6 expression, so that the already elevated levels of IL-6 were significantly more pronounced after the stressor plus LPS treatments.

The variations of the IL-1 receptor did not coincide with that of IL-1 β itself. Specifically, although a small increase of IL-1R1 was elicited by LPS, this effect did not reach significance ($p=.08$). Moreover, unlike the effects in the PFC, the stressor did not influence hippocampal IL-1R1, nor did the effects of the stressor interact with the strain or LPS treatments in affecting receptor expression (data not shown). In contrast, to these findings (as well as effects evident within the PFC), the expression of IL-6R did not differ as a function of any of the variables examined. Finally, the expression of the TNF- α receptor was also found to be greater in C57BL/6ByJ than in BALB/cByJ mice, F

(1,42)=47.33, $p<.001$. However, the effects of LPS and the stressor on this receptor's expression were limited and did not interact with the strain of mouse.

To facilitate comprehension of the wide ranging effects of LPS as a function of the various treatments, Table 2.1 presents a summary of the effects of the social stressor, LPS and the combination of LPS and the social stressor on sickness behaviours, plasma corticosterone and cytokines, as well as PFC and hippocampal mRNA expression of various cytokines. Treatment with LPS influenced both strains of mice, but for some outcome measures, such as LPS-induced changes of sickness and corticosterone, they were more pronounced in BALB/cByJ mice than in the C57BL/6ByJ. As well, the sickness and corticosterone reactions were further enhanced in the BALB/cByJ mice when LPS was administered on a stressor backdrop. In other respects, however, such as circulating cytokine levels as well as cytokine mRNA expression, greater effects were apparent in C57BL/6ByJ mice than in similarly treated BALB/cByJ mice. Finally, whereas stressors augmented the actions of LPS and on PFC cytokine expression and hippocampal IL-6 expression were increased, the stressor treatment attenuated the effects of LPS on hippocampal IL-1 β and TNF- α mRNA expression. We make particular note of the fact that in this experiment tissue samples were collected at a single time point following the treatments. Yet, the peak effects of different cytokines in blood differ from one another, and it is equally possible that brain mRNA changes for the cytokines are differentially expressed in relation to the time following treatment. Thus, the data represented in Table 2.1 need to be considered with this caveat in mind.

Table 2.1
Summary of LPS and social stressor effects in two strains of mice

	C57BL/ByJ				BALB/cByJ		
	Stress	LPS	Stress + LPS		Stress	LPS	Stress + LPS
Sickness	-	↑↑	↑↑↑	<	-	↑↑	↑↑↑
Corticosterone	↑↑	↑↑	↑↑	<	↑↑	↑↑	↑↑
Plasma IL-6	-	↑↑	↑↑↑	>	-	↑↑	↑↑↑
Plasma TNF- α	-	↑↑	↑↑	>	-	↑↑	↑↑
Plasma IL-10	-	↑↑	↑↑↑	=	-	↑↑	↑↑↑
PFC IL-1 β	-	↑↑	↑↑	>	-	↑↑	-
PFC IL-6	-	↑↑	↑↑↑	>	-	-	-
PFC TNF- α	-	↑↑	↑↑	=	-	-	-
Hippocampus IL-1 β	-	↑↑	↑	>	-	↑↑	-
Hippocampus IL-6	-	↑↑	↑↑↑	>	-	-	↑↑↑
Hippocampus TNF- α	-	↑↑	-	>	-	↑↑	-

↑↑ increase due to stress or LPS reflected by significant main effect. ↑↑↑ synergistic increase associated with combined LPS plus stressor treatment reflected by a significant interaction between the treatment variables. ↑ increase in response to LPS plus the stressor, but less than elicited by LPS alone (i.e. desensitization effect reflected by a significant treatment interaction). - No change relative to untreated animals. > or < significantly larger effect of LPS in C57BL/6ByJ compared to BALB/cByJ mice.

Experiment 3: effects of poly I:C and social disturbance on sickness behaviours, plasma corticosterone and plasma cytokine levels, and brain cytokine mRNA expression

Sickness and plasma corticosterone

Sickness behaviour associated with poly I:C could be differentiated from the effects of LPS described earlier. Although sickness was elicited by poly I:C, $F(1,40)=59.63$, $p<.001$, and was more pronounced in BALB/cByJ than in C57BL/6ByJ mice, $F(1,40)=39.78$, $p<.001$, as depicted in Figure 2.5 (left panel) the stressor treatment did not influence sickness elicited by poly I:C.

The differentiation between the effects of LPS and poly I:C was also evident with respect to the plasma corticosterone changes. The level of plasma corticosterone varied as

a function of the strain×poly I:C treatment, $F(1,38)=6.77$, $p<.01$. The follow-up tests indicated that poly I:C produced a significant increase of corticosterone in BALB/cByJ mice (see Figure 2.5), whereas in C57BL/6ByJ mice the magnitude of the effect was less clearly pronounced ($p<.05$). In neither strain was the stressor found to enhance the effects of poly I:C.

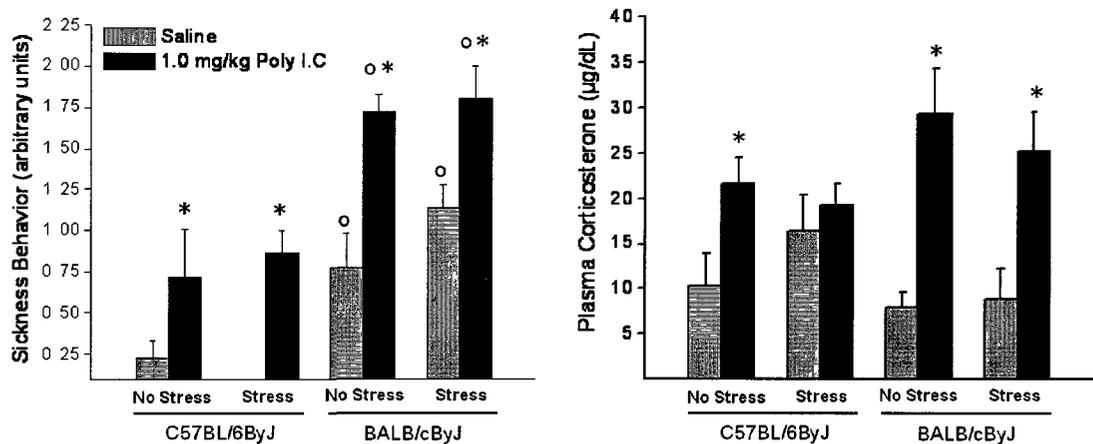


Figure 2.5. Mean (\pm SEM) sickness score (left panel) and plasma corticosterone concentrations (right panel) among non-stressed and stressed C57BL/6ByJ and BALB/cByJ mice that had been treated with Poly I:C or saline. * $p < .05$ relative to saline treated mice. ^o $p < .05$ relative to similarly treated C57BL/6ByJ mice. ^{*} $p < .05$ relative to mice of the same strain.

Plasma cytokine variations

The different effects of poly I:C relative to the effects of LPS were also apparent with regard to the changes of plasma cytokines associated with these treatments. Figure 2.6 depicts the plasma cytokine changes associated with the poly I:C treatments in the two strains of mice as a function of the stressor treatments. Whereas the levels of IL-6 were not affected by the stressor, the levels of this cytokine varied as a function of the strain×poly I:C treatment, $F(1,40)=9.60$, $p<.01$. The follow-up comparisons confirmed that poly I:C increased circulating IL-6 in C57BL/6ByJ mice ($p<.01$), but had no such

effect in the BALB/cByJ strain. Furthermore, unlike the effect seen in relation to LPS, the stressor did not significantly affect the plasma IL-6 levels changes elicited by poly I:C.

The levels of TNF- α also varied as a function of the strain \times poly I:C as well as the stressor \times poly I:C interactions, F 's (1,40)=6.05, 7.84 p 's<.02 and .01, respectively. The strain \times stressor \times poly I:C interaction did not reach statistical significance. Once again, however, because a priori predictions had been made concerning the differential effects of the treatment in the two strains, follow-up comparisons were conducted of the simple effects comprising this interaction. As seen in Figure 2.6, and confirmed by follow-up tests, poly I:C increased TNF- α in both strains. In the BALB/cByJ mice the TNF- α levels were comparable irrespective of the stressor condition. In contrast, in C57BL/6ByJ mice a marked elevation of TNF- α was apparent in non-stressed mice treated with poly I:C, but this outcome was very much reduced by exposure to the social stressor (p <.05).

The levels of the anti-inflammatory cytokine IL-10 varied as a function of the strain \times stressor \times poly I:C treatment interaction, F (1,40)=10.02, p =.01. In BALB/cByJ mice poly I:C did not influence the levels of IL-10 regardless of whether or not mice had been stressed. However, in C57BL/6ByJ mice a pronounced increase of IL-10 was apparent following poly I:C treatment. As seen in Figure 2.6 (bottom), the effects of poly I:C were apparent in C57BL/6ByJ mice irrespective of whether they had experienced the social stressor. However, among those mice that had been stressed the poly I:C effects on this cytokine were exceptionally variable. The effects of the stressor on IL-10 changes induced by poly I:C contrasts with the effects of the stressor in LPS-treated mice of Experiment 1 where the increased levels of IL-10 were markedly exaggerated.

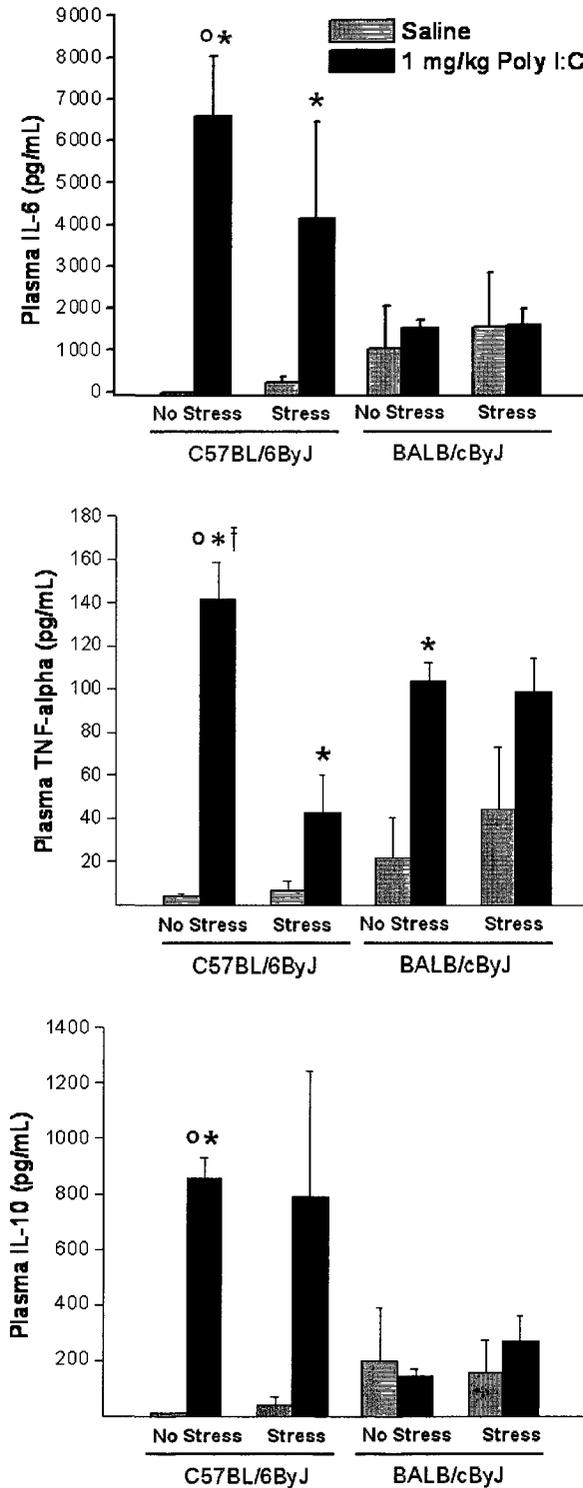


Figure 2.6. Mean (\pm SEM) plasma IL-6 (top panel), TNF- α (middle panel) and IL-10 (bottom panel) among C57BL/6ByJ and BALB/cByJ mice as a function of the stressor condition and Poly I:C treatment. * $p < .05$ relative to saline treated mice. ^o $p < .05$ relative to similarly treated BALB/cByJ mice. [†] $p < .05$ relative to similarly treated stressed mice of the same strain.

Cytokine mRNA expression in brain

Prefrontal cortex

As depicted in Figure 2.7 (top, left), within the PFC the poly I:C treatment increased the mRNA expression of IL-1 β , $F(1,38)=63.95$, $p<.001$, which was also higher in BALB/cByJ than in C57BL/6ByJ mice, $F(1,38)=32.25$, $p<.001$. The stressor treatment, however, did not influence IL-1 β mRNA expression, nor did it interact with the effects of the strain of mouse.

The expression of IL-6 varied as a function of the strain \times stressor \times poly I:C treatment, $F(1,36)=4.54$, $p<.05$. The follow-up comparisons confirmed that in the absence of a stressor poly I:C increased IL-6 expression among C57BL/6ByJ mice, whereas a comparable outcome was not apparent in BALB/cByJ mice. However, whereas the stressor did not increase the effect of poly I:C in the C57BL/6ByJ strain, it markedly increased the effect of poly I:C in BALB/cByJ mice.

The profile of TNF- α mRNA expression was very much like that of IL-6, with the mRNA expression of this cytokine varying as a function of the strain \times stress \times poly I:C treatment, $F(1,37)=3.82$, $p<.05$. As in the case of IL-6, the expression of TNF- α was increased in C57BL/6ByJ mice, but less so in those animals that had been stressed. Overall, the expression of the cytokine was greater in the BALB/cByJ mice and was increased still further by poly I:C in stressed mice ($p<.05$), even though poly I:C itself did not increase the expression of this cytokine in mice that had not been stressed.

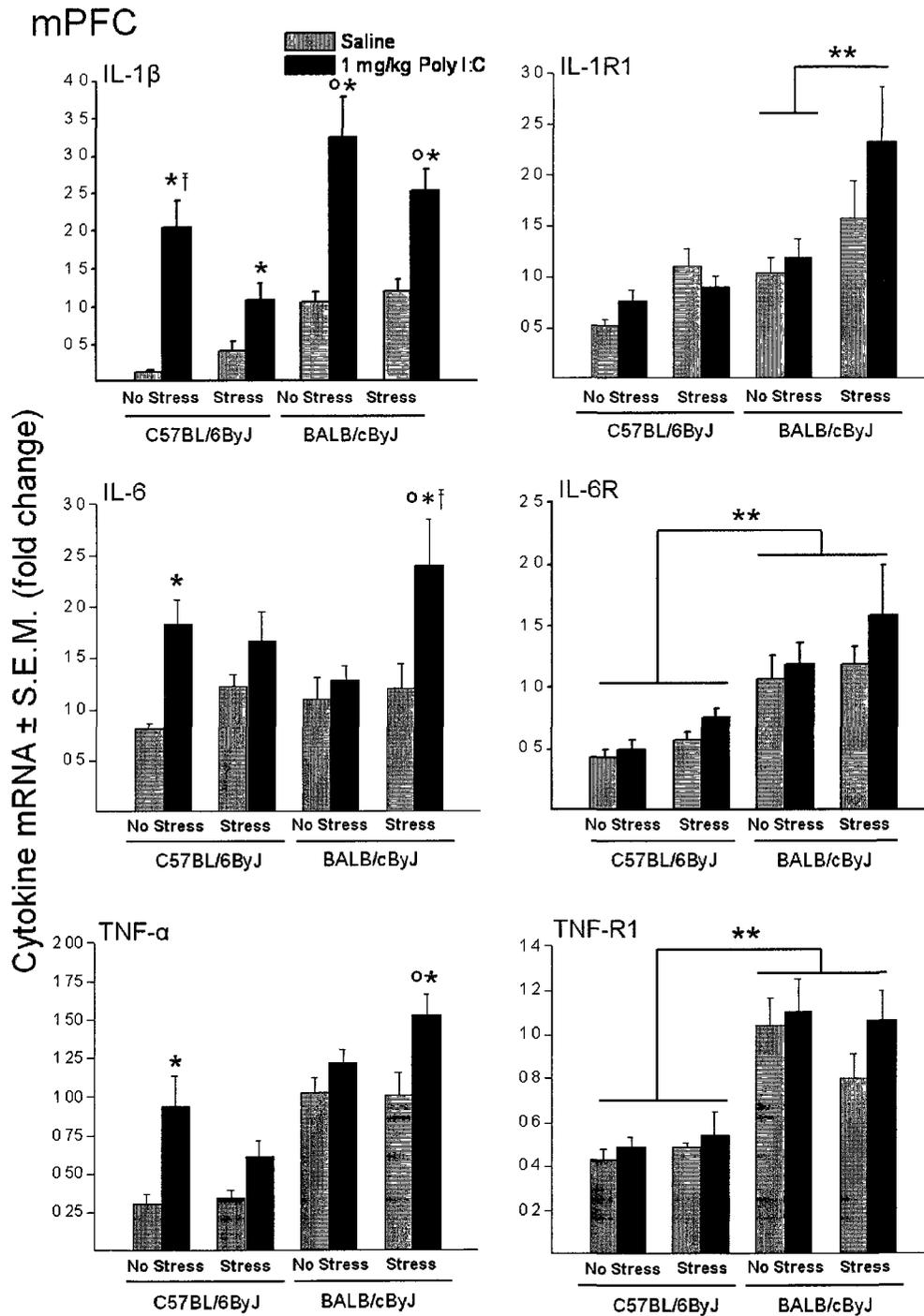


Figure 2.7. Mean (\pm SEM) IL-1 β , IL-6 and TNF- α (left hand panels) and their respective receptors (right hand panels) within the prefrontal cortex of stressed and non-stressed C57BL/6ByJ and BALB/cByJ mice that were treated with either Poly I:C or saline. Data are presented as fold changes. * $p < .05$ relative to saline treated mice. [†] $p < .05$ relative to similarly treated BALB/cByJ mice. ^{††} $p < .05$ relative to similarly treated mice of the same strain. ^{**} $p < .05$ relative to non-stressed mice.

The mRNA expression of the receptors for IL-1, IL-6 and TNF- α was higher in BALB/cByJ than in C57BL/6ByJ mice, F's (1,38)=14.69, 26.73, and 48.91, p's<.001. As well, the stressor increased IL-1R mRNA expression, F (1,38)=10.30, p<.002, and to a marginal extent also increased IL-6R expression, F (1,38)=2.91, p=.09, but had no effect whatever on TNF- α receptor mRNA expression. Treatment with poly I:C did not affect expression of any of the receptors.

Hippocampus

Only some of the effects elicited by the stressor and poly I:C treatments in the PFC were recapitulated in the hippocampus. Specifically, poly I:C increased IL-1 β , IL-6 and TNF- α in both strains, F's (1,38)=7.34, 6.35 and 5.17, p's<.02, and the stressor treatment reduced the effect on IL-1 β and IL-6 in BALB/cByJ, but such an outcome was not evident in C57BL/6ByJ mice, F's (1,38)=5.10 and 9.07, p<.05. Moreover, as depicted in Figure 2.8, the effects of the treatments on TNF- α were relatively variable and the effects of the treatments on mRNA expression of this cytokine was not as pronounced as those of the other cytokines. Still it seemed that among C57BL/6ByJ mice the increased TNF- α mRNA expression provoked by poly I:C was prevented if mice had been stressed. In contrast, poly I:C. itself had little effect in BALB/cByJ mice in the absence of stressor, but expression of this cytokine increased in LPS-treated mice that had been treated with a stressor (p<.05).

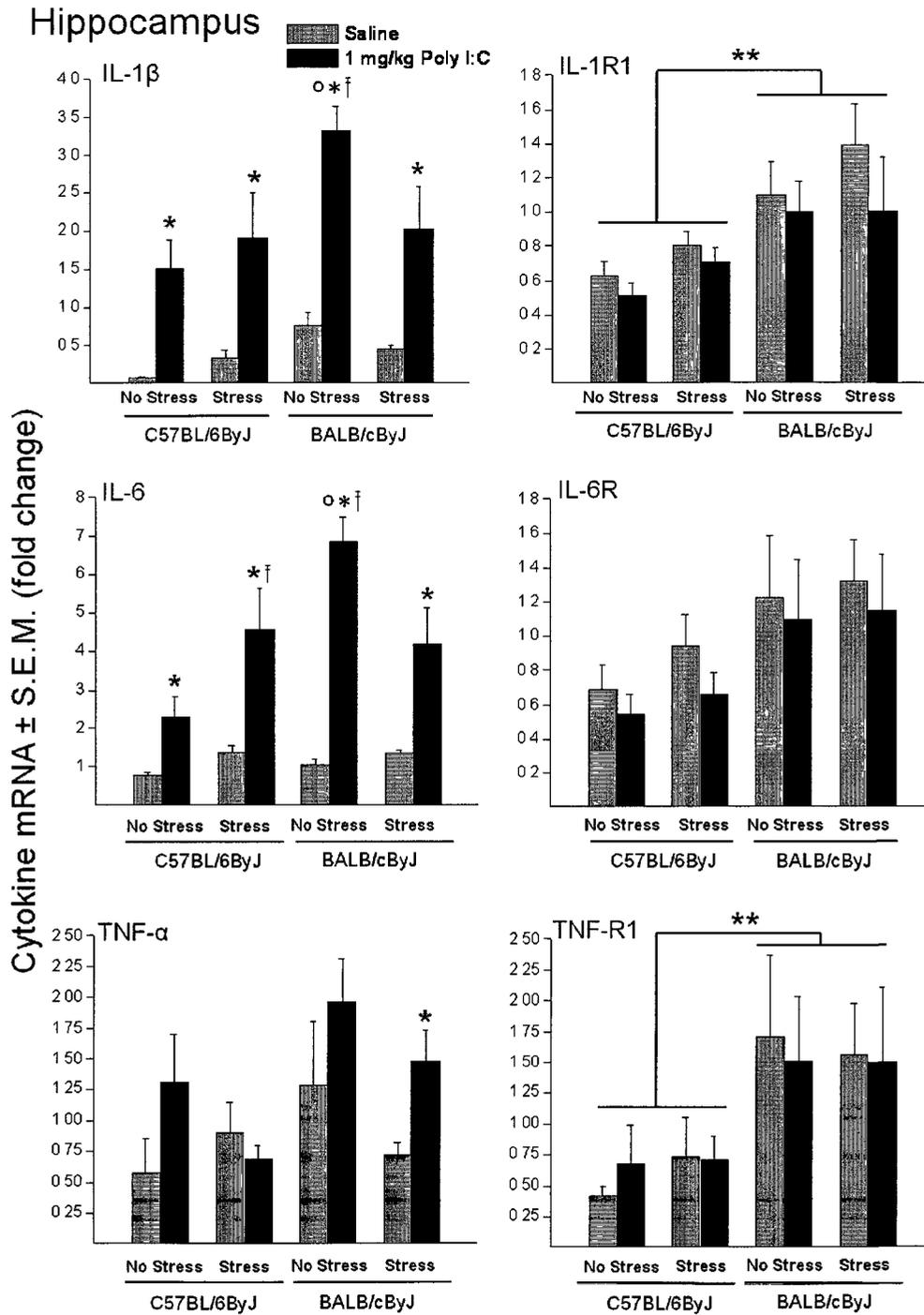


Figure 2.8. Mean (\pm SEM) IL-1 β , IL-6 and TNF- α (left hand panels) and their respective receptors (right hand panels) within the hippocampus of stressed and non-stressed C57BL/6ByJ and BALB/cByJ mice that were treated with either Poly I:C or saline. Data are presented as fold changes. * p < .05 relative to saline treated mice. ^o p < .05 relative to similarly treated BALB/cByJ mice. [†] p < .05 relative to similarly treated stressed mice of the same strain. ** p < .05 relative to non-stressed mice.

Analysis of the receptor mRNA expression of these cytokines indicated that IL-1R, IL-6R and TNF- α R expression was greater in BALB/cByJ than C57BL/6ByJ mice, F 's (1,38)=12.48, 7.08, and 8.46, p 's<.01. Neither poly I:C nor the stressor treatment, influenced the cytokine receptor expression.

In view of the complexity of the data set, Table 2.2 is meant to provide a broad summary of the effects of poly I:C on biological and behavioural outcomes in the two strains of mice. As seen in this table, poly I:C produced a small rise of sickness behaviours that was not enhanced by the stressor, and poly I:C also increased plasma corticosterone that was somewhat limited in the C57BL/6ByJ mice that had been exposed to the stressor. In the main, the synergistic action of the stressor treatment and LPS depicted earlier (in Table 2.1), was not evident when poly I:C was administered on the stressor background.

The strains of mice differed markedly in their cytokine responses to poly I:C. Circulating IL-6 and TNF- α , as well as IL-10 was increased by poly I:C among C57BL/6ByJ mice, but had modest effects in BALB/cByJ mice, as only TNF- α was found to increase. Finally, as in the response to LPS treatment, administration of poly I:C increased cytokine mRNA expression in the PFC, but these effects were modestly reduced by the stressor in C57BL/6ByJ mice, whereas the effects on IL-6 and TNF- α were only evident in BALB/cByJ mice that had been stressed. Within the hippocampus, poly I:C alone elicited greater effects on IL-1 β and IL-6 in BALB/cByJ than in C57BL/6ByJ mice, but the effects in BALB/cByJ mice were reduced by the stressor.

Table 2.2
Summary of Poly I:C and social stressor effects in two strains of mice

	C57BL/ByJ				BALB/cByJ		
	Stress	Poly I:C	Stress + Poly I:C		Stress	Poly I:C	Stress + Poly I:C
Sickness	-	↑↑	↑↑	<	-	↑↑	↑↑
Corticosterone	-	↑↑	-	<	-	↑↑	↑↑
Plasma IL-6	-	↑↑	↑↑	>	-	-	-
Plasma TNF- α	-	↑↑	↑	=	-	↑↑	↑↑
Plasma IL-10	-	↑↑	↑↑	>	-	-	-
PFC IL-1 β	-	↑↑	↑↑	<	-	↑↑	↑↑
PFC IL-6	-	↑↑	-	=	-	-	↑↑↑
PFC TNF- α	-	↑↑	↑	<	-	-	↑↑↑
Hippocampus IL-1 β	-	↑↑	↑↑	<	-	↑↑	↑
Hippocampus IL-6	-	↑↑	↑↑↑	<	-	↑↑	↑
Hippocampus TNF- α	-	↑↑	-	<	-	-	↑↑

↑↑ increase due to stress or poly I:C reflected by significant main effect. ↑↑↑ synergistic increase associated with combined poly I:C plus stressor treatment reflected by a significant interaction between the treatment variables. ↑ increase in response to poly I:C plus the stressor, but less than elicited by poly I:C alone (i.e. desensitization effect reflected by a significant treatment interaction). - No change relative to untreated animals. > or < significantly larger effect of poly I:C in C57BL/6ByJ compared to BALB/cByJ mice.

Discussion

Social disturbances act as profound stressors that, among other things, influence circulating and brain cytokine activity (Audet et al., 2010; Bartolomucci et al., 2001, 2003; Bailey et al., 2007; Stefanski, 2001) and augment the impact of immunological challenges on neurobiological processes (i.e. Audet et al., 2010; Avitsur et al., 2001, 2005; Gibb et al., 2008; Merlot et al., 2003; Quan et al., 2001). For instance, among non-inbred mice, LPS (as well as poly I:C and interferon- α) administered on the backdrop of a psychosocial stressor (disruption of the social hierarchy) markedly increased sickness behaviours and circulating corticosterone and cytokine levels (Anisman et al., 2007a; Gandhi et al., 2007; Gibb et al., 2008). In view of the pronounced inter-individual variability frequently associated with stressors (Anisman et al., 2008b), the present investigation assessed the impact of a psychosocial stressor on behavioural and cytokine responses in strains of mice that have been reported to be differentially reactive to stressors. To this end, we assessed the actions of different immunogenic challenges (LPS and poly I:C), taking into consideration that C57BL/6ByJ are particularly Th1 responsive, whereas BALB/cByJ mice are more TH2 responsive. A direct comparison between the effects of LPS, which primarily affects macrophages and B cells and poly I:C, which primarily affects Th1 (Manetti et al., 1995; Pruett et al., 2003; Town et al., 2006) is unwarranted given that dose response curves were not performed. Our intent instead was to assess whether the effects of stressors would differentially influence the impact of these challenges in these strains of mice.

As frequently reported (Dunn and Vickers, 1994; Ellis et al., 2006; Fortier et al., 2004; Gandhi et al., 2007; Gibb et al., 2008; Katafuchi et al., 2003), poly I:C and LPS

increased circulating pro-inflammatory cytokine levels, as well as plasma corticosterone concentration. Although the effects of poly I:C on plasma corticosterone and sickness behaviours, at least at the doses used, were less pronounced than those of LPS, the effects of both treatments were greater in BALB/cByJ than in C57BL/6ByJ mice. Moreover, LPS, but not poly I:C, synergistically augmented the impact of the social stressor upon sickness behaviour and plasma corticosterone levels. In effect, it seems that stressors may be acting on processes that are unique to those activated by LPS, and not poly I:C.

It has been reported that both stressors and LPS increase circulating pro-inflammatory cytokine levels (Dantzer, 2001; Goujon et al., 1995; LeMay et al., 1990; Johnson et al., 2002; Pugh et al., 1999), and that the combination of these treatments had additive or synergistic effects (Gibb et al., 2008). In the present investigation, the profile of circulating cytokine levels varied with the stressor conditions and was very different from that evident with respect to corticosterone or sickness behaviours. Although the stressor augmented the effects of LPS on circulating IL-6 and IL-10 levels in both strains, this outcome appeared more robust in C57BL/6ByJ mice even though the stress response (corticosterone and anxiety) in this strain is generally less pronounced than in BALB/cByJ mice. In contrast, no such effect was observed for TNF- α , and indeed, the stressor reduced the circulating levels of this cytokine in BALB/cByJ mice. At this point, it is uncertain why LPS and stressor exposure differentially influenced circulating IL-6 and TNF- α . However, it is notable that production of TNF- α elicited by LPS occurs earlier than that of IL-6 (Givalois et al., 1994), and it is possible that temporal changes associated with the treatments might favor detection of IL-6 changes.

As expected, given that C57BL/6ByJ are generally more Th1 responsive, poly I:C provoked greater effects on IL-6, TNF- α and IL-10 in this strain than in BALB/cByJ mice. In fact, in BALB/cByJ mice only plasma TNF- α levels were at all increased by poly I:C. Moreover, in marked contrast to the stressor augmentation of the LPS effects, the psychosocial stressor actually limited the effects of poly I:C on TNF- α and IL-10, without affecting IL-6 levels. It will be recalled that LPS acts through activation of a toll-like receptor (TLR)-4-dependent pathway, whereas poly I:C induces its effects by stimulating TLR-3 receptors (Pruett et al., 2003; Town et al., 2006). As alluded to earlier, it may be that stressors differentially influence these TLR pathways leading to varied interactions with the two immune challenges.

Beyond their peripheral actions, stressors and immune challenges may influence central cytokine mRNA expression or protein levels (Gibb et al., 2008; Nguyen et al., 1998). In this regard, it appeared that the influence of LPS on central cytokine expression differed appreciably from its peripheral actions, and it also appeared that the observed effects varied between the PFC and hippocampus. As previously observed (Datta and Opp, 2008; Staikos et al., 2008; Teeling et al., 2007; Turrin et al., 2001), LPS increased IL-1 β , IL-6 and TNF- α mRNA expression in both the PFC and the hippocampus, but as summarized in Table 2.1, these effects were significantly greater in C57BL/cByJ than in BALB/cByJ mice. Within the PFC and hippocampus, the stressor enhanced the LPS provoked IL-6 mRNA expression in C57BL/6ByJ mice, and to a lesser extent this was also the case in the BALB/cByJ strain. However, as we previously observed in stressed CD-1 mice (Gibb et al., 2008), the impact of LPS on hippocampal IL-1 β and TNF- α was curtailed when administered in the context of the stressor. Furthermore, IL-6R, IL-1R1

and TNF-R1 mRNA expression within the PFC was increased by LPS, primarily among C57BL/cByJ mice; however, these receptor changes were not apparent within the hippocampus.

Like LPS, poly I:C increased the central mRNA expression of IL-1 β , TNF- α , and IL-6. However, whereas the effects of LPS, particularly within the hippocampus, were greater in C57BL/6ByJ than in BALB/cByJ mice, the opposite profile was apparent in response to poly I:C. Furthermore, whereas some of the effects of the LPS were enhanced in the context of stressor exposure, the effects of poly I:C on brain cytokine expression were frequently reduced by the stressor experience, particularly in the PFC of C57BL/6ByJ mice and the hippocampus of BALB/cByJ mice.

It is possible that the suppression of IL-1 β and TNF- α observed with combined LPS and stressor exposure stemmed from the appreciably elevated circulating corticosterone levels elicited by these conjoint treatments (Nadeau and Rivest, 2003; Barnes, 1998). Indeed, given the relatively high density of glucocorticoid receptors within the hippocampus, variations of cytokine functioning within this region might be expected. However, this explanation does not explain why the central cytokine changes elicited by the stressor plus LPS treatment were more pronounced in C57BL/6ByJ mice, especially as the effects on corticosterone were markedly greater in the BALB/cByJ strain. Furthermore, this would not account for why the effects of LPS on IL-1 β and TNF- α gene expression were diminished by the stressor, whereas the actions on IL-6 were actually enhanced. Other negative feedback processes exist that could affect central cytokine production, such as “suppressors of cytokine signaling” (SOCS) that operate through JAK/STAT pathways (Planas et al., 2006; Wang and Campbell, 2002), but

sufficient data are not available concerning the conjoint actions of stressors and immune challenges on SOCS3 production.

It may be significant that IL-6, unlike IL-1 β and TNF- α , can act as both an inflammatory cytokine and as an anti-inflammatory (e.g., Yasukawa et al., 2003) and might thus be uniquely affected by combined exposure to different classes of insults. Moreover, it is notable that while IL-1 β and TNF- α predominately act by liberating the intracellular transcription factor NF κ B, IL-6 signaling is largely mediated by the JAK-STAT pathway. Interestingly, NF κ B mediated signaling following TLR activation was reported to be influenced by psychosocial stressors (Bailey et al., 2007), raising the possibility that this signaling pathway might be especially sensitive to the impact of immune and psychological challenges. Along these lines, the combined LPS and stressor exposure could promote concomitant activation of multiple NF κ B and STAT pathways, which could then additively, synergistically, or even antagonistically interact to modulate functional outcomes. Indeed, it has been suggested that these pathways play a pivotal role in depressive symptoms that arise in response to inflammatory signals (e.g., Pace and Miller, 2009).

Inasmuch as sickness behaviours are provoked by direct or indirect central actions of cytokines (Konsman et al., 2002), it might seem incongruous that the stressor augmented sickness elicited by LPS, but limited central IL-1 β and TNF- α mRNA expression provoked by the endotoxin. It is possible that the stressor-provoked enhancement of sickness associated with LPS treatment is related to stressors increasing blood brain barrier permeability (Skultéyová et al., 1998), thereby permitting greater cytokine entry into the brain. Further, peripheral LPS administration may stimulate

cytokines release from endothelial cells, thereby increasing their central appearance without an increase of de novo synthesis (Verma et al., 2006). Of course, in the present investigation we only assessed cytokine mRNA expression in the PFC and hippocampus, and the sickness behaviours may be more aligned with cytokine changes in other brain regions, such as the hypothalamus.

Conclusion

Following an inflammatory challenge the temporal changes of IL-1 β , IL-6 and TNF- α may be distinct from one another (Bobrowski et al., 2005). Thus, the use of a single time point used in the present investigation might not have reflected peak variations in some of the cytokines. Accordingly, the differences observed across cytokines with respect to the presence or absence of an additive or synergistic effect between a stressor and LPS treatment might reflect the time of assessment.

It was clear that LPS and a psychosocial stressor had additive or synergistic effects on sickness behaviours, plasma corticosterone and IL-6, TNF- α and IL-10, but different effects on central cytokine mRNA expression, depending on the brain region examined. Moreover, these effects were strain dependent. Whereas the effects of the treatments on sickness and on corticosterone were more pronounced in BALB/cByJ mice, the effects on circulating cytokines and on central cytokine mRNA expression were more pronounced in the C57BL/6ByJ strain. Thus, the greater stressor reactivity of BALB/cByJ mice (with regards to behaviour and corticosterone), and their presumed vulnerability to psychological disturbances in response to stressors, might not translate to greater cytokine alterations in this strain. Nevertheless, the differential effects of stressors

and immune challenges on central and peripheral cytokine processes in these strains of mice offers the opportunity to assess their respective contributions to psychopathological outcomes (e.g., depressive illness) that have been linked to activation of inflammatory processes.

CHAPTER 3: The effects of minocycline pretreatment on endotoxin-induced behavioural, neurochemical and central cytokine alterations

It has been demonstrated thus far that stressors and immunogenic agents can synergistically increase sickness behaviour, circulating corticosterone and cytokines and central cytokine mRNA expression. In Chapter 1, we demonstrated that this synergy was dependent upon the chronicity of the stressor, and the profile of responses varied over time following the treatments. In addition, Chapter 2 indicated that the interaction between stressors and immunogenic agents is also moderated by the stressor reactivity and the underlying immune characteristics of the mouse. As activation of the immune system appears to be related to the development of behavioural disturbances, in Chapter 3, we assessed whether inhibiting the inflammatory immune response with the antibiotic minocycline would attenuate the behavioural disturbances, elevations of circulating corticosterone and central cytokine mRNA expression provoked by a bacterial endotoxin.

Abstract

Neuroinflammation has been implicated as a possible instigator of psychiatric illnesses, such as major depression and anxiety disorders. It has been demonstrated that immune activation and the subsequent release of cytokines, signaling molecules of the immune system, promote several brain neurochemical changes, which may then favor the evolution of behavioural disturbances. In the present investigation, we assessed whether the behavioural and neurochemical alterations normally provoked by an immunogenic challenge could be precluded by pretreatment with an anti-inflammatory agent, namely the semi-synthetic second generation tetracycline minocycline. To this end, in a first study using male and female CD-1 mice, we assessed whether pretreatment with minocycline could attenuate lipopolysaccharide- (LPS)-induced locomotor and exploratory disturbances in an open field; behaviours thought to be associated with anxiety-like behaviour. In a second study, we assessed whether pretreatment could also attenuate LPS-induced sickness behaviour, and elevations of circulating corticosterone and central cytokine (IL-1 β , IL-6 and TNF- α) mRNA expression in the medial prefrontal cortex and hippocampus, brain regions involved in the development of affective disorders. Together, the experiments indicated that minocycline abrogated the LPS-induced central cytokine mRNA expression, as well as the anxiety-like behaviour in an open field, whereas, the combined minocycline and LPS treatments markedly enhanced sickness behaviour and plasma corticosterone. As it is an already widely prescribed antibiotic, and easily crosses the blood-brain barrier, minocycline could provide a novel therapeutic adjunct for individuals suffering from affective disorders associated with

elevated inflammatory immune system activity, although additional studies should be conducted to further investigate its stressor-like effects.

Introduction

The development of major depressive disorder has been linked to the activation of the inflammatory immune system (Anisman, 2009; Dantzer, 2009; Loftis, Huckans, & Morasco, 2010; Maes, et al., 2009; Miller, et al., 2009). Indeed, depressed patients frequently exhibit elevated levels of circulating inflammatory mediators, such as proinflammatory cytokines (Maes, 1995; Maes, et al., 2009; Mossner, et al., 2007; Piletz, et al., 2009), and antidepressant medication can function to decrease their expression (Basterzi, et al., 2005; Janssen, et al. 2010; Piletz, et al., 2009). Furthermore, chronic inflammatory conditions, such as rheumatoid arthritis, cancer, and cardiovascular disease, are typically accompanied by higher levels of cytokines, and these levels have been positively correlated with severity of depression (Yirmiya, et al., 1999). Although it is difficult to ascertain whether these inflammatory abnormalities are a cause of the psychiatric symptoms, or merely a symptom, administration of pro-inflammatory cytokines (e.g. interferon- α for immunotherapy) or cytokine inducers (e.g. bacterial endotoxin or inoculations) can provoke general malaise and depressive affect (Brydon, et al., 2009; Harrison, et al., 2009; Reichenberg, et al., 2001; Wright, et al., 2005).

Paralleling clinical research, studies using animal models have demonstrated that the administration of proinflammatory cytokines, or cytokine inducers, can elicit a variety of behavioural effects commonly seen in depression, including anhedonia and despair (Borowski, et al., 1998; Frenois, et al., 2007; Merali, et al., 2003; Moreau, et al., 2008). Furthermore, cytokines are associated with the development of sickness behaviour, a general term used to describe the constellation of symptoms geared toward survival and recovery from illness (Dantzer, et al., 2008; Gibb, et al., 2009). Although sickness can be

dissociated from depressive-like behaviour, it has been argued that they share many similarities (e.g. social withdrawal, decrease in exploratory activity, decreased reactivity to food and reward), and that sickness behaviour may represent some of the neurovegetative features of depression (Anisman, 2009; Castanon, Leonard, Neveu, & Yirmiya, 2002; Dantzer, et al., 2008). In conjunction with these behavioural abnormalities, cytokine administration in animals elicits neurochemical effects similar to those seen in depressive disorder, including neurotransmitter disturbances in several brain regions, disruptions of the hypothalamic pituitary adrenal (HPA) axis, and altered neuroplasticity and neurogenesis (Anisman, Merali, & Hayley, 2008; Khairova, et al., 2009; Miller, et al., 2009; Pace & Miller, 2009). Considering the potential involvement of inflammation in the pathophysiology of affective disorders, it is of interest to investigate whether cytokine-induced behavioural and neurochemical alterations can be precluded with the pretreatment with an anti-inflammatory agent.

Minocycline is a semi-synthetic second generation tetracycline that readily crosses the blood brain barrier, and has been studied extensively for its anti-inflammatory and neuroprotective properties. This antibiotic has been used successfully to study many different animal models of neurodegenerative diseases, including Alzheimer's disease (Choi, et al., 2007), Parkinson's disease (Wu, et al., 2002), Huntingtons chorea (M. Chen, et al., 2000; Wang, et al., 2003), multiple sclerosis (Popovic, et al., 2002) and amyotrophic lateral sclerosis (Zhu, et al., 2002), as well as traumatic brain injuries (Lee, et al., 2003; for a complete review see Kim et al., 2009). Aside from its antimicrobial activity, minocycline exerts potent anti-inflammatory effects by inhibiting the activation of microglial cells, which subsequently inhibits the production and release of

inflammatory factors, such as proinflammatory cytokines, chemokines and reactive oxygen species (Kim & Suh, 2009; Stirling, Koochesfahani, Steeves, & Tetzlaff, 2005). Minocycline also increases circulating IL-10, an anti-inflammatory cytokine, which serves to diminish proinflammatory responses (Lee, et al., 2003). Although its many mechanisms of action have yet to be fully elucidated, the potential of minocycline as an antidepressant treatment has shown promising results in animal models, with decreased depressive-like behaviour resulting from the antibiotic administered alone, or synergistically with traditional antidepressants or glutamate antagonists (Molina-Hernandez, Tellez-Alcantara, Perez-Garcia, Olivera-Lopez, & Jaramillo-Jaimes, 2008a, 2008b).

Considering its potent anti-inflammatory properties and neuroprotective capacity, the aim of the following studies was to assess whether pretreatment with minocycline could inhibit lipopolysaccharide (LPS)-induced behavioural and biological disturbances in male and female CD1 mice. LPS, part of the outer membrane of gram-negative bacteria, is known to be a potent inducer of inflammatory mediators and to provoke a constellation of behavioural and neurochemical disturbances (Bay-Richter, Janelidze, Hallberg, & Brundin, 2011; Gibb, et al., 2008; Harden, du Plessis, Loram, Poole, & Laburn, 2011; Yirmiya, 1996). As such, the first study assessed whether pretreatment with minocycline could attenuate endotoxin induced anxiety-like behaviour in an open field. A second study investigated whether minocycline would abrogate the sickness behaviour and neurochemical alterations normally provoked by LPS, including increased circulating corticosterone and pro-inflammatory cytokine mRNA expression within the medial prefrontal cortex (mPFC) and hippocampus; brain regions thought to be involved

in the development of depression (Koolschijn, van Haren, Lensvelt-Mulders, Hulshoff Pol, & Kahn, 2009).

Materials and Methods

Subjects

Male and female CD-1 mice were obtained from Charles River Canada (St. Constance, Quebec) at approximately 6-8 weeks of age. Mice were housed 4 per cage in standard polypropylene cages (27cm x 21cm x 14cm), by sex, and were permitted approximately 2 weeks to acclimatize to the vivarium prior to serving as experimental subjects. In order to minimize the potential disruption of isolating mice for locomotor assessment on test day, mice were individually housed 7 days prior to the beginning of the study. The vivarium was maintained on a 12 h light/dark (8am-8pm) cycle in a temperature (21°C) controlled room with food and water freely available. All experimental procedures were conducted between 8am and 8pm to reduce variability associated with diurnal rhythms. All procedures complied with the guidelines set by the Canadian Council on Animal Care and were approved by the Carleton University Animal Care Committee.

Procedures

Experiment 1: Effects of pretreatment with minocycline on LPS-induced behavioural disturbances in male and female CD-1 mice

Male and female mice (n = 8/group) were pretreated with intraperitoneal (i.p.) injections of minocycline (45 mg/kg) or vehicle (phosphate-buffered saline; PBS), on

each of 7 days. Minocycline hydrochloride (Sigma, St. Louis, MO) was dissolved in sterile PBS (pH 7.4; heated gently to ensure complete dissolution), and solutions were prepared fresh daily. On the sixth day of pretreatment following injection, animals were relocated to the behavioural testing room overnight to minimize the disruption of a new environment. On the seventh day of the experiment, animals received their final i.p. injection of minocycline or PBS, followed 30 min later by i.p. administration of LPS (10 μ g; Sigma L-3755 from *Escherichia coli* serotype O26:B6) or saline. Animals were immediately returned to their home cages, where they were monitored for locomotor activity for 120 min using the Micromax photodetection system (Columbus, OH). Mice were then run through the open field paradigm (see General Methods) for a period of 5 min. The behaviours of interest were the latency to enter the central portion of the arena and the time spent in center arena, as these have been shown to reflect anxiety-like behaviour (Belzung & Griebel, 2001).

Experiment 2: Effects of pretreatment with minocycline on LPS-induced alterations of sickness behaviour, circulating corticosterone and central cytokine mRNA expression

Male and female mice (n = 6/group) were treated identically to Experiment 1. However, as preliminary studies had indicated that 3 days of minocycline pretreatment (as opposed to 7) was sufficient to attenuate inflammatory effects (Henry, et al., 2008; O'Connor, et al., 2009), and considering the potential nociceptive effects of the drug (see Nessler, et al., 2002), animals were only given the treatment on three occasions, including the test day. Following LPS or Saline administration, animals were monitored for sickness behaviour every 15 mins (see General Methods) up to 1.5hrs. Animals were

then sacrificed and blood was collected to analyze corticosterone, and brains were rapidly removed and placed on a stainless steel brain matrix ($2.5 \times 3.75 \times 2.0$ cm) that was positioned on a block of ice. The matrix had a series of slots spaced approximately 500 μm apart that guided razor blades to provide coronal brain sections. Once a brain was sliced, tissue from the mPFC and hippocampus were collected, by micropunch, following the mouse atlas of Franklin and Paxinos (1997). Cytokine (IL-1 β , IL-6, and TNF- α) mRNA expression was determined using reverse transcription-quantitative polymerase chain reaction (RT-QPCR) analysis.

General Methods

Open Field

The open field paradigm is routinely used to assess anxiety-like behaviour and motor activity in mice (Belzung & Griebel, 2001; Swiergiel & Dunn, 2007). Animals were placed into a specific corner of a clear polypropylene open field (48.0 x 38.0 x 20.0 cm). Behaviour was recorded for 5 min using an overhead mounted video camera. The latency to enter the center square as well as the total amount of time spent in the unprotected center were recorded. These behaviours are thought to reflect anxiety-like behaviour (Belzung & Griebel, 2001). The open field was cleaned thoroughly with 70% ethanol following the testing of each animal.

Sickness behaviour

Sickness behaviour was scored for 10 sec epochs at 15 min intervals over the 90 min period following the injection of LPS. To this end, the presence or absence of the

following symptoms was recorded: lethargy (demonstrated by diminished locomotion and exploratory activities; curled body posture), ptosis (drooping eyelids), and piloerection (ruffled and greasy fur, typically at the neck). Animals were rated on a 4-point scale with respect to the number of symptoms present (0 = no sickness symptoms, 1 = 1 symptom, 2 = 2 symptoms, 3 = three symptoms). This procedure was found to provide less than 10% variability between raters blind to the treatment mice received and was highly correlated with other methods of scoring sickness (e.g., assessing severity of each symptom independently; see (Gandhi, et al., 2007).

Plasma Corticosterone Analysis

Following decapitation, trunk blood was collected in tubes containing 10 µg of EDTA, centrifuged for 15 min at 3600 RPM, and the plasma was stored at -80 °C for subsequent corticosterone determination using a commercial radioimmunoassay RIA kit (ICN Biomedicals, CA). For each study, corticosterone levels were determined, in duplicate, in a single run to avoid inter-assay variability, and intra-assay variability was less than 10%.

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

mRNA from the mPFC and hippocampus was isolated and purified by standard methodologies employing Trizol according to the manufacturer's protocol (Invitrogen; Burlington, ON, Canada). The mRNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen; Burlington, Ontario), and aliquots of this reaction were used in simultaneous QPCR reactions.

BioRad MyIQ real time thermocycler was used to collect the data. For QPCR, SYBR green detection was used according to the manufacturer's protocol (Bio-Rad). 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 software. All primer pairs had a minimum of 90% percent efficiency.

Primers that amplify Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and Beta-Actin mRNA were used as a control to normalize the data. Primer sequences were as follows: GAPDH, Forward: GGTCGGTGTGAACGGATTTG, Reverse: TGCCGTTGGAGTCATACTG, Beta-Actin, Forward: GAACCCTAAGGCCAACCGTG, Reverse: GGTACGACCAGAGGCATACAGG; Mus IL-1 β , Forward: TGTCTGAAGCAGCTATGGCAAC: Reverse: CTGCCTGAAGCTCTTGTTGATG; Mus IL-6. Forward: TCTTGGGACTGATGCTGGTG, Reverse: CAGAATTGCCATTGCACAACCTC; Mus TNF- α , Forward: CTC AGCCTCTTCTCATTCCTGC Reverse: GCCATAGAACTGATGAGAGGG. To compensate for inter-individual variability that ordinarily exists within the assay, the expression of each species was normalized by subtracting its Ct from the housekeeping Ct. Following the procedure described by (Livak & Schmittgen, 2001), normalized brain mRNA expression values were converted to fold changes relative to controls.

Statistical Analyses

In each experiment, the outcome measures were analyzed through between-groups analyses of variance (ANOVA). As such, sickness behaviour, locomotor activity, latency to enter and time spent in the center of the open field, plasma corticosterone, as well as the mRNA expression of cytokines were all analyzed independently. In the case of sickness behaviour and locomotor activity, a composite score was created by averaging all the time points, which was then used in the ANOVA. 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.

Results

Experiment 1 – Pretreatment with minocycline attenuated endotoxin-induced anxiety-like behaviour, but not locomotor disturbances in male and female mice

Within the open field paradigm, the latency to enter center square and time spent in centre square varied as function of the Minocycline x LPS interaction, $F_s(1, 56) = 11.14$ and 4.53 , $p_s < 0.01$ and 0.05 , respectively (see Figure 3.1). Follow-up analyses revealed that LPS increased the latency to enter the center square in both males and females, and that minocycline completely abolished this effect. Similarly, LPS significantly reduced the time spent in the center square, an outcome that was prevented by the administration of minocycline. These changes were not dependent on motor effects, however, as the LPS-induced decrease in home cage activity, $F(1, 56) = 15.21$, p

< .001, was not significantly altered by the pretreatment of minocycline ($p > .6$), see Figure 3.2.

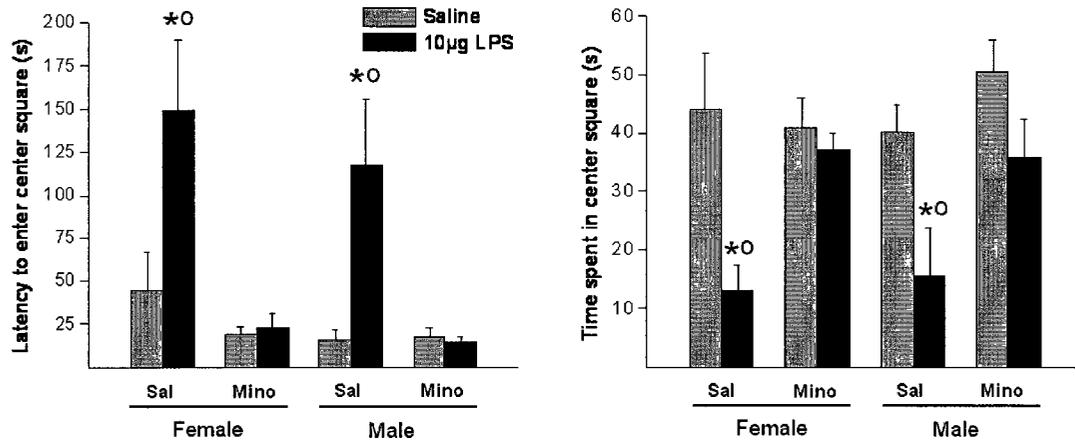


Figure 3.1. Mean (\pm SEM) latency to enter center square (left panel) and time spent in center square (right panel) among male and female mice that had been pre-treated with minocycline or saline and subsequently administered LPS or saline. * $p < .05$ relative to saline treated mice. $^{\circ}p < .05$ relative to LPS-treated mice that had been given minocycline.

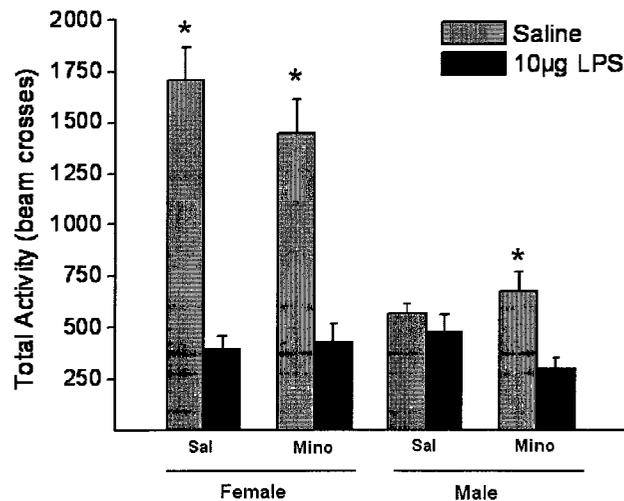


Figure 3.2. Mean (\pm SEM) total locomotor activity among LPS or Saline treated male and female mice that were pre-treated with either minocycline or Saline. * $p < .05$ relative to LPS treated mice.

Experiment 2: Minocycline effectively inhibited LPS-induced mRNA expression of pro-inflammatory cytokines in both the mPFC and hippocampus, whereas sickness behaviour and circulating corticosterone were synergistically enhanced by the combined treatments

Total sickness behaviour varied as a function of the Minocycline x LPS interaction, $F(1, 40) = 8.89$, $p < .01$; see Figure 3.3 (left panel). Follow-up comparisons of the simple effects revealed that LPS provoked sickness symptoms in females, although the effects of the endotoxin were non-significant in males. Interestingly, when LPS administration was preceded by minocycline, sickness behaviour was markedly enhanced in both genders, suggesting synergistic effects between the antibiotic and bacterial endotoxin.

Plasma corticosterone was influenced by the Minocycline x LPS and the Gender x LPS interactions, $F_s(1, 40) = 7.12$ and 4.46 , $p_s < .05$, respectively. Follow-up comparisons indicated that LPS markedly increased circulating corticosterone, and this outcome was more pronounced in females than males. Furthermore, the effects of the LPS treatment on corticosterone were further augmented by the minocycline treatment, as seen in Figure 3.3 (right panel).

Central Cytokine mRNA Expression

Prefrontal cortex

IL-1 β mRNA expression varied as a function of the Minocycline x LPS interaction, $F(1, 37) = 14.63$, $p < .001$. As shown in Figure 3.4 (upper panel), and confirmed by multiple comparisons, LPS significantly increased IL-1 β mRNA expression in both genders, and these effects were blunted by pretreatment of minocycline.

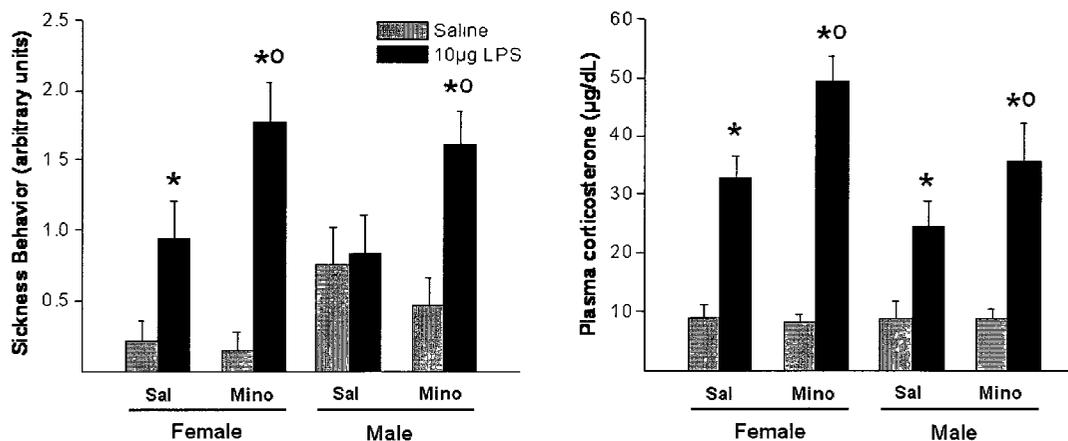


Figure 3.3. Mean (\pm SEM) sickness score (left panel) and plasma corticosterone concentrations (right panel) among male and female mice that had been pre-treated with minocycline or saline and subsequently administered LPS or saline. * $p < .05$ relative to saline treated mice. $^{\circ}p < .05$ relative to endotoxin-treated mice that had not received pre-treatment with the antibiotic.

The interaction of LPS, Minocycline treatment and gender did not reach statistical significance, likely owing to the small N involved in the study. However, an a priori prediction had been made concerning this interaction given previous reports indicating that stressors and LPS differentially influenced male and female mice (Dalla, et al., 2011). Thus, follow up tests were conducted of the simple effects that comprised this interaction. As seen in Figure 3.4, the administration of minocycline in absence of LPS significantly increased levels of IL-1 β , but this only occurred in male mice, perhaps reflecting a stressor-like effect of the minocycline, although it is uncertain why this was unique to males.

Similar to the effects on IL-1 β , IL-6 mRNA expression varied as a function of the Minocycline x LPS interaction, $F(1, 36) = 9.24$, $p < .01$; Figure 3.4 middle panel. Follow-up comparisons revealed that LPS increased IL-6 mRNA expression in both genders, and these effects were precluded with pretreatment with minocycline. However,

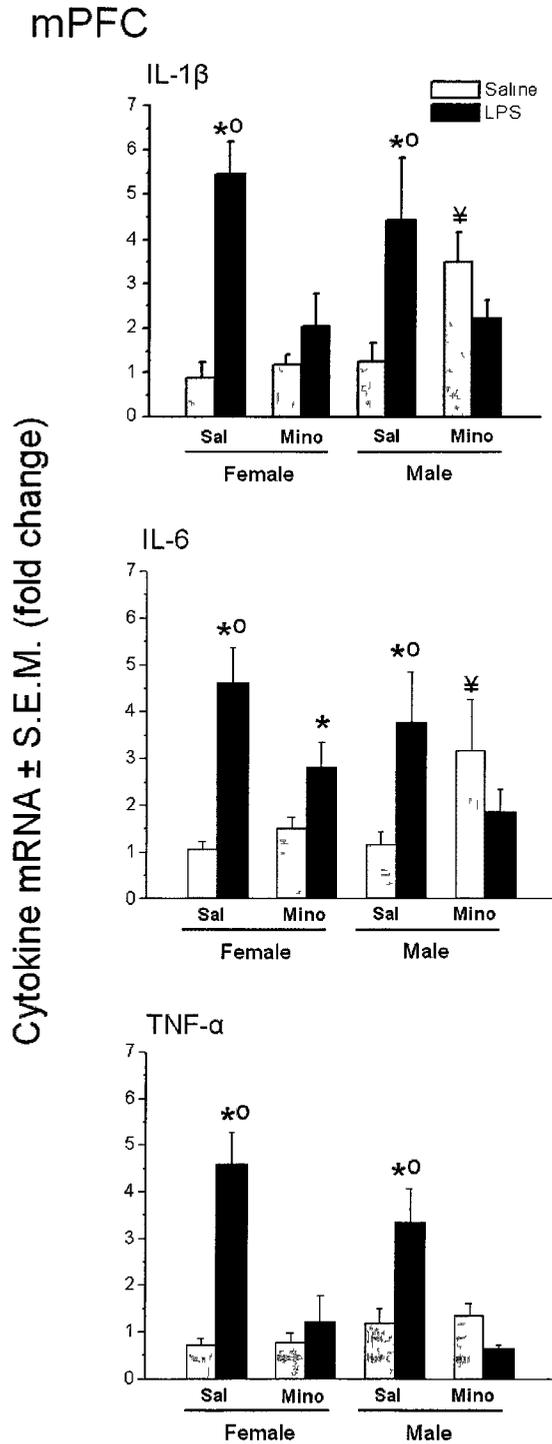


Figure 3.4. Mean (\pm SEM) IL-1 β , IL-6 and TNF- α mRNA expression within the prefrontal cortex of male and female mice that were given pre-treatment with minocycline or saline followed by the administration of LPS or saline. Data are presented as fold changes. * $p < .05$ relative to saline treated mice. ° $p < .05$ relative to endotoxin-treated mice that received pre-treatment with minocycline. ≠ $p < .05$ relative to saline treated male mice pre-treated with saline.

as in the case of IL-1 β expression, minocycline alone modestly increased cytokine expression, but only in males.

TNF- α expression was influenced by both the Gender x LPS and Minocycline x LPS interactions, $F_s(1, 36) = 4.20$ and 20.29 , $p_s < .05$ and $.0001$, respectively. Analyses of the simple main effects revealed that LPS produced much greater expression of TNF- α in females, and minocycline abrogated these effects in both genders (see Figure 3.4, lower panel).

Hippocampus

Consistent with the effects provoked in the mPFC, IL-1 β mRNA expression in the hippocampus was influenced by the Minocycline x LPS interaction, $F(1, 40) = 15.56$, $p < .01$. As shown in Figure 3.5 (upper panel), and confirmed by multiple comparisons, LPS increased IL-1 β expression in both males and females, and this effect was attenuated by pretreatment with minocycline. However, unlike the effect in the PFC, minocycline alone did not increase IL-1 β expression in males.

Interleukin-6 varied as a function of both the Gender x LPS and Minocycline x LPS interactions, $F_s(1, 38) = 5.08$ and 7.95 , $p_s < .05$ and $.01$, respectively; Figure 3.5 (middle panel). Post-hoc comparisons revealed that LPS provoked a greater IL-6 response in females, compared to males, although the LPS effects were significant in both genders. Furthermore, minocycline diminished these effects.

Hippocampus

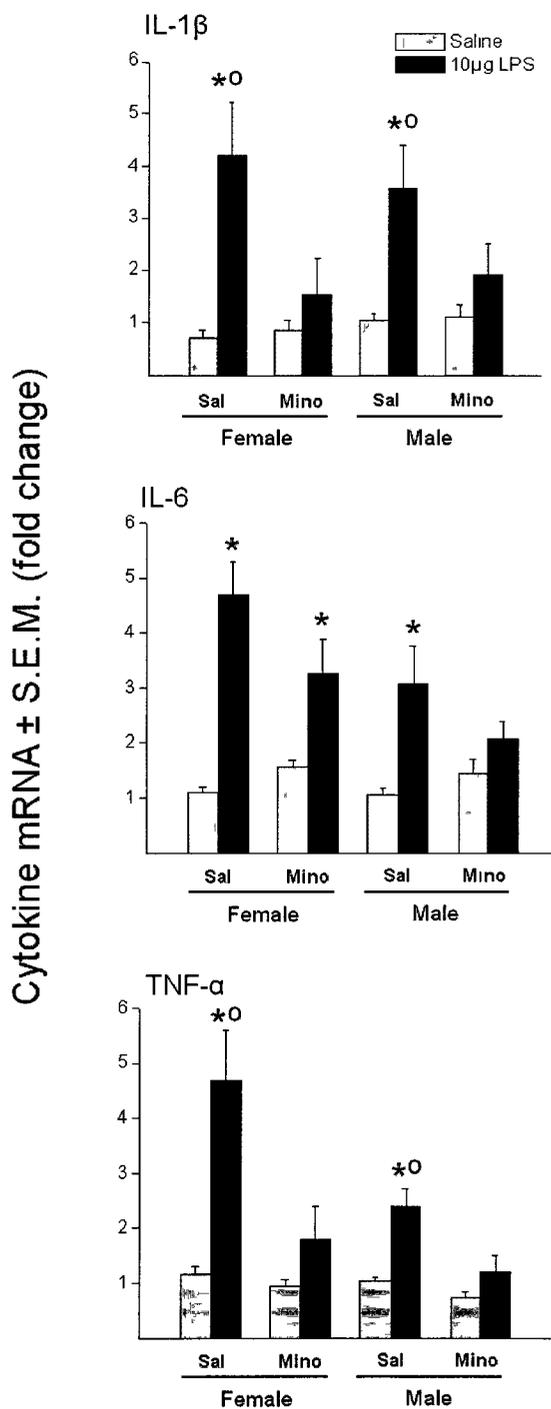


Figure 3.5. Mean (\pm SEM) IL-1 β , IL-6 and TNF- α mRNA expression within the hippocampus of male and female mice that were given pre-treatment with minocycline or saline followed by the administration of LPS or saline. Data are presented as fold changes. * $p < .05$ relative to saline treated mice. ° $p < .05$ relative to endotoxin-treated mice that received pre-treatment with minocycline.

TNF- α was similarly influenced and varied as a function of both the Gender x LPS and Minocycline x LPS interactions, $F_{s(1, 38)} = 4.48$ and 8.90 , $p < .05$ and $.01$, respectively. As shown in Figure 3.5 (lower panel), LPS significantly increased this cytokine in both genders, and this effect was more pronounced in female mice. Furthermore, the endotoxin-induced elevations were eliminated by pretreatment with the antibiotic in both genders.

Discussion

As activation of the inflammatory immune system may be related to the development of anxiety and depressive disorders (Anisman, 2009; Dantzer, 2009; Loftis et al., 2010; Maes, 2009, Miller 2009), it was of interest to assess whether minocycline, a commonly prescribed antibiotic that readily crosses the BBB, could inhibit the behavioural and central effects of a bacterial endotoxin. Interestingly, although sickness behaviour and plasma corticosterone were strikingly enhanced by the combined minocycline and LPS treatments, the antibiotic successfully abolished LPS-induced anxiety-like behaviour and elevations of pro-inflammatory cytokines in the mPFC and hippocampus.

The acute administration of an endotoxin has consistently been shown to increase levels of cytokines in otherwise healthy individuals, an effect associated with mild sickness symptoms and higher scores on depression rating scales (Harrison et al., 2009, Reichenberg et al., 2001; Wright et al., 2005). Animals exposed to LPS exhibit parallel outcomes, reflected by increased sickness behaviour, social withdrawal, anhedonia, as well as reduced exploratory behaviour (Borowski et al., 1998, Frenois et al., 2007; Moreau et al., 2008; Merali et al., 2003). In line with this, the acute administration of LPS in the current study markedly induced the development of anxiety-like behaviour in an open field and sickness behaviour in mice, an effect that was more pronounced in females than males. Interestingly, although pretreatment with minocycline reduced the occurrence of behavioural disturbances in the open field test, sickness behaviour was unaltered and even enhanced by the antibiotic.

Even though sickness behaviour is not fully congruent with depression, the two share many overlapping features, and it has been suggested that sickness behaviour may be mimicking the neurovegetative symptoms of depression (Castanon et al., 2002, Dantzer et al., 2008, Loftis et al., 2010). It has further been postulated that depression may be a maladaptive form of sickness behaviour, resulting from intense and/or prolonged inflammation and the subsequent neurochemical disturbances, especially in vulnerable populations, such as the elderly or those with preexisting medical conditions and/or a predisposition toward depression (Dantzer et al., 2008; Henry et al., 2008). In this regard, it is significant that minocycline failed to abrogate the LPS-induced sickness behaviour in the present study, although previous reports showed that minocycline decreased endotoxin-induced depressive-like behaviours in the forced swim and tail suspension tests (O'Connor et al., 2009), and reductions of sucrose consumption and social exploration (Henry et al., 2008). One possibility is that the effects of minocycline on sickness behaviour are only apparent after several hours, and were missed due to the fact that animals were sacrificed only 1.5 hrs post-LPS. In fact, one study demonstrated that although minocycline did not completely abolish sickness, it did facilitate recovery beginning at 4 hrs post-endotoxin administration (Henry et al., 2008). It is also possible that sickness behaviour may initially be more related to the systemic effects of the LPS, including peripheral cytokine release and circulating corticosterone, rather than central cytokine production. In this regard, it is interesting that the sickness behaviour paralleled corticosterone levels, which were markedly enhanced by the LPS and minocycline co-administration. As minocycline is known for its nociceptive effects (Nessler et al., 2002),

the injection may have acted as a stressor, which can potentiate LPS-induced sickness behaviour and inflammatory effects (Gibb et al., 2008; Johnson et al., 2002).

Depression is frequently co-morbid with other psychiatric conditions, such as anxiety disorders (Gorman, 1996). As such, it was of interest to determine whether minocycline would also attenuate anxiety-like behaviour in an open field. The open field is a widely used paradigm to assess anxiety-like behaviour (Swiergiel & Dunn, 2007), and is a validated animal model used to test the efficiency of anxiolytic medications (Choleris et al., 2001). The administration of a bacterial endotoxin typically reduces exploratory behaviour and increases the latency to enter the central portion of the test arena (Cunningham et al., 2009; Lacosta et al., 1999; Swiergiel et al., 2007). In part, this outcome is no doubt due to sickness provoked by the endotoxin, but anxiety elicited by the treatment might also contribute to this outcome above and beyond the effects of sickness. In line with the suggestion that LPS increases anxiety, acute LPS treatment significantly increased the latency to enter the center arena of the open field and decreased the amount of time spent in this area; behaviours thought to represent anxiety-like behaviour (Belzung & Griebel, 2001). Both of these behaviours were precluded with the pretreatment with minocycline. These effects appear to be independent of motor disturbances and sickness, as minocycline failed to prevent LPS-induced reductions of locomotor activity. Moreover, if sickness was primarily responsible for the behaviour in the open field, there would be no reason to expect that LPS treated mice would spend less time in the central portion of the open field, a behaviour that, in contrast, is associated with elevated anxiety. It is thus possible that different mechanisms are responsible for exploratory and locomotor behaviours, and that minocycline is only effective in

attenuating endotoxin-induced exploratory alterations. Taken together, these findings suggest that beyond its potential antidepressant-like effects, minocycline should also be investigated as prospective treatment for other psychiatric conditions.

The behavioural effects induced by the bacterial endotoxin have been attributed, in part, to increased peripheral and central release of proinflammatory cytokines (Dantzer et al., 2008; Koonsman et al., 2002). In this regard, following exposure to LPS, pattern recognition toll-like receptors (TLR)-4 and -2 are activated and provoke the production and release of a cascade of cytokines (such as IL-1 β , IL-6 and TNF- α) which serve as an acute phase response to counteract the threat (Cognasse et al., 2007; Lu et al., 2008). These cytokines subsequently exert their actions in the brain, both directly and indirectly through second messengers. In the present investigation, in addition to preventing the development of some of the behavioural disturbances, minocycline also prevented endotoxin-induced central mRNA expression of pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α) within the mPFC and hippocampus; brain regions which have been implicated in the development of stress-related behavioural disturbances. This is consistent with previous reports where minocycline decreased IL-1 β and TNF- α mRNA expression in whole brains 24 hours post endotoxin administration (O'Connor et al., 2009), as well as IL-1 β and IL-6 mRNA expression in hippocampal and parahippocampal brain regions 4 hours post-administration (Henry et al., 2008). This decrease in cytokine activity is likely responsible for the prevention of exploratory alterations, but it will be necessary to confirm this using specific antagonists directed at these brain regions.

Minocycline may exert its anti-inflammatory actions directly by inhibiting macrophage and microglial activation, or indirectly by influencing second messenger

systems. For instance, minocycline was found to inhibit both the P38 MAP Kinase (Stirling et al., 2005) and NF κ B (Nikodemova, Duncan, & Watters, 2006) pathways. Both pathways are considered mediators of inflammation, and are involved in the production of cytokines (Nadjar et al., 2005; Vermullen et al., 2006). Furthermore, their inhibition can reduce cytokine-induced behavioural effects (Loftis et al., 2010; Nadjar et al., 2005; Nikodemova et al., 2006). In addition to modulating microglial activation, minocycline may also be exerting its anti-inflammatory effects through its actions on cyclooxygenase (COX) activity and prostaglandin production (Yrjanheikki et al., 1999; Kim et al., 2004; Kuang et al., 2009). COX enzymes act by converting arachidonic acid into prostaglandin endoperoxide H₂, leading to the synthesis of prostaglandins, considered to be secondary mediators of inflammation (Aid, Langenbach, & Bosetti, 2008). Both LPS and IL-1 β have been shown to increase this enzyme's expression in brain endothelial cells, which corresponded with depressive-like symptoms (Dunn, Swiergiel, Zhang, & Quan, 2006). Furthermore, several reports indicated that selective COX-2 inhibitors, such as indomethacin and ibuprofen (de Paiva et al., 2010; Teeling et al., 2009; Teeling et al., 2007) as well as nonselective inhibitors such as celecoxib (Swiergiel & Dunn, 2002), were able to attenuate the behavioural effects associated with an acute immune challenge. In this regard, the reduction of behavioural symptoms in the current investigation may have been due, in part, to minocycline's inhibitory actions on microglial cell activation and prostaglandin production.

The behavioural effects of minocycline have also been attributed, in part, to its inhibitory effects on indoleamine 2,3-dioxygenase (IDO), the rate limiting enzyme in the kynurenine pathway. IDO converts tryptophan into kynurenine and quinolinic acid and

high levels of activity can lead to decreased synthesis of serotonin (Morita et al., 2002). Proinflammatory cytokines, such as IL-1 β and TNF- α , as well as bacterial endotoxins have been shown to increase the tryptophan degrading enzyme IDO (Lestage et al., 2002; Muller et al., 2007). It is thought that the depressogenic effects of immunotherapy are due to IDO activity and the resulting tryptophan depletion (Capuron et al., 2002; Capuron & Miller, 2004; Musselman et al., 2001). A related possibility is that a shift in activation of the IDO-kynurenine pathway promotes the production of neurotoxic metabolites, namely 3-hydroxy kynurenine and quinolinic acid, and the NMDA antagonist, kynurenic acid. Owing to their neurotoxic actions, these products could engender the development of pathology (Dantzer, O'Connor, Lawson, & Kelley, 2011; Maes, Leonard, Myint, Kubera, & Verkerk, 2011). As well, a direct role of kynurenine in depressive-like behaviour is also possible (see O'Connor et al., 2009). Interestingly, minocycline has been shown to decrease mRNA expression of IDO (Henry et al., 2008; O'Connor et al., 2009), and similarly, the administration of an IDO antagonist produced comparable antidepressant-like effects (O'Connor et al., 2009).

Dysregulation of the HPA axis is one of the most consistent biomarkers of depression, and has been hypothesized to play a major role in its pathophysiology (Chrousos, 2009; Gillespie & Nemeroff, 2005; Holsboer, 2001; Marques, Silverman, & Sternberg, 2009). Furthermore, LPS, and the subsequent release of cytokines, has consistently been shown to increase corticotropin-releasing hormone and plasma levels of corticosterone (Brebner et al., 2000; Gibb et al., 2008), just as stressors have such effects. Indeed, it was suggested that an immune challenge might be considered as a stressor by the brain (Anisman & Merali, 1999). Interestingly, although minocycline was able to

prevent cytokine production in the brain, it failed to prevent LPS-induced corticosterone release, and actually synergistically enhanced circulating corticosterone. The distinct possibility exists that, as previously mentioned, minocycline exerts a degree of stress, as it causes some discomfort (see Nessler et al., 2002), and induces an increase of endocrine activity even in the absence of an endotoxin (Deak et al., 2005). It was also shown in the current study that minocycline, in absence of the LPS, increased IL-1 β and IL-6 cytokine expression in the mPFC of male mice, possibly indicating a stressor effect on central cytokine production. As depression is associated with neuroendocrine disturbances, it is interesting that the sickness behaviour paralleled circulating corticosterone, rather than central cytokine mRNA expression.

Conclusion

The present study highlights the potential of minocycline in modifying LPS-induced behavioural variations. Although clinical research is limited, preliminary studies have demonstrated that minocycline, when co-administered with tricyclic antidepressants, led to significant and long-lasting improvements of depressive symptoms in patients suffering from rheumatoid arthritis (Levine et al., 1996). Animal models have shown similar results, where minocycline delivered systemically or infused directly into the nucleus accumbens, had antidepressant effects in the forced swim test (Molina-Hernandez et al., 2008a, 2008b). These effects were synergistically enhanced when combined with several glutamate antagonists and classic antidepressant medication (Molina-Hernandez et al., 2008a, 2008b). In the present study, minocycline abolished anxiety-like behaviour in the open field test and attenuated the central cytokine mRNA

alterations ordinarily elicited by LPS. As it is an already widely prescribed antibiotic (Kim et al., 2009), and easily crosses the blood-brain barrier, minocycline could potentially serve as a therapeutic adjunct for affective disorders associated with elevated inflammatory immune system activity.

General Discussion

Traditionally, theories of MDD have focused on the involvement of monoamines in its pathophysiology. The reason for this is two-fold. First, antidepressant treatments specifically target and enhance monoamine neurotransmission. Second, stressful experiences can elicit reductions of these neurochemicals in humans and animals, and depressive affect (in animal models) has been associated with these reductions (Beekman, et al., 2005; Fuchs & Flugge, 2003; Keeney, et al., 2006). Although monoamine theories of depression have not been entirely abandoned, the fact remains that only about 65% of patients treated with 5-HT or NE/5-HT acting antidepressants display alleviation of depression, and even when the drug is “effective”, symptom remission is not always complete (Moncrieff & Kirsch, 2005). Furthermore, simply increasing the availability of serotonin is not sufficient to abate the symptoms of the disorder immediately, as the therapeutic effects of antidepressants are only observed after 2-3 weeks (Blier, 2001). In view of the limitations of the monoamine-based hypotheses, several alternative substrates have been proposed as potential mediators of major depressive illness. These have included inflammatory factors, various neuropeptides (e.g., CRH), glucocorticoids and several growth factors (e.g., BDNF).

In the past decade, it has become clear that activation of the inflammatory immune system may have repercussions on brain functioning, including neurotransmitter secretion and effects on growth factors, and it has been suggested that inflammatory factors could potentially influence depressive states (Anisman, et al., 2008; Dantzer, et al., 2008; Miller, et al., 2009). Activation of inflammatory immune processes can also be related to stressful encounters, as these adverse events directly or indirectly affect

immune functioning (Bartolomucci & Leopardi, 2009), and conversely, the brain may be interpreting inflammation much like a stressor (Anisman et al., 2008). Of the numerous inflammatory factors, cytokines in particular have been shown to be associated with depressive affect and neurochemical changes. It is important to underscore, however, that although the focus of inflammatory cytokine involvement in depression has been concerned with effects of immune activation, it has been reported that *de novo* synthesis of cytokines also occurs in brain, primarily in glial cells but may also occur in neurons (Rivest, 2009), and could have effects on brain functioning.

The role cytokines play in the brain is not entirely clear. It was previously thought that they were solely secreted in the brain following trauma, infections and disease, however, it is now known that cytokines (such as IL-1 β , IL-6 and TNF- α) are naturally expressed in low levels within the brains of healthy individuals, and are important in synaptic plasticity and LTP (Khairova, et al., 2009). Although basal levels are critical for normal functioning, it has been debated whether elevated levels are neuroprotective, neurodestructive, or whether they play a dual role (Pascoe, Crewther, Carey, & Crewther, 2011; Rivest, 2009; Stoll, Jander, & Schroeter, 2002). A large number of different insults (e.g. seizure activity, infections, or trauma) all produce increased cytokine activity in brain (Rivest, 2009). Whether they are acting in a beneficial (e.g. eliminating debris or infection) or detrimental capacity is uncertain, but one thing that is certain is that cytokine activation in the brain is often accompanied by depressive-like states. For instance, the post-stroke period is often accompanied by depression, possibly owing to elevated cytokine levels that follow an ischemic attack (Pascoe et al., 2011). In addition, cerebrovascular occlusion in an animal stroke model was associated with the

development of anhedonia, which could be attenuated with IL-1ra, thus implicating pro-inflammatory cytokines in post-stroke depression (Craft, & DeVries, 2006).

Commensurate with this, immunogenic agents, administered peripherally or centrally, also provoke increased central cytokine synthesis and secretion, and these are often associated with the development of behavioural disturbances, including sickness behaviour, decreased social and exploratory behaviour, anhedonia, and learned helplessness (for a review see Gibb, et al., 2009).

Consistent with the hypothesis that immunogenic agents can lead to behavioural disturbances, the results of the present investigation demonstrated that when a bacterial endotoxin or viral mimic was administered peripherally, pro-inflammatory cytokine mRNA expression was increased in the brain, and this was accompanied by elevations of circulating corticosterone and the development of sickness behaviour. Interestingly, when LPS, and to a lesser degree poly I:C, was combined with acute stressor exposure, these effects were exaggerated. However, as seen in Chapter 1, the synergistic effects of stressor and endotoxin exposure were dependent upon the chronicity of the stressor used, the brain region examined and the timing of the outcome measures. For instance, both acute and chronic stressor exposure elicited marked sickness behaviour and corticosterone levels when combined with LPS, an effect that peaked relatively early following the endotoxin treatment (1.5 hrs). Furthermore, acute stressor exposure potentiated LPS-induced inflammatory cytokine expression in the CNS, though this effect was only visible at 3 hrs post LPS, when levels of sickness and corticosterone were approaching those of controls. Unlike acute stressor exposure, however, the effects of chronic stressors on central cytokine mRNA expression were diminished, an effect more

pronounced in the PFC, perhaps reflecting an adaptation to the inflammatory effects of the stressor.

It is unclear whether central cytokine alterations were functioning in a neuroprotective or neurodestructive capacity; however, it is interesting that the observed cytokine variations did not invariably parallel those of the sickness behaviour, indicating that the emergence of the behavioural disturbances were not secondary to enhanced central cytokine expression. In fact, the emergence and severity of sickness behaviour were more closely aligned with levels of circulating corticosterone, suggesting that sickness behaviour may be more related to the peripheral effects of the treatments on the brain, rather than central cytokine synthesis, although the data do not speak to this directly. Regardless of the processes involved, it is evident that stressors can potentiate the inflammatory effects of an immune challenge.

There is considerable inter-individual variability in the impact of stressors, likely owing to factors related to individual characteristics, such as age, gender, genetic and biological vulnerability, as well as characteristics of the stressor itself, such as severity, chronicity and controllability (Anisman & Matheson, 2005; Anisman et al., 2008). Indeed, an entire area of study has evolved dedicated to the biopsychosocial and genetic factors surrounding stressor 'resilience', and how resilience can be a protective factor against the development of affective disorders (for a review, see Feder, Nestler & Charney, 2009).

Of the biological determinants that seem to moderate the impact of stressors and vulnerability to depression, hyperactivity of the HPA axis in response to stressors appears to be one of the most profound (Capuron, Raison, Musselman, Lawson, Nemeroff, &

Miller, 2003; Feder et al., 2009; Southwick, Vythilingam, & Charney, 2005). For instance, in patients treated with IFN- α , a cytokine known to produce depressive symptoms, exaggerated HPA activity following the initial injection was associated with an increased vulnerability to develop depression with ongoing treatment (Capuron et al., 2003). Furthermore, depressed patients that had experienced early life stressors had higher levels of circulating cortisol, and a heightened reactivity to subsequent stressor exposure, compared to non-depressed controls who had experienced similar life events (Heim, Newport, Bonsall, Miller, & Nemeroff, 2001; Heim, Newport, Heit, Graham, Wilcox, Bonsall, et al. 2000). These findings demonstrate that stressor reactivity not only modifies reactions to an immune challenge, but may also influence the development of depressive states.

In the case of animal models, inter-individual variability in emotionality has traditionally been studied using strains of rodents differing in their responses to stressors. In line with the hypothesis that stressor reactivity can influence the alterations provoked by an immunogenic challenge, results from Chapter 2 demonstrated that sickness behaviour and elevations of corticosterone elicited by a bacterial endotoxin, and to a lesser extent poly I:C, were exaggerated when combined with an acute social stressor; effects only apparent in the stressor reactive BALB/cByJ strain of mouse. Conversely, alterations of circulating cytokine levels, along with central cytokine mRNA expression, were also additively enhanced by the combined treatments, but this was only apparent in the more resilient C57BL/6ByJ mice. Thus, it seems that the cytokine changes, HPA activity and sickness operate through independent mechanisms, and were differentially affected by the combined treatments, and the stressor reactivity of the strains.

Humans tend to display differing immunogenic profiles, namely the balance between cell-mediated (Th1) and extra-cellular immunity (Th2), which can alter their responses to subsequent stressor exposure (Elenkov, 2008). Similarly, the strains of mice used in the present study also displayed different immune characteristics, with BALB/cByJ mice being highly Th2 responsive and C57BL/6ByJ mice exhibiting greater Th1 responses (Watanabe, et al., 2004). In this regard, it is interesting that LPS, which primarily elicits a Th2 response, and poly I:C, which elicits a Th1 response, differentially influenced sickness behaviour, neuroendocrine and cytokine activity in these strains. This suggests that the effects of immune challenges are not only influenced by stressor reactivity, but also on the underlying immune characteristics of the individual. It remains to be established how the effects of immunogenic challenges play out in these strains when sophisticated behavioural models of depression are used.

If cytokines are mediating the development of depression, it would be expected that reducing cytokine activity with anti-inflammatory medication could have anti-depressant-like effects. Preliminary studies have shown that this is indeed the case. For instance, recent work with COX-inhibitors demonstrated that the efficiency of antidepressant medication in depressed patients could be enhanced when combined with NSAIDs, such as celecoxib and acetylsalicylic acid, which are non-selective and target both COX-1 and COX-2 enzymes (Mendlewicz, et al., 2006; Muller, et al., 2006). Likewise, animal studies have revealed that selective COX-2 inhibitors, such as indomethacin and ibuprofen (de Paiva, et al., 2010; Teeling, et al., 2009; Teeling, et al., 2007) as well as nonselective inhibitors such as celecoxib (Swiergiel & Dunn, 2002), reduced the behavioural effects associated with an acute immune challenge.

In addition to the potential antidepressant-like effects of NSAIDs, recent attention has focused on the synthetic tetracycline antibiotic minocycline that exerts potent anti-inflammatory effects by inhibiting the activation of microglial cells, which subsequently inhibits cytokine release (Kim & Suh, 2009; Stirling, et al., 2005). Although clinical research is limited, preliminary studies have demonstrated that minocycline, when co-administered with tricyclic antidepressants, led to significant and long-lasting improvements of depressive symptoms in patients suffering from rheumatoid arthritis (Levine et al., 1996). Animal models showed similar results, with decreased depressive-like behaviour resulting from the antibiotic administered alone, or synergistically with traditional antidepressants or glutamate antagonists (Molina-Hernandez, et al., 2008a, 2008b). It was further demonstrated that minocycline attenuated depressive-like behaviour, as well as elevations of circulating cytokine levels and central cytokine mRNA expression in endotoxin-treated mice (Henry et al., 2008; O'Connor et al., 2009). Commensurate with these findings, results from Chapter 3 showed that minocycline attenuated LPS-induced anxiety-like behaviour in an open field, and abolished cytokine mRNA expression in the PFC and hippocampus. However, minocycline appeared to have a stressful effect, as the combined LPS and minocycline treatments additively enhanced circulating corticosterone and sickness behaviour. Thus, although minocycline may present a novel therapeutic adjunct for cytokine-induced depressive behaviour, its potential stressor-like effects should be taken into consideration.

Limitations

Major depressive disorder is a heterogeneous disorder, comprising several subtypes (e.g. dysthymia, typical and atypical depression, unipolar and bipolar disorders), that are diagnosed based on the presence of a cluster of symptoms and clinical characteristics. It is, therefore, difficult to come up with a unifying theory of depression, considering that the subtypes not only have overlapping features, but may represent diverse underlying pathophysiology and endophenotypes (Anisman et al., 2008). Furthermore, although increased inflammatory markers have been found among depressed patients (Dowlati, et al. 2010; Mossner, et al., 2007; Zorrilla, et al., 2001), this has not always been consistent, and appears to vary according to the subtype of depression examined (Anisman, Ravindran, Griffiths, & Merali, 1999; Kaestner, et al., 2005; Maes, et al., 1997; Marques-Deak, et al. 2007). It has been suggested that cytokine-induced depression should be its own subtype of depression, as its clinical manifestations can be dissociated from those of traditional MDD. For instance, it was demonstrated cytokine-induced depression was associated with more weight loss and psychomotor retardation, and lower feelings of guilt compared to traditional depression (Capuron et al., 2009). It has further been found that depression with an inflammatory component was more treatment-resistant than depression not associated with inflammation (Maes et al., 1997). In this regard, the results of the current study may only be appropriate to study the pathophysiology of depression with an inflammatory component.

The main behavioural outcome assessed in the present studies involved the scoring of sickness behaviour, as it has been suggested that it mimics some of the

neurovegetative symptoms of depression (Castanon et al., 2002, Dantzer et al., 2008, Loftis et al., 2010). However, it should be noted that these 'neurovegetative' symptoms are not consistent with any single subtype of depression. For instance, the hypersomnia and lethargy characteristic of sickness behaviour are classic symptoms of atypical depression, whereas anorexia and weight loss are more adherent to the typical melancholic type of depression. Furthermore, although there is considerable overlap between sickness behaviour and depressive-like behaviour, the timing of these two conditions can be readily dissociated, with sickness symptoms emerging quickly following the administration of an acute or chronic treatment, and depressive symptoms emerging later, and persisting for several days following exposure (Frenois, et al., 2007; Moreau, et al., 2008). That said, it can be argued that sickness behaviour is not an adequate measure of depressive-like behaviour, and that different processes may be responsible for the emergence of the two conditions. Interestingly, through fMRI studies, sickness behaviour was shown to be associated more with basal ganglia activity (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008; Capuron, Pagnoni, Demetrasvili, Lawson, Fornwalt, Woolwine, et al., 2007), whereas depressive-like behaviour is subserved by other regions, such as the prefrontal cortex, hippocampus and dorsal anterior cingulate cortex (Koolschijn, et al., 2009). Inasmuch as sickness behaviour may reflect some aspects of depression and is a convenient measure, further studies should be conducted assessing the interaction of cytokines and stressors using more validated models of depression, such as those based on brain stimulation reward.

Conclusion

The release of pro-inflammatory cytokines in response to trauma, pathogen invasion or psychosocial stressors is part of a well-orchestrated, adaptive response needed for survival (Rivest, 2009). However, when the immune system becomes chronically activated or uncontrolled, as in the case of patients receiving immunotherapy or with chronic medical illnesses or psychosocial stress, cytokine secretion and circulation may become maladaptive and eventually pathological, leading to behavioural disturbances. The present investigation demonstrated that the behavioural, endocrine and neuroinflammatory effects of immunogenic agents, such as a bacterial endotoxin or viral mimics, were enhanced when administered following stressor exposure. These synergistic effects were dependent upon the chronicity of the stressor, timing of the outcome measures, as well as the stressor reactivity and immune profile of the mouse. Furthermore, when pre-treated with the anti-inflammatory agent minocycline, some of these endotoxin-induced effects were precluded. As alluded to repeatedly, major depressive disorder is likely a biochemically heterogeneous disorder, involving both serial and parallel pathways (Anisman et al., 2008). Ultimately, it likely will be necessary to consider the additive, subtractive or interactive effects of multiple systems to define the biological characteristics underlying this constellation of disorders. To this end, an endophenotypic approach will be necessary to define the relations between particular neurochemical processes, specific genetic factors, and individual symptoms. This approach will also be fundamental to the development of individualized treatment protocols.

References

- Adachi, M., Barrot, M., Autry, A. E., Theobald, D., & Monteggia, L. M. (2008). Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. *Biol Psychiatry*, 63(7), 642-649.
- Aid, S., Langenbach, R., & Bosetti, F. (2008). Neuroinflammatory response to lipopolysaccharide is exacerbated in mice genetically deficient in cyclooxygenase-2. *J Neuroinflammation*, 5, 17.
- Alleva, E., & Francia, N. (2009). Psychiatric vulnerability: suggestions from animal models and role of neurotrophins. *Neurosci Biobehav Rev*, 33(4), 525-536.
- Almawi, W. Y., & Melemedjian, O. K. (2002). Negative regulation of nuclear factor-kappaB activation and function by glucocorticoids. *J Mol Endocrinol*, 28(2), 69-78.
- Andre, C., O'Connor, J. C., Kelley, K. W., Lestage, J., Dantzer, R., & Castanon, N. (2008). Spatio-temporal differences in the profile of murine brain expression of proinflammatory cytokines and indoleamine 2,3-dioxygenase in response to peripheral lipopolysaccharide administration. *J Neuroimmunol*, 200(1-2), 90-99.
- Anguelova, M., Benkelfat, C., & Turecki, G. (2003). A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: I. Affective disorders. *Mol Psychiatry*, 8(6), 574-591.
- Anisman, H. (2009). Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. *J Psychiatry Neurosci*, 34(1), 4-20.

- Anisman, H., Gibb, J., & Hayley, S. (2008). Influence of continuous infusion of interleukin-1beta on depression-related processes in mice: corticosterone, circulating cytokines, brain monoamines, and cytokine mRNA expression. *Psychopharmacology (Berl)*, 199(2), 231-244.
- Anisman, H., Hayley, S., Kelly, O., Borowski, T., & Merali, Z. (2001). Psychogenic, neurogenic, and systemic stressor effects on plasma corticosterone and behavior: mouse strain-dependent outcomes. *Behav Neurosci*, 115(2), 443-454.
- Anisman, H., Kokkinidis, L., Borowski, T., & Merali, Z. (1998). Differential effects of interleukin (IL)-1beta, IL-2 and IL-6 on responding for rewarding lateral hypothalamic stimulation. *Brain Res*, 779(1-2), 177-187.
- Anisman, H., & Matheson, K. (2005). Stress, depression, and anhedonia: caveats concerning animal models. *Neurosci Biobehav Rev*, 29(4-5), 525-546.
- Anisman, H., Merali, Z., & Hayley, S. (2008). Neurotransmitter, peptide and cytokine processes in relation to depressive disorder: comorbidity between depression and neurodegenerative disorders. *Prog Neurobiol*, 85(1), 1-74.
- Anisman, H., Merali, Z., & Stead, J. D. (2008). Experiential and genetic contributions to depressive- and anxiety-like disorders: clinical and experimental studies. *Neurosci Biobehav Rev*, 32(6), 1185-1206.
- Anisman, H., & Merali, Z. (1999). Understanding stress: characteristics and caveats. *Alcohol Res Health*. 23(4), 241-249.
- Anisman, H., Poulter, M. O., Gandhi, R., Merali, Z., & Hayley, S. (2007a). Interferon-alpha effects are exaggerated when administered on a psychosocial stressor

backdrop: cytokine, corticosterone and brain monoamine variations. *J Neuroimmunol*, 186(1-2), 45-53.

- Anisman, H., Prakash, P., Merali, Z., & Poulter, M. O. (2007b). Corticotropin releasing hormone receptor alterations elicited by acute and chronic unpredictable stressor challenges in stressor-susceptible and resilient strains of mice. *Behav Brain Res*, 181(2), 180-190.
- Anisman, H., Ravindran, A.V., Griffiths, J., & Merali, Z. (1999). Endocrine and cytokine correlates of major depression and dysthymia with typical or atypical features. *Mol Psychiatry*, 4(2), 182-188.
- Antonijevic, I. A. (2006). Depressive disorders -- is it time to endorse different pathophysiologies? *Psychoneuroendocrinology*, 31(1), 1-15.
- Armario, A., Vallès, A., Dal-Zotto, S., Márquez, C., & Belda, X. (2004). A single exposure to severe stressors causes long-term desensitisation of the physiological response to the homotypic stressor. *Stress*, 7(3), 157-172.
- Audet, M. C., & Anisman, H. (2009). Neuroendocrine and neurochemical impact of aggressive social interactions in submissive and dominant mice: implications for stress-related disorders. *Int J Neuropsychopharmacol*, 1-12.
- Audet, M-C., Mangano, E.N., & Anisman, H. (2010). Behavior and pro-inflammatory cytokine variations among submissive and dominant mice engaged in aggressive encounters: moderation by corticosterone reactivity. *Frontiers Neurosci*, 4, 156.
- Autry, A. E., Adachi, M., Cheng, P., & Monteggia, L. M. (2009). Gender-specific impact of brain-derived neurotrophic factor signaling on stress-induced depression-like behavior. *Biol Psychiatry*, 66(1), 84-90.

- Avital, A., Goshen, I., Kamsler, A., Segal, M., Iverfeldt, K., Richter-Levin, G., et al. (2003). Impaired interleukin-1 signaling is associated with deficits in hippocampal memory processes and neural plasticity. *Hippocampus*, 13(7), 826-834.
- Avitsur, R., Kavelaars, A., Heijnen, C., & Sheridan, J. F. (2005). Social stress and the regulation of tumor necrosis factor-alpha secretion. *Brain Behav Immun*, 19(4), 311-317.
- Avitsur, R., Stark, J. L., & Sheridan, J. F. (2001). Social stress induces glucocorticoid resistance in subordinate animals. *Horm Behav*, 39(4), 247-257.
- Aydemir, O., Deveci, A., & Taneli, F. (2005). The effect of chronic antidepressant treatment on serum brain-derived neurotrophic factor levels in depressed patients: a preliminary study. *Prog Neuropsychopharmacol Biol Psychiatry*, 29(2), 261-265.
- Babcock, T. A., & Carlin, J. M. (2000). Transcriptional activation of indoleamine dioxygenase by interleukin 1 and tumor necrosis factor alpha in interferon-treated epithelial cells. *Cytokine*, 12(6), 588-594.
- Bailey, M. T., Avitsur, R., Engler, H., Padgett, D. A., & Sheridan, J. F. (2004). Physical defeat reduces the sensitivity of murine splenocytes to the suppressive effects of corticosterone. *Brain Behav Immun*, 18(5), 416-424.
- Bailey, M.T., Engler, H., Powell, N.D., Padgett, D.A., & Sheridan, J.F. (2007). Repeated social defeat increases the bactericidal activity of splenic macrophages through a Toll-like receptor-dependent pathway. *Am J Physiol Regul Integr Comp Physiol*, 293, R1180-1190.

- Bailey, M. T., Kinsey, S. G., Padgett, D. A., Sheridan, J. F., & Leblebicioglu, B. (2009). Social stress enhances IL-1beta and TNF-alpha production by Porphyromonas gingivalis lipopolysaccharide-stimulated CD11b+ cells. *Physiol Behav*, 98(3), 351-358.
- Baldwin, A.S. Jr. (1996). The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol*, 14, 649-83.
- Bambico, F. R., Nguyen, N. T., & Gobbi, G. (2009). Decline in serotonergic firing activity and desensitization of 5-HT1A autoreceptors after chronic unpredictable stress. *Eur Neuropsychopharmacol*, 19(3), 215-228.
- Banks, W. A., Farr, S. A., & Morley, J. E. (2002). Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. *Neuroimmunomodulation*, 10(6), 319-327.
- Barnes, P.J. (1998). Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond)*, 94, 557-572.
- Barrientos, R. M., Sprunger, D. B., Campeau, S., Higgins, E. A., Watkins, L. R., Rudy, J. W., et al. (2003). Brain-derived neurotrophic factor mRNA downregulation produced by social isolation is blocked by intrahippocampal interleukin-1 receptor antagonist. *Neuroscience*, 121(4), 847-853.
- Bartolomucci, A. (2005). Resource loss and stress-related disease: is there a link? *Med Sci Monit*, 11(5), RA147-154.
- Bartolomucci, A., & Leopardi, R. (2009). Stress and depression: preclinical research and clinical implications. *PLoS ONE*, 4(1), e4265.

- Bartolomucci, A., Palanza, P., Gaspani, L., Limiroli, E., Panerai, A. E., Ceresini, G., et al. (2001). Social status in mice: behavioral, endocrine and immune changes are context dependent. *Physiol Behav*, 73(3), 401-410.
- Bartolomucci, A., Palanza, P., Parmigiani, S., Pederzani, T., Merlot, E., Neveu, P. J., et al. (2003). Chronic psychosocial stress down-regulates central cytokines mRNA. *Brain Res Bull*, 62(3), 173-178.
- Bartolomucci, A., Palanza, P., Sacerdote, P., Panerai, A. E., Sgoifo, A., Dantzer, R., et al. (2005). Social factors and individual vulnerability to chronic stress exposure. *Neurosci Biobehav Rev*, 29(1), 67-81.
- Bartolomucci, A., Pederzani, T., Sacerdote, P., Panerai, A. E., Parmigiani, S., & Palanza, P. (2004). Behavioral and physiological characterization of male mice under chronic psychosocial stress. *Psychoneuroendocrinology*, 29(7), 899-910.
- Basterzi, A. D., Aydemir, C., Kisa, C., Aksaray, S., Tuzer, V., Yazici, K., et al. (2005). IL-6 levels decrease with SSRI treatment in patients with major depression. *Hum Psychopharmacol*, 20(7), 473-476.
- Bauer, M. E., Papadopoulos, A., Poon, L., Perks, P., Lightman, S. L., Checkley, S., et al. (2003). Altered glucocorticoid immunoregulation in treatment resistant depression. *Psychoneuroendocrinology*, 28(1), 49-65.
- Bay-Richter, C., Janelidze, S., Hallberg, L., & Brundin, L. (2011). Changes in behaviour and cytokine expression upon a peripheral immune challenge. *Behav Brain Res*. 222, 193-199.

- Beekman, M., Flachskamm, C., & Linthorst, A. C. (2005). Effects of exposure to a predator on behaviour and serotonergic neurotransmission in different brain regions of C57bl/6N mice. *Eur J Neurosci*, 21(10), 2825-2836.
- Belzung, C., & Griebel, G. (2001). Measuring normal and pathological anxiety-like behaviour in mice: a review. *Behav Brain Res*, 125(1-2), 141-149.
- Belzung, C., El Hage, W., Moindrot, N., & Griebel, G. (2001). Behavioral and neurochemical changes following predatory stress in mice. *Neuropharmacology*, 41, 400-408.
- Berton, O., Durand, M., Aguerre, S., Mormede, P., & Chaouloff, F. (1999). Behavioral, neuroendocrine and serotonergic consequences of single social defeat and repeated fluoxetine pretreatment in the Lewis rat strain. *Neuroscience*, 92(1), 327-341.
- Bierhaus, A., Wolf, J., Andrassy, M., Rohleder, N., Humpert, P.M., Petrov, D., et al. (2003). A mechanism converting psychosocial stress into mononuclear cell activation. *Proc Natl Acad Sci USA*. 100, 1920-1925.
- Black, P. H., & Garbutt, L. D. (2002). Stress, inflammation and cardiovascular disease. *J Psychosom Res*, 52(1), 1-23.
- Blanchard, D. C., Sakai, R. R., McEwen, B., Weiss, S. M., & Blanchard, R. J. (1993). Subordination stress: behavioral, brain, and neuroendocrine correlates. *Behav Brain Res*, 58(1-2), 113-121.
- Blanchard, R. J., McKittrick, C. R., & Blanchard, D. C. (2001). Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiol Behav*, 73(3), 261-271.

- Blandino, P., Jr., Barnum, C. J., & Deak, T. (2006). The involvement of norepinephrine and microglia in hypothalamic and splenic IL-1beta responses to stress. *J Neuroimmunol*, 173(1-2), 87-95.
- Blier, P. (2001). Pharmacology of rapid-onset antidepressant treatment strategies. *J Clin Psychiatry*, 62 Suppl 15, 12-17.
- Bluthe, R. M., Castanon, N., Pousset, F., Bristow, A., Ball, C., Lestage, J., et al. (1999). Central injection of IL-10 antagonizes the behavioural effects of lipopolysaccharide in rats. *Psychoneuroendocrinology*, 24(3), 301-311.
- Bluthe, R. M., Dantzer, R., & Kelley, K. W. (1992). Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rat. *Brain Res*, 573(2), 318-320.
- Bobrowski, W.F., McDuffie, J.E., Sobocinski, G., Chupka, J., Olle, E., Bowman, A., et al. (2005). Comparative methods for multiplex analysis of cytokine protein expression in plasma of lipopolysaccharide-treated mice. *Cytokine*, 32, 194-198.
- Borowski, T., Kokkinidis, L., Merali, Z., & Anisman, H. (1998). Lipopolysaccharide, central in vivo biogenic amine variations, and anhedonia. *Neuroreport*, 9(17), 3797-3802.
- Brebner, K., Hayley, S., Zacharko, R., Merali, Z., & Anisman, H. (2000). Synergistic effects of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha: central monoamine, corticosterone, and behavioral variations. *Neuropsychopharmacology*, 22(6), 566-580.

- Brenner, G. J., Cohen, N., & Moynihan, J. A. (1994). Similar immune response to nonlethal infection with herpes simplex virus-1 in sensitive (BALB/c) and resistant (C57BL/6) strains of mice. *Cell Immunol*, 157(2), 510-524.
- Brenner, G. J., & Moynihan, J. A. (1997). Stressor-induced alterations in immune response and viral clearance following infection with herpes simplex virus-type 1 in BALB/c and C57BL/6 mice. *Brain Behav Immun*, 11(1), 9-23.
- Brinks, V., van der Mark, M., de Kloet, R., & Oitzl, M. (2007). Emotion and Cognition in High and Low Stress Sensitive Mouse Strains: A Combined Neuroendocrine and Behavioral Study in BALB/c and C57BL/6J Mice. *Front Behav Neurosci*, 1, 8.
- Brown, S. M., Henning, S., & Wellman, C. L. (2005). Mild, short-term stress alters dendritic morphology in rat medial prefrontal cortex. *Cereb Cortex*, 15(11), 1714-1722.
- Brydon, L., Walker, C., Wawrzyniak, A., Whitehead, D., Okamura, H., Yajima, J., et al. (2009). Synergistic effects of psychological and immune stressors on inflammatory cytokine and sickness responses in humans. *Brain Behav Immun*, 23(2), 217-224.
- Brydon, L., Harrison, N.A., Walker, C., Steptoe, A., & Critchley, H.D. (2008). Peripheral inflammation is associated with altered substantia nigra activity and psychomotor slowing in humans. *Biol Psychiatry*, 63(11), 1022-1029.
- Bueller, J. A., Aftab, M., Sen, S., Gomez-Hassan, D., Burmeister, M., & Zubieta, J. K. (2006). BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. *Biol Psychiatry*, 59(9), 812-815.

- Calcagni, E., & Elenkov, I. (2006). Stress system activity, innate and T helper cytokines, and susceptibility to immune-related diseases. *Ann N Y Acad Sci*, 1069, 62-76.
- Capuron, L., Fornwalt, F. B., Knight, B. T., Harvey, P. D., Ninan, P. T., & Miller, A. H. (2009). Does cytokine-induced depression differ from idiopathic major depression in medically healthy individuals? *J Affect Disord*.
- Capuron, L., & Miller, A. H. (2004). Cytokines and psychopathology: lessons from interferon-alpha. *Biol Psychiatry*, 56(11), 819-824.
- Capuron, L., Neuraeter, G., Musselman, D. L., Lawson, D. H., Nemeroff, C. B., Fuchs, D., et al. (2003). Interferon-alpha-induced changes in tryptophan metabolism. relationship to depression and paroxetine treatment. *Biol Psychiatry*, 54(9), 906-914.
- Capuron, L., Raison, C.L., Musselman, D.L., Lawson, D.H., Nemeroff, C.B., & Miller, A.H. (2003). Association of exaggerated HPA axis response to the initial injection of interferon-alpha with development of depression during interferon-alpha therapy. *Am J Psychiatry*, 160(7), 1342-1345.
- Capuron, L., Ravaut, A., Neveu, P. J., Miller, A. H., Maes, M., & Dantzer, R. (2002). Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Mol Psychiatry*, 7(5), 468-473.
- Capuron, L., Pagnoni, G., Demetrashvili, M.F., Lawson, D.H., Fornwalt, F.B., Woolwine, B., et al. (2007). Basal ganglia hypermetabolism and symptoms of fatigue during interferon-alpha therapy. *Neuropsychopharmacology*, 32(11), 2384-2392.

- Carobrez, S. G., Gasparotto, O. C., Buwalda, B., & Bohus, B. (2002). Long-term consequences of social stress on corticosterone and IL-1beta levels in endotoxin-challenged rats. *Physiol Behav*, 76(1), 99-105.
- Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*, 301(5631), 386-389.
- Castanon, N., Leonard, B. E., Neveu, P. J., & Yirmiya, R. (2002). Effects of antidepressants on cytokine production and actions. *Brain Behav Immun*, 16(5), 569-574.
- Chen, B., Dowlatshahi, D., MacQueen, G. M., Wang, J. F., & Young, L. T. (2001). Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiatry*, 50(4), 260-265.
- Chen, M., Ona, V. O., Li, M., Ferrante, R. J., Fink, K. B., Zhu, S., et al. (2000). Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med*, 6(7), 797-801.
- Chizzolini, C., Chicheportiche, R., Burger, D., & Dayer, J. M. (1997). Human Th1 cells preferentially induce interleukin (IL)-1beta while Th2 cells induce IL-1 receptor antagonist production upon cell/cell contact with monocytes. *Eur J Immunol*, 27(1), 171-177.
- Choi, Y., Kim, H. S., Shin, K. Y., Kim, E. M., Kim, M., Park, C. H., et al. (2007). Minocycline attenuates neuronal cell death and improves cognitive impairment in Alzheimer's disease models. *Neuropsychopharmacology*, 32(11), 2393-2404.

- Choleris, E., Thomas, A.W., Kavaliers, M., & Prato, F.S. (2001). A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. *Neurosci Biobehav Rev*, 25(3), 235-260.
- Chourbaji, S., Hellweg, R., Brandis, D., Zorner, B., Zacher, C., Lang, U. E., et al. (2004). Mice with reduced brain-derived neurotrophic factor expression show decreased choline acetyltransferase activity, but regular brain monoamine levels and unaltered emotional behavior. *Brain Res Mol Brain Res*, 121(1-2), 28-36.
- Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nat Rev Endocrinol*, 5(7), 374-381.
- Cognasse, F., Lafarge, S., Chavarin, P., Acquart, S., & Garraud, O. (2007). Lipopolysaccharide induces sCD40L release through human platelets TLR4, but not TLR2 and TLR9. *Intensive Care Med*. 33(2), 382-384.
- Connor, T. J., Brewer, C., Kelly, J. P., & Harkin, A. (2005). Acute stress suppresses pro-inflammatory cytokines TNF-alpha and IL-1 beta independent of a catecholamine-driven increase in IL-10 production. *J Neuroimmunol*, 159(1-2), 119-128.
- Craft, T.K., & DeVries, A.C. (2006). Role of IL-1 in poststroke depressive-like behavior in mice. *Biol Psychiat*, 60, 812-818.
- Crawley, J. N., Belknap, J. K., Collins, A., Crabbe, J. C., Frankel, W., Henderson, N., et al. (1997). Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology (Berl)*, 132(2), 107-124.

- Cunha, A. B., Frey, B. N., Andreazza, A. C., Goi, J. D., Rosa, A. R., Goncalves, C. A., et al. (2006). Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. *Neurosci Lett*, 398(3), 215-219.
- Cunningham, C., Champion, S., Teeling, J., Felton, L., & Perry, V. H. (2007). The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). *Brain Behav Immun*, 21(4), 490-502.
- Cunningham, C., Champion, S., Lunnon, K., Murray, C.L., Woods, J.F., Deacon, R.M., Rawlins, J.N., Perry, V.H., et al. (2009). Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. *Biol Psychiatry*. 65(4), 304-312.
- Dalla, C., Pitychoutis, P.M., Kokras, N., & Papadopoulou-Daifoti, Z. (2011). Sex differences in response to stress and expression of depressive-like behaviours in the rat. *Curr Top Behav Neurosci*, 8, 97-118.
- Dantzer, R. (2001). Cytokine-induced sickness behavior: mechanisms and implications. *Ann N Y Acad Sci*, 933, 222-234.
- Dantzer, R. (2009). Cytokine, sickness behavior, and depression. *Immunol Allergy Clin North Am*, 29(2), 247-264.
- Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*, 9(1), 46-56.

- Dantzer, R., O'Connor, J.C., Lawson, M.A., & Kelley, K.W. (2011). Inflammation-associated depression: From serotonin to kynurenine. *Psychoneuroendocrinology*, 36, 426-436.
- Datta, S.C., & Opp, M.R. (2008). Lipopolysaccharide-induced increases in cytokines in discrete mouse brain regions are detectable using Luminex xMAP technology. *J Neurosci Methods*, 175, 199-124.
- Day, H. E., Curran, E. J., Watson, S. J., Jr., & Akil, H. (1999). Distinct neurochemical populations in the rat central nucleus of the amygdala and bed nucleus of the stria terminalis: evidence for their selective activation by interleukin-1beta. *J Comp Neurol*, 413(1), 113-128.
- Deak, T., Bellamy, C., & Bordner, K. A. (2005). Protracted increases in core body temperature and interleukin-1 following acute administration of lipopolysaccharide: implications for the stress response. *Physiol Behav*, 85(3), 296-307.
- de Pablos, R.M., Villaran, R.F., Arguelles, S., Herrera, A.J., Venero, J.L., Ayala, A., Cano, J., Machado, A., et al. (2006). Stress Increases Vulnerability to Inflammation in the Rat Prefrontal Cortex. *J. Neurosci.* 26(21), 5709-5719.
- de Paiva, V.N., Lima, S.N., Fernandes, M.M., Soncini, R., Andrade, C.A., & Giusti-Paiva, A. (2010). Prostaglandins mediate depressive-like behaviour induced by endotoxin in mice. *Behav Brain Res*, 215(1), 146-51.
- Deveci, A., Aydemir, O., Taskin, O., Taneli, F., & Esen-Danaci, A. (2007). Serum BDNF levels in suicide attempters related to psychosocial stressors: a comparative study with depression. *Neuropsychobiology*, 56(2-3), 93-97.

- Dong-Newsom, P., Powell, N. D., Bailey, M. T., Padgett, D. A., & Sheridan, J. F. (2009). Repeated social stress enhances the innate immune response to a primary HSV-1 infection in the cornea and trigeminal ganglia of Balb/c mice. *Brain Behav Immun*.
- Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., et al. (2010). A meta-analysis of cytokines in major depression. *Biol Psychiat*, 67, 446-57.)
- Dulawa, S. C., Holick, K. A., Gundersen, B., & Hen, R. (2004). Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology*, 29(7), 1321-1330.
- Duman, R. S., & Monteggia, L. M. (2006). A neurotrophic model for stress-related mood disorders. *Biol Psychiatry*, 59(12), 1116-1127.
- Dunn, A. J., Swiergiel, A. H., Zhang, H., & Quan, N. (2006). Reduced ingestion of sweetened milk induced by interleukin-1 and lipopolysaccharide is associated with induction of cyclooxygenase-2 in brain endothelia. *Neuroimmunomodulation*, 13(2), 96-104.
- Dunn, A.J., & Vickers, S.L. (1994). Neurochemical and neuroendocrine responses to Newcastle disease virus administration in mice. *Brain Res*, 645, 103-112.
- Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., et al. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, 112(2), 257-269.

- Elenkov, I. J. (2008). Neurohormonal-cytokine interactions: implications for inflammation, common human diseases and well-being. *Neurochem Int*, 52(1-2), 40-51.
- Ellis, S., Mouihate, A., & Pittman, Q.J. (2006). Neonatal programming of the rat neuroimmune response: stimulus specific changes elicited by bacterial and viral mimetics. *J. Physiol.* 571, 695-701.
- Engler, H., Bailey, M. T., Engler, A., Stiner-Jones, L. M., Quan, N., & Sheridan, J. F. (2008). Interleukin-1 receptor type 1-deficient mice fail to develop social stress-associated glucocorticoid resistance in the spleen. *Psychoneuroendocrinology*, 33(1), 108-117.
- Engler, H., Engler, A., Bailey, M. T., & Sheridan, J. F. (2005). Tissue-specific alterations in the glucocorticoid sensitivity of immune cells following repeated social defeat in mice. *J Neuroimmunol*, 163(1-2), 110-119.
- Espinosa-Oliva, A.M., de Pablos, R.M., Villarán, R.F., Argüelles, S., Venero, J.L., Machado, A., et al. (2011). Stress is critical for LPS-induced activation of microglia and damage in the rat hippocampus. *Neurobiol Aging*, 32(1), 85-102.
- Evans, D. L., Charney, D. S., Lewis, L., Golden, R. N., Gorman, J. M., Krishnan, K. R., et al. (2005). Mood disorders in the medically ill: scientific review and recommendations. *Biol Psychiatry*, 58(3), 175-189.
- Feder, A., Nestler, E.J., & Charney, D.S. (2009). Psychobiology and molecular genetics of resilience. *Nat Rev Neurosci*, 10(6), 446-57.
- Flugge, G. (1995). Dynamics of central nervous 5-HT_{1A}-receptors under psychosocial stress. *J Neurosci*, 15(11), 7132-7140.

- Flugge, G., Ahrens, O., & Fuchs, E. (1997). Beta-adrenoceptors in the tree shrew brain. II. Time-dependent effects of chronic psychosocial stress on [125I]iodocyanopindolol bindings sites. *Cell Mol Neurobiol*, 17(4), 417-432.
- Fortier, M. E., Kent, S., Ashdown, H., Poole, S., Boksa, P., & Luheshi, G. N. (2004). The viral mimic, polyinosinic:polycytidylic acid, induces fever in rats via an interleukin-1-dependent mechanism. *Am J Physiol Regul Integr Comp Physiol*, 287(4), R759-766.
- Frank, M.G., Baratta, M.V., Sprunger, D.B., Watkins, L.R., & Maier, S.F. (2007). Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. *Brain Behav Immun*. 21, 47-59.
- Frank, M.G., Miguel, Z.D., Watkins, L.R., & Maier, S.F. (2010). Prior exposure to glucocorticoids sensitizes the neuroinflammatory and peripheral inflammatory responses to E. Coli lipopolysaccharide. *Brain Behav Immun*. 24, 19-30.
- Frenois, F., Moreau, M., O'Connor, J., Lawson, M., Micon, C., Lestage, J., et al. (2007). Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. *Psychoneuroendocrinology*, 32(5), 516-531.
- Fuchs, E., & Flugge, G. (2003). Chronic social stress: effects on limbic brain structures. *Physiol Behav*, 79(3), 417-427.
- Fujigaki, H., Saito, K., Fujigaki, S., Takemura, M., Sudo, K., Ishiguro, H., et al. (2006). The signal transducer and activator of transcription 1alpha and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-

dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB pathways, and synergistic effect of several proinflammatory cytokines. *J Biochem*, 139(4), 655-662.

Gandhi, R., Hayley, S., Gibb, J., Merali, Z., & Anisman, H. (2007). Influence of poly I:C on sickness behaviors, plasma cytokines, corticosterone and central monoamine activity: moderation by social stressors. *Brain Behav Immun*, 21(4), 477-489.

Gatt, J. M., Nemeroff, C. B., Dobson-Stone, C., Paul, R. H., Bryant, R. A., Schofield, P. R., et al. (2009). Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. *Mol Psychiatry*.

Gervasoni, N., Aubry, J. M., Bondolfi, G., Osiek, C., Schwald, M., Bertschy, G., et al. (2005). Partial normalization of serum brain-derived neurotrophic factor in remitted patients after a major depressive episode. *Neuropsychobiology*, 51(4), 234-238.

Gibb, J., Audet, M. C., Hayley, S., & Anisman, H. (2009). Neurochemical and behavioral responses to inflammatory immune stressors. *Front Biosci (Schol Ed)*, 1, 275-295.

Gibb, J., Hayley, S., Gandhi, R., Poulter, M. O., & Anisman, H. (2008). Synergistic and additive actions of a psychosocial stressor and endotoxin challenge: Circulating and brain cytokines, plasma corticosterone and behavioral changes in mice. *Brain Behav Immun*, 22(4), 573-589.

Gillespie, C. F., & Nemeroff, C. B. (2005). Hypercortisolemia and depression. *Psychosom Med*, 67 Suppl 1, S26-28.

- Givalois, L., Dornand, J., Mekaouche, M., Solier, M.D., Bristow, A.F., Ixart, G., Siaud, P., Assenmacher, I., Barbanel, G., et al. (1994). Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. *Am. J. Physiol.* 267, R164-170.
- Godbout, J. P., Berg, B. M., Krzyszton, C., & Johnson, R. W. (2005). Alpha-tocopherol attenuates NFkappaB activation and pro-inflammatory cytokine production in brain and improves recovery from lipopolysaccharide-induced sickness behavior. *J Neuroimmunol*, 169(1-2), 97-105.
- Goehler, L. E., Erisir, A., & Gaykema, R. P. (2006). Neural-immune interface in the rat area postrema. *Neuroscience*, 140(4), 1415-1434.
- Gonul, A. S., Akdeniz, F., Taneli, F., Donat, O., Eker, C., & Vahip, S. (2005). Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. *Eur Arch Psychiatry Clin Neurosci*, 255(6), 381-386.
- Gorman, J.M. (1996). Comorbid depression and anxiety spectrum disorders. *Depress Anxiety*, 4(4), 160-168.
- Goshen, I., Kreisel, T., Ben-Menachem-Zidon, O., Licht, T., Weidenfeld, J., Ben-Hur, T., 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.
- Goujon, E., Parnet, P., Laye, S., Combe, C., Kelley, K. W., & Dantzer, R. (1995). Stress downregulates lipopolysaccharide-induced expression of proinflammatory cytokines in the spleen, pituitary, and brain of mice. *Brain Behav Immun*, 9(4), 292-303.

- Griebel, G., Belzung, C., Perrault, G., Sanger, D.J. (2000). Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology*, 148, 164-170.
- Guan, Z., & Fang, J. (2006). Peripheral immune activation by lipopolysaccharide decreases neurotrophins in the cortex and hippocampus in rats. *Brain Behav Immun*, 20(1), 64-71.
- Haenisch, B., Bilkei-Gorzo, A., Caron, M. G., & Bonisch, H. (2009). Knockout of the norepinephrine transporter and pharmacologically diverse antidepressants prevent behavioral and brain neurotrophin alterations in two chronic stress models of depression. *J Neurochem*, 111(2), 403-416.
- Harden, L.M., du Plessis, I., Roth, J., Loram, L.C., Poole, S., & Laburn, H.P. (2011). Differences in the relative involvement of peripherally released interleukin (IL)-6, brain IL-1 β and prostanoids in mediating lipopolysaccharide-induced fever and sickness behavior. *Psychoneuroendocrinology*, 36(5), 608-622.
- Hariri, A. R., Goldberg, T. E., Mattay, V. S., Kolachana, B. S., Callicott, J. H., Egan, M. F., et al. (2003). Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci*, 23(17), 6690-6694.
- Harrison, N. A., Brydon, L., Walker, C., Gray, M. A., Steptoe, A., Dolan, R. J., et al. (2009). Neural origins of human sickness in interoceptive responses to inflammation. *Biol Psychiatry*, 66(5), 415-422.
- Hart, B. L. (1988). Biological basis of the behavior of sick animals. *Neurosci Biobehav Rev*, 12(2), 123-137.

- Hayley, S., Borowski, T., Merali, Z., & Anisman, H. (2001). Central monoamine activity in genetically distinct strains of mice following a psychogenic stressor: effects of predator exposure. *Brain Res*, 892(2), 293-300.
- Hayley, S., Brebner, K., Lacosta, S., Merali, Z., & Anisman, H. (1999). Sensitization to the effects of tumor necrosis factor- α : neuroendocrine, central monoamine, and behavioral variations. *J Neurosci*, 19(13), 5654-5665.
- Hayley, S., Mangano, E., Strickland, M., & Anisman, H. (2008). Lipopolysaccharide and a social stressor influence behaviour, corticosterone and cytokine levels: divergent actions in cyclooxygenase-2 deficient mice and wild type controls. *J Neuroimmunol*, 197(1), 29-36.
- Heim, C., Newport, D.J., Bonsall, R., Miller, A.H., & Nemeroff, C.B. (2001). Altered pituitary-adrenal axis responses to provocative challenge tests in adult survivors of childhood abuse. *Am J Psychiatry*, 158, 575–581.
- Heim, C., Newport, D.J., Heit, S., Graham, Y.P., Wilcox, M., Bonsall, R., et al. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA*, 284, 592–597.
- Heinrich, P.C., Behrmann, I., Haan, S., Hermanns, H.M., Müller-Newen, G., & Schaper, F. (2003). Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J*, 374(Pt 1), 1–20.
- Henry, C. J., Huang, Y., Wynne, A., Hanke, M., Himler, J., Bailey, M. T., et al. (2008). Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. *J Neuroinflammation*, 5, 15.

- Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci*, 20(2), 78-84.
- Ho, B. C., Milev, P., O'Leary, D. S., Librant, A., Andreasen, N. C., & Wassink, T. H. (2006). Cognitive and magnetic resonance imaging brain morphometric correlates of brain-derived neurotrophic factor Val66Met gene polymorphism in patients with schizophrenia and healthy volunteers. *Arch Gen Psychiatry*, 63(7), 731-740.
- Hofer, M., Pagliusi, S. R., Hohn, A., Leibrock, J., & Barde, Y. A. (1990). Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *EMBO J*, 9(8), 2459-2464.
- Holsboer, F. (2001). Stress, hypercortisolism and corticosteroid receptors in depression: implications for therapy. *J Affect Disord*, 62(1-2), 77-91.
- Hoshaw, B. A., Malberg, J. E., & Lucki, I. (2005). Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. *Brain Res*, 1037(1-2), 204-208.
- Hu, B., Hissong, B. D., & Carlin, J. M. (1995). Interleukin-1 enhances indoleamine 2,3-dioxygenase activity by increasing specific mRNA expression in human mononuclear phagocytes. *J Interferon Cytokine Res*, 15(7), 617-624.
- Hu, Z.Q., Zhao, W.H., Shimamura, T. (2006). Mechanisms of strain-dependent development of mast cells from mouse splenocytes. *Immunol Cell Biol*, 84, 184-191.
- Huang, T. L., & Lee, C. T. (2007). T-helper 1/T-helper 2 cytokine imbalance and clinical phenotypes of acute-phase major depression. *Psychiatry Clin Neurosci*, 61(4), 415-420.

- Hutchinson, A. J., Chou, C. L., Israel, D. D., Xu, W., & Regan, J. W. (2009). Activation of EP2 prostanoid receptors in human glial cell lines stimulates the secretion of BDNF. *Neurochem Int*, 54(7), 439-446.
- Ibarguen-Vargas, Y., Surget, A., Vourc'h, P., Leman, S., Andres, C. R., Gardier, A. M., et al. (2009). Deficit in BDNF does not increase vulnerability to stress but dampens antidepressant-like effects in the unpredictable chronic mild stress. *Behav Brain Res*, 202(2), 245-251.
- Jacobsen, J. P., & Mork, A. (2004). The effect of escitalopram, desipramine, electroconvulsive seizures and lithium on brain-derived neurotrophic factor mRNA and protein expression in the rat brain and the correlation to 5-HT and 5-HIAA levels. *Brain Res*, 1024(1-2), 183-192.
- Jacobsen, J. P., & Mork, A. (2006). Chronic corticosterone decreases brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. *Brain Res*, 1110(1), 221-225.
- Janssen, D. G., Caniato, R. N., Verster, J. C., & Baune, B. T. A. (2010). A psychoneuroimmunological review on cytokines involved in antidepressant treatment response. *Hum Psychopharmacol*, 25(3), 201-215.
- Johnson, J. D., Campisi, J., Sharkey, C. M., Kennedy, S. L., Nickerson, M., Greenwood, B. N., et al. (2005). Catecholamines mediate stress-induced increases in peripheral and central inflammatory cytokines. *Neuroscience*, 135(4), 1295-1307.
- Johnson, J.D., O'Connor, K.A., Deak, T., Stark, M., Watkins, L.R., Maier, S.F., et al. (2002). Prior stressor exposure sensitizes LPS-induced cytokine production. *Brain Behav. Immun.* 16, 461-476.

- Juric, D. M., Loncar, D., & Carman-Krzan, M. (2008). Noradrenergic stimulation of BDNF synthesis in astrocytes: mediation via alpha1- and beta1/beta2-adrenergic receptors. *Neurochem Int*, 52(1-2), 297-306.
- Kaestner, F., Hettich, M., Peters, M., Sibrowski, W., Hetzel, G., Ponath, G., et al. (2005). Different activation patterns of proinflammatory cytokines in melancholic and non-melancholic major depression are associated with HPA axis activity. *J Affect Disord*, 87(2-3), 305-311.
- Karege, F., Perret, G., Bondolfi, G., Schwald, M., Bertschy, G., & Aubry, J. M. (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res*, 109(2), 143-148.
- Karege, F., Vaudan, G., Schwald, M., Perroud, N., & La Harpe, R. (2005). Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. *Brain Res Mol Brain Res*, 136(1-2), 29-37.
- Katafuchi, T., Kondo, T., Yasaka, T., Kubo, K., Take, S., Yoshimura, M., et al. (2003). Prolonged effects of polyribonucleosinic: polyribocytidylic acid on spontaneous running wheel activity and brain interferon-alpha mRNA in rats: a model for immunologically induced fatigue. *Neuroscience* 120, 837-845.
- Kaufman, J., Yang, B. Z., Douglas-Palumberi, H., Houshyar, S., Lipschitz, D., Krystal, J. H., et al. (2004). Social supports and serotonin transporter gene moderate depression in maltreated children. *Proc Natl Acad Sci U S A*, 101(49), 17316-17321.
- Kaufman, J., Yang, B. Z., Douglas-Palumberi, H., Grasso, D., Lipschitz, D., Houshyar, S., et al. (2006). Brain-derived neurotrophic factor-5-HTTLPR gene interactions

and environmental modifiers of depression in children. *Biol Psychiatry*, 59(8), 673-680.

Keeney, A., Jessop, D. S., Harbuz, M. S., Marsden, C. A., Hogg, S., & Blackburn-Munro, R. E. (2006). Differential effects of acute and chronic social defeat stress on hypothalamic-pituitary-adrenal axis function and hippocampal serotonin release in mice. *J Neuroendocrinol*, 18(5), 330-338.

Keeney, A. J., Hogg, S., & Marsden, C. A. (2001). Alterations in core body temperature, locomotor activity, and corticosterone following acute and repeated social defeat of male NMRI mice. *Physiol Behav*, 74(1-2), 177-184.

Kemeny, M. E., & Schedlowski, M. (2007). Understanding the interaction between psychosocial stress and immune-related diseases: a stepwise progression. *Brain Behav Immun*, 21(8), 1009-1018.

Khairova, R. A., Machado-Vieira, R., Du, J., & Manji, H. K. (2009). A potential role for pro-inflammatory cytokines in regulating synaptic plasticity in major depressive disorder. *Int J Neuropsychopharmacol*, 12(4), 561-578.

Kim, H. S., & Suh, Y. H. (2009). Minocycline and neurodegenerative diseases. *Behav Brain Res*, 196(2), 168-179.

Kim, J. M., Stewart, R., Kim, S. W., Yang, S. J., Shin, I. S., Kim, Y. H., et al. (2007). Interactions between life stressors and susceptibility genes (5-HTTLPR and BDNF) on depression in Korean elders. *Biol Psychiatry*, 62(5), 423-428.

Kim, S., Lee, S., Ryu, S., Suk, J., & Park, C. (2002). Comparative analysis of the anxiety-related behaviors in four inbred mice. *Behav Processes*, 60(2), 181-190.

- Kim, Y. K., Lee, H. P., Won, S. D., Park, E. Y., Lee, H. Y., Lee, B. H., et al. (2007). Low plasma BDNF is associated with suicidal behavior in major depression. *Prog Neuropsychopharmacol Biol Psychiatry*, 31(1), 78-85.
- Kim, S.S., Kong, P.J., Kim, B.S., Sheen, D.H., Nam, S.Y., Chun, W., et al. (2004). Inhibitory action of minocycline on lipopolysaccharide-induced release of nitric oxide and prostaglandin E2 in BV2 microglial cells. *Arch Pharm Res*. 27(3), 314-318.
- Konsman, J. P., Parnet, P., & Dantzer, R. (2002). Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci*, 25(3), 154-159.
- Koolhaas, J.M., Meerlo, P., De Boer, S.F., Strubbe, J.H., & Bohus, B. (1997). The temporal dynamics of the stress response. *Neurosci Biobehav Rev*, 21(6), 775-82.
- Koolschijn, P. C., van Haren, N. E., Lensvelt-Mulders, G. J., Hulshoff Pol, H. E., & Kahn, R. S. (2009). Brain volume abnormalities in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. *Hum Brain Mapp*, 30(11), 3719-3735.
- Koponen, E., Rantamaki, T., Voikar, V., Saarelainen, T., MacDonald, E., & Castren, E. (2005). Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. *Cell Mol Neurobiol*, 25(6), 973-980.
- Korte, S. M., Buwalda, B., Meijer, O., De Kloet, E. R., & Bohus, B. (1995). Socially defeated male rats display a blunted adrenocortical response to a low dose of 8-OH-DPAT. *Eur J Pharmacol*, 272(1), 45-50.

- Krishnan, V., & Nestler, E. J. (2008). The molecular neurobiology of depression. *Nature*, 455(7215), 894-902.
- Kronfol, Z., & Remick, D. G. (2000). Cytokines and the brain: implications for clinical psychiatry. *Am J Psychiatry*, 157(5), 683-694.
- Kruszewska, B., Felten, S. Y., & Moynihan, J. A. (1995). Alterations in cytokine and antibody production following chemical sympathectomy in two strains of mice. *J Immunol*, 155(10), 4613-4620.
- Kuang, X., Scofield, V.L., Yan, M., Stoica, G., Liu, N., Wong, P.K., et al. (2009). Attenuation of oxidative stress, inflammation and apoptosis by minocycline prevents retrovirus-induced neurodegeneration in mice. *Brain Res.* 1286, 174-184.
- Kuroda, E., Noguchi, J., Doi, T., Uematsu, S., Akira, S., Yamashita, U. (2007). IL-3 is an important differentiation factor for the development of prostaglandin E2-producing macrophages between C57BL/6 and BALB/c mice. *Eur J Immunol*, 37, 2185-2195.
- Lacosta, S., Merali, Z., & Anisman, H. (1999). Behavioral and neurochemical consequences of lipopolysaccharide in mice: anxiogenic-like effects. *Brain Res.* 818(2), 291-303.
- Lee, B. H., Kim, H., Park, S. H., & Kim, Y. K. (2007). Decreased plasma BDNF level in depressive patients. *J Affect Disord*, 101(1-3), 239-244.
- Lee, S. M., Yune, T. Y., Kim, S. J., Park, D. W., Lee, Y. K., Kim, Y. C., et al. (2003). Minocycline reduces cell death and improves functional recovery after traumatic spinal cord injury in the rat. *J Neurotrauma*, 20(10), 1017-1027.

- LeMay, L.G., Vander, A.J., & Kluger, M.J. (1990). The effects of psychological stress on plasma interleukin-6 activity in rats. *Physiol. Behav.* 47, 957-961.
- Leonard, B. E., & Myint, A. (2009). The psychoneuroimmunology of depression. *Hum Psychopharmacol*, 24(3), 165-175.
- Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274(5292), 1527-1531.
- Lestage, J., Verrier, D., Palin, K., & Dantzer, R. (2002). The enzyme indoleamine 2,3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen. *Brain Behav Immun.* 16(5), 596-601.
- Levine, J., Cholestoy, A., & Zimmerman, J. (1996). Possible antidepressant effect of minocycline. *Am J Psychiatry.* 153(4), 582.
- Linthorst, A. C., Flachskamm, C., Muller-Preuss, P., Holsboer, F., & Reul, J. M. (1995). Effect of bacterial endotoxin and interleukin-1 beta on hippocampal serotonergic neurotransmission, behavioral activity, and free corticosterone levels: an in vivo microdialysis study. *J Neurosci*, 15(4), 2920-2934.
- Linthorst, A. C., & Reul, J. M. (1998). Brain neurotransmission during peripheral inflammation. *Ann N Y Acad Sci*, 840, 139-152.
- Linthorst, A. C., & Reul, J. M. (2008). Stress and the brain: solving the puzzle using microdialysis. *Pharmacol Biochem Behav*, 90(2), 163-173.

- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, 25(4), 402-408.
- Loftis, J. M., Huckans, M., & Morasco, B. J. (2010). Neuroimmune mechanisms of cytokine-induced depression: current theories and novel treatment strategies. *Neurobiol Dis*, 37(3), 519-533.
- Lu, Y.C., Yeh, W.C., & Ohashi, P.S. (2008). LPS/TLR4 signal transduction pathway. *Cytokine*. 42(2), 145-151.
- MacQueen, G. M., Campbell, S., McEwen, B. S., Macdonald, K., Amano, S., Joffe, R. T., et al. (2003). Course of illness, hippocampal function, and hippocampal volume in major depression. *Proc Natl Acad Sci U S A*, 100(3), 1387-1392.
- Maes, M. (1995). Evidence for an immune response in major depression: a review and hypothesis. *Prog Neuropsychopharmacol Biol Psychiatry*, 19(1), 11-38.
- Maes, M., Yirmiya, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., et al. (2009). The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab Brain Dis*, 24(1), 27-53.
- Maes, M., Leonard, B.E., Myint, A.M., Kubera, M., & Verkerk, R. (2011). The new '5-HT' hypothesis of depression: Cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. *Prog Neuropsychopharmacol Biol Psychiat*, 35, 702-21.

- Maes, M., Bosmans, E., De Jongh, R., Kenis, G., Vandoolaeghe, E., & Neels, H. (1997). Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. *Cytokine*, 9(11), 853-858.
- Magarinos, A. M., McEwen, B. S., Flugge, G., & Fuchs, E. (1996). Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *J Neurosci*, 16(10), 3534-3540.
- Mamounas, L. A., Blue, M. E., Siuciak, J. A., & Altar, C. A. (1995). Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. *J Neurosci*, 15(12), 7929-7939.
- Manetti, R., Annunziato, F., Tomasevic, L., Gianni, V., Parronchi, P., Romagnani, S., et al. (1995). Polyinosinic acid: polycytidylic acid promotes T helper type 1-specific immune responses by stimulating macrophage production of interferon-alpha and interleukin-12. *Eur J Immunol*, 25(9), 2656-2660.
- Marano, C. M., Phatak, P., Vemulapalli, U. R., Sasan, A., Nalbandyan, M. R., Ramanujam, S., et al. (2007). Increased plasma concentration of brain-derived neurotrophic factor with electroconvulsive therapy: a pilot study in patients with major depression. *J Clin Psychiatry*, 68(4), 512-517.
- Marques, A. H., Silverman, M. N., & Sternberg, E. M. (2009). Glucocorticoid dysregulations and their clinical correlates. *Ann N Y Acad Sci*, 1179, 1-18.
- Marques-Deak, A.H., Neto, F.L., Dominguez, W.V., Solis, A.C., Kurcgant, D., Sato, F., et al. (2007). Cytokine profiles in women with different subtypes of major depressive disorder. *J Psychiatr Res*, 41(1-2), 152-159.

- Mathers, C. D., Ezzati, M., & Lopez, A. D. (2007). Measuring the burden of neglected tropical diseases: the global burden of disease framework. *PLoS Negl Trop Dis*, 1(2), e114.
- McEwen, B. S. (2008). Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol*, 583(2-3), 174-185.
- McGeer, P. L., & McGeer, E. G. (1995). The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Res Brain Res Rev*, 21(2), 195-218.
- McKay, L.I., & Cidlowski, J.A. (2000). CBP (CREB binding protein) integrates NF-kappaB (nuclear factor-kappaB) and glucocorticoid receptor physical interactions and antagonism. *Mol Endocrinol* 14, 1222-1234.
- McKittrick, C. R., Blanchard, D. C., Blanchard, R. J., McEwen, B. S., & Sakai, R. R. (1995). Serotonin receptor binding in a colony model of chronic social stress. *Biol Psychiatry*, 37(6), 383-393.
- Meerlo, P., Easton, A., Bergmann, B.M., Turek, F.W. (2001). Restraint increases prolactin and REM sleep in C57Bl/6J mice but not in BALB/cJ mice. *Am J Physiol Regul Integr Comp Physiol*, 281, R846-854.
- Mendlewicz, J., Kriwin, P., Oswald, P., Souery, D., Alboni, S., & Brunello, N. (2006). Shortened onset of action of antidepressants in major depression using acetylsalicylic acid augmentation: a pilot open-label study. *Int Clin Psychopharmacol*, 21(4), 227-231.

- Merali, Z., Brennan, K., Brau, P., & Anisman, H. (2003). Dissociating anorexia and anhedonia elicited by interleukin-1beta: antidepressant and gender effects on responding for "free chow" and "earned" sucrose intake. *Psychopharmacology (Berl)*, 165(4), 413-418.
- Merali, Z., Lacosta, S., & Anisman, H. (1997). Effects of interleukin-1beta and mild stress on alterations of norepinephrine, dopamine and serotonin neurotransmission: a regional microdialysis study. *Brain Res*, 761(2), 225-235.
- Merlot, E., Moze, E., Dantzer, R., & Neveu, P. J. (2003). Importance of fighting in the immune effects of social defeat. *Physiol Behav*, 80(2-3), 351-357.
- Merlot, E., Moze, E., Dantzer, R., & Neveu, P. J. (2004). Cytokine production by spleen cells after social defeat in mice: activation of T cells and reduced inhibition by glucocorticoids. *Stress*, 7(1), 55-61.
- Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry*, 65(9), 732-741.
- Miller, G. E., Chen, E., Sze, J., Marin, T., Arevalo, J. M., Doll, R., et al. (2008). A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-kappaB signaling. *Biol Psychiatry*, 64(4), 266-272.
- Miyamoto, S., & Verma, I. M. (1995). Rel/NF-kappa B/I kappa B story. *Adv Cancer Res*, 66, 255-292.
- Molina-Hernandez, M., Tellez-Alcantara, N. P., Perez-Garcia, J., Olivera-Lopez, J. I., & Jaramillo-Jaimes, M. T. (2008a). Antidepressant-like actions of minocycline

combined with several glutamate antagonists. *Prog Neuropsychopharmacol Biol Psychiatry*, 32(2), 380-386.

Molina-Hernandez, M., Tellez-Alcantara, N. P., Perez-Garcia, J., Olivera-Lopez, J. I., & Jaramillo-Jaimes, M. T. (2008b). Desipramine or glutamate antagonists synergized the antidepressant-like actions of intra-nucleus accumbens infusions of minocycline in male Wistar rats. *Prog Neuropsychopharmacol Biol Psychiatry*, 32(7), 1660-1666.

Molteni, R., Calabrese, F., Cattaneo, A., Mancini, M., Gennarelli, M., Racagni, G., et al. (2009). Acute stress responsiveness of the neurotrophin BDNF in the rat hippocampus is modulated by chronic treatment with the antidepressant duloxetine. *Neuropsychopharmacology*, 34(6), 1523-1532.

Moncrieff, J., & Kirsch, I. (2005). Efficacy of antidepressants in adults. *BMJ*, 331(7509), 155-157.

Montag, C., Weber, B., Fliessbach, K., Elger, C., & Reuter, M. (2009). The BDNF Val66Met polymorphism impacts parahippocampal and amygdala volume in healthy humans: incremental support for a genetic risk factor for depression. *Psychol Med*, 1-9.

Monteggia, L. M., Barrot, M., Powell, C. M., Berton, O., Galanis, V., Gemelli, T., et al. (2004). Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc Natl Acad Sci U S A*, 101(29), 10827-10832.

Monteggia, L. M., Luikart, B., Barrot, M., Theobald, D., Malkovska, I., Nef, S., et al. (2007). Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biol Psychiatry*, 61(2), 187-197.

- Moreau, M., Andre, C., O'Connor, J. C., Dumich, S. A., Woods, J. A., Kelley, K. W., et al. (2008). Inoculation of *Bacillus Calmette-Guerin* to mice induces an acute episode of sickness behavior followed by chronic depressive-like behavior. *Brain Behav Immun*, 22(7), 1087-1095.
- Morita, I. (2002). Distinct functions of COX-1 and COX-2. *Prostaglandins Other Lipid Mediat*, 68-69, 165-175.
- Mossner, R., Mikova, O., Koutsilieri, E., Saoud, M., Ehlis, A. C., Muller, N., et al. (2007). Consensus paper of the WFSBP Task Force on Biological Markers: biological markers in depression. *World J Biol Psychiatry*, 8(3), 141-174.
- Muller, N., & Schwarz, M. J. (2007). The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. *Mol Psychiatry*, 12(11), 988-1000.
- Muller, N., Schwarz, M. J., Dehning, S., Douhe, A., Cerovecki, A., Goldstein-Muller, B., et al. (2006). The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Mol Psychiatry*, 11(7), 680-684.
- Munhoz, C.D., Lepsch, L.B., Kawamoto, E.M., Malta, M.B., de Sa Lima, L., Werneck Avellar, M.C., Sapolsky, R.M., Scavone, C., et al. (2006). Chronic Unpredictable Stress Exacerbates Lipopolysaccharide-Induced Activation of Nuclear Factor- κ B in the Frontal Cortex Hippocampus via Glucocorticoid Secretion. *J. Neurosci*. 26(14), 3813-3820.

- Murakami, S., Imbe, H., Morikawa, Y., Kubo, C., & Senba, E. (2005). Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neurosci Res*, 53(2), 129-139.
- Murali, R., Hanson, M. D., & Chen, E. (2007). Psychological stress and its relationship to cytokines and inflammatory diseases. In N. P. Plotnikoff, R. E. Faith, A. J. Murgu & R. A. Good (Eds.), *Cytokines: Stress and Immunity* (2nd ed., pp. 29-49). Boca Raton: CRC Press: Taylor & Francis Group.
- Musselman, D. L., Lawson, D. H., Gumnick, J. F., Manatunga, A. K., Penna, S., Goodkin, R. S., et al. (2001). Paroxetine for the prevention of depression induced by high-dose interferon alfa. *N Engl J Med*, 344(13), 961-966.
- Myint, A. M., Schwarz, M. J., Steinbusch, H. W., & Leonard, B. E. (2009). Neuropsychiatric disorders related to interferon and interleukins treatment. *Metab Brain Dis*, 24(1), 55-68.
- Nadeau, S., & Rivest, S. (2003). Glucocorticoids play a fundamental role in protecting the brain during innate immune response. *J. Neurosci.* 23, 5536-5544.
- Nadjar, A., Bluthe, R. M., May, M. J., Dantzer, R., & Parnet, P. (2005). Inactivation of the cerebral NFkappaB pathway inhibits interleukin-1beta-induced sickness behavior and c-Fos expression in various brain nuclei. *Neuropsychopharmacology*, 30(8), 1492-1499.
- Nair, A., & Bonneau, R.H. (2006). Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. *J. Neuroimmunol.* 171, 72-85.

- Nemeroff, C. B., Krishnan, K. R., Reed, D., Leder, R., Beam, C., & Dunnick, N. R. (1992). Adrenal gland enlargement in major depression. A computed tomographic study. *Arch Gen Psychiatry*, 49(5), 384-387.
- Nessler, S., Dodel, R., Bittner, A., Reuss, S., Du, Y., Hemmer, B., et al. (2002). Effect of minocycline in experimental autoimmune encephalomyelitis. *Ann Neurol*, 52(5), 689-690; author reply 690.
- Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of depression. *Neuron*, 34(1), 13-25.
- Nguyen, K. T., Deak, T., Will, M. J., Hansen, M. K., Hunsaker, B. N., Fleshner, M., et al. (2000). Timecourse and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1beta protein response to acute stress. *Brain Res*, 859(2), 193-201.
- Nguyen, K. T., Deak, T., Owens, S.M., Kohno, T., Fleshner, M., Watkins, L.R., Maier, S.F., et al. (1998). Exposure to acute stress induces brain interleukin-1 β protein in the rat. *J. Neurosci.* 19, 2799-2805.
- Nibuya, M., Morinobu, S., & Duman, R. S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci*, 15(11), 7539-7547.
- Nikodemova, M., Duncan, I.D., & Watters, J.J. (2006). Minocycline exerts inhibitory effects on multiple mitogen-activated protein kinases and IkappaBalpha degradation in a stimulus-specific manner in microglia. 96(2), 314-323.
- Norcross, M., Mathur, P., Enoch, A. J., Karlsson, R. M., Brigman, J. L., Cameron, H. A., et al. (2008). Effects of adolescent fluoxetine treatment on fear-, anxiety- or

- stress-related behaviors in C57BL/6J or BALB/cJ mice. *Psychopharmacology* (Berl), 200(3), 413-424.
- Nutt, D. J. (2002). The neuropharmacology of serotonin and noradrenaline in depression. *Int Clin Psychopharmacol*, 17 Suppl 1, S1-12.
- O'Connor, J. C., Lawson, M. A., Andre, C., Moreau, M., Lestage, J., Castanon, N., et al. (2009). Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry*, 14(5), 511-522.
- O'Connor, K. A., Johnson, J. D., Hansen, M. K., Wieseler Frank, J. L., Maksimova, E., Watkins, L. R., et al. (2003). Peripheral and central proinflammatory cytokine response to a severe acute stressor. *Brain Res*, 991(1-2), 123-132.
- O'Connor, T. M., O'Halloran, D. J., & Shanahan, F. (2000). The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. *QJM*, 93(6), 323-333.
- Pace, T. W., Hu, F., & Miller, A. H. (2007). Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. *Brain Behav Immun*, 21(1), 9-19.
- Pace, T. W., & Miller, A. H. (2009). Cytokines and glucocorticoid receptor signaling. *Ann N Y Acad Sci*, 1179, 86-105.
- Pace, T.W.W., Mletzko, T.C., Alagbe, O., Musselman, D.L., Nemeroff, C.B., Miller, A.H., & Heim, C.M. (2006). Increased Stress-Induced Inflammatory Responses in Male Patients With Major Depression and Increased Early Life Stress. *Am J Psychiatry*, 163, 1630-1633.

- Parker, K. J., Schatzberg, A. F., & Lyons, D. M. (2003). Neuroendocrine aspects of hypercortisolism in major depression. *Horm Behav*, 43(1), 60-66.
- Pascoe, M.C., Crewther, S.G., Carey, L.M., & Crewther, D.P. (2011). Inflammation and depression: why poststroke depression may be the norm and not the exception. *Int J Stroke*, 6, 128-35.
- Pezawas, L., Verchinski, B. A., Mattay, V. S., Callicott, J. H., Kolachana, B. S., Straub, R. E., et al. (2004). The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. *J Neurosci*, 24(45), 10099-10102.
- Piccinni, A., Del Debbio, A., Medda, P., Bianchi, C., Roncaglia, I., Veltri, A., et al. (2009). Plasma Brain-Derived Neurotrophic Factor in treatment-resistant depressed patients receiving electroconvulsive therapy. *Eur Neuropsychopharmacol*, 19(5), 349-355.
- Piletz, J. E., Halaris, A., Iqbal, O., Hoppensteadt, D., Fareed, J., Zhu, H., et al. (2009). Pro-inflammatory biomarkers in depression: treatment with venlafaxine. *World J Biol Psychiatry*, 10(4), 313-323.
- Pizarro, J. M., Lumley, L. A., Medina, W., Robison, C. L., Chang, W. E., Alagappan, A., et al. (2004). Acute social defeat reduces neurotrophin expression in brain cortical and subcortical areas in mice. *Brain Res*, 1025(1-2), 10-20.
- Planas, A.M., Gorina, R., & Chamorro, A. (2006). Signalling pathways mediating inflammatory responses in brain ischaemia. *Biochem. Soc. Trans.* 4, 1267-1270.

- Popovic, N., Schubart, A., Goetz, B. D., Zhang, S. C., Linington, C., & Duncan, I. D. (2002). Inhibition of autoimmune encephalomyelitis by a tetracycline. *Ann Neurol*, 51(2), 215-223.
- Powell, N. D., Bailey, M. T., Mays, J. W., Stiner-Jones, L. M., Hanke, M. L., Padgett, D. A., et al. (2009). Repeated social defeat activates dendritic cells and enhances Toll-like receptor dependent cytokine secretion. *Brain Behav Immun*, 23(2), 225-231.
- Prakash, P., Merali, Z., Kolajova, M., Tannenbaum, B. M., & Anisman, H. (2006). Maternal factors and monoamine changes in stress-resilient and susceptible mice: cross-fostering effects. *Brain Res*, 1111(1), 122-133.
- Pruett, S.B., Fan, R., & Zheng, Q. (2003). Acute ethanol administration profoundly alters poly 1:C-induced cytokine expression in mice by a mechanism that is not dependent on corticosterone. *Life Sci*. 72, 1825-1839.
- Pugh, C.R., Nguyen, K. T., Gonyea, J.L., Fleshner, M., Watkins, L.R., Maier, S.F., Rudy, J.W., et al. (1999). Role of interleukin-1 beta in impairment of contextual fear conditioning caused by social isolation. *Behav. Brain Res*. 106, 109-118.
- Quan, N., Avitsur, R., Stark, J. L., He, L., Lai, W., Dhabhar, F., et al. (2003). Molecular mechanisms of glucocorticoid resistance in splenocytes of socially stressed male mice. *J Neuroimmunol*, 137(1-2), 51-58.
- Quan, N., Avitsur, R., Stark, J. L., He, L., Shah, M., Caligiuri, M., et al. (2001). Social stress increases the susceptibility to endotoxic shock. *J Neuroimmunol*, 115(1-2), 36-45.

- Quan, N., & Banks, W. A. (2007). Brain-immune communication pathways. *Brain Behav Immun*, 21(6), 727-735.
- Quan, N., He, L., Lai, W., Shen, T., & Herkenham, M. (2000). Induction of I κ B α mRNA expression in the brain by glucocorticoids: a negative feedback mechanism for immune-to-brain signaling. *J. Neurosci.* 20, 6473-6477.
- Raison, C. L., Borisov, A. S., Majer, M., Drake, D. F., Pagnoni, G., Woolwine, B. J., et al. (2009). Activation of central nervous system inflammatory pathways by interferon-alpha: relationship to monoamines and depression. *Biol Psychiatry*, 65(4), 296-303.
- Raison, C. L., Capuron, L., & Miller, A. H. (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol*, 27(1), 24-31.
- Raison, C. L., Dantzer, R., Kelley, K. W., Lawson, M. A., Woolwine, B. J., Vogt, G., et al. (2009). CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. *Mol Psychiatry*.
- Ravnkilde, B., Videbech, P., Clemmensen, K., Egander, A., Rasmussen, N. A., & Rosenberg, R. (2002). Cognitive deficits in major depression. *Scand J Psychol*, 43(3), 239-251.
- Razzoli, M., Carboni, L., & Arban, R. (2009). Alterations of behavioral and endocrinological reactivity induced by 3 brief social defeats in rats: relevance to human psychopathology. *Psychoneuroendocrinology*, 34(9), 1405-1416.

- Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., Morag, A., et al. (2001). Cytokine-associated emotional and cognitive disturbances in humans. *Arch Gen Psychiatry*, 58(5), 445-452.
- Reul, J. M., & Holsboer, F. (2002). Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. *Curr Opin Pharmacol*, 2(1), 23-33.
- Richwine, A. F., Sparkman, N. L., Dilger, R. N., Buchanan, J. B., & Johnson, R. W. (2009). Cognitive deficits in interleukin-10-deficient mice after peripheral injection of lipopolysaccharide. *Brain Behav Immun*, 23(6), 794-802.
- Rivest, S. (2003). Molecular insights on the cerebral innate immune system. *Brain Behav Immun*, 17(1), 13-19.
- Rivest, S. (2009). Regulation of innate immune responses in the brain. *Nat Rev Immunol*, 9, 429-439.
- Rosenkranz, M. A. (2007). Substance P at the nexus of mind and body in chronic inflammation and affective disorders. *Psychol Bull*, 133(6), 1007-1037.
- Rubin, R. T., Phillips, J. J., Sadow, T. F., & McCracken, J. T. (1995). Adrenal gland volume in major depression. Increase during the depressive episode and decrease with successful treatment. *Arch Gen Psychiatry*, 52(3), 213-218.
- Rygula, R., Abumaria, N., Flugge, G., Fuchs, E., Ruther, E., & Havemann-Reinecke, U. (2005). Anhedonia and motivational deficits in rats: impact of chronic social stress. *Behav Brain Res*, 162(1), 127-134.
- Rygula, R., Abumaria, N., Flugge, G., Hiemke, C., Fuchs, E., Ruther, E., et al. (2006). Citalopram counteracts depressive-like symptoms evoked by chronic social stress in rats. *Behav Pharmacol*, 17(1), 19-29.

- Saarelainen, T., Hendolin, P., Lucas, G., Koponen, E., Sairanen, M., MacDonald, E., et al. (2003). Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *J Neurosci*, 23(1), 349-357.
- Sabban, E.L., Serova, L.I. (2007). Influence of prior experience with homotypic or heterotypic stressor on stress reactivity in catecholaminergic systems. *Stress*, 10(2), 137-143.
- Sapolsky, R. M. (2000). Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry*, 57(10), 925-935.
- Schiepers, O. J., Wichers, M. C., & Maes, M. (2005). Cytokines and major depression. *Prog Neuropsychopharmacol Biol Psychiatry*, 29(2), 201-217.
- Schule, C., Baghai, T. C., Eser, D., Hafner, S., Born, C., Herrmann, S., et al. (2009). The combined dexamethasone/CRH Test (DEX/CRH test) and prediction of acute treatment response in major depression. *PLoS One*, 4(1), e4324.
- Schweizer, M. C., Henniger, M. S., & Sillaber, I. (2009). Chronic mild stress (CMS) in mice: of anhedonia, 'anomalous anxiolysis' and activity. *PLoS One*, 4(1), e4326.
- Sen, S., Duman, R., & Sanacora, G. (2008). Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biol Psychiatry*, 64(6), 527-532.
- Shalev, I., Lerer, E., Israel, S., Uzefovsky, F., Gritsenko, I., Mankuta, D., et al. (2009). BDNF Val66Met polymorphism is associated with HPA axis reactivity to psychological stress characterized by genotype and gender interactions. *Psychoneuroendocrinology*, 34(3), 382-388.

- Shanks, N., & Anisman, H. (1993). Escape deficits induced by uncontrollable foot-shock in recombinant inbred strains of mice. *Pharmacol Biochem Behav*, 46(3), 511-517.
- Shanks, N., Griffiths, J., & Anisman, H. (1994). Norepinephrine and serotonin alterations following chronic stressor exposure: mouse strain differences. *Pharmacol Biochem Behav*, 49(1), 57-65.
- Sheline, Y. I. (2000). 3D MRI studies of neuroanatomic changes in unipolar major depression: the role of stress and medical comorbidity. *Biol Psychiatry*, 48(8), 791-800.
- Sheline, Y. I., Gado, M. H., & Kraemer, H. C. (2003). Untreated depression and hippocampal volume loss. *Am J Psychiatry*, 160(8), 1516-1518.
- Sheline, Y. I., Wang, P. W., Gado, M. H., Csernansky, J. G., & Vannier, M. W. (1996). Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci U S A*, 93(9), 3908-3913.
- Shelton, R. C., Claiborne, J., Sidoryk-Wegrzynowicz, M., Reddy, R., Aschner, M., Lewis, D. A., et al. (2010). Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. *Mol Psychiatry*.
- Sheridan, J. F., Stark, J. L., Avitsur, R., & Padgett, D. A. (2000). Social disruption, immunity, and susceptibility to viral infection. Role of glucocorticoid insensitivity and NGF. *Ann N Y Acad Sci*, 917, 894-905.
- Shimizu, E., Hashimoto, K., Okamura, N., Koike, K., Komatsu, N., Kumakiri, C., et al. (2003). Alterations of serum levels of brain-derived neurotrophic factor (BDNF)

in depressed patients with or without antidepressants. *Biol Psychiatry*, 54(1), 70-75.

Shintani, F., Nakaki, T., Kanba, S., Sato, K., Yagi, G., Shiozawa, M., et al. (1995).

Involvement of interleukin-1 in immobilization stress-induced increase in plasma adrenocorticotrophic hormone and in release of hypothalamic monoamines in the rat. *J Neurosci*, 15(3 Pt 1), 1961-1970.

Shirayama, Y., Chen, A. C., Nakagawa, S., Russell, D. S., & Duman, R. S. (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J Neurosci*, 22(8), 3251-3261.

Shively, C. A., Grant, K. A., Ehrenkauf, R. L., Mach, R. H., & Nader, M. A. (1997).

Social stress, depression, and brain dopamine in female cynomolgus monkeys.

Ann N Y Acad Sci, 807, 574-577.

Siuciak, J. A., Boylan, C., Fritsche, M., Altar, C. A., & Lindsay, R. M. (1996). BDNF

increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. *Brain Res*, 710(1-2), 11-20.

Siuciak, J. A., Clark, M. S., Rind, H. B., Whittemore, S. R., & Russo, A. F. (1998).

BDNF induction of tryptophan hydroxylase mRNA levels in the rat brain. *J*

Neurosci Res, 52(2), 149-158.

Siuciak, J. A., Lewis, D. R., Wiegand, S. J., & Lindsay, R. M. (1997). Antidepressant-

like effect of brain-derived neurotrophic factor (BDNF). *Pharmacol Biochem*

Behav, 56(1), 131-137.

Skultetyova, I., Tokarev, D., & Jezova, D. (1998). Stress-induced increase in blood-brain

- barrier permeability in control and monosodium glutamate-treated rats. *Brain Res. Bull.* 45, 175-178.
- Song, C., Merali, Z., & Anisman, H. (1999). Variations of nucleus accumbens dopamine and serotonin following systemic interleukin-1, interleukin-2 or interleukin-6 treatment. *Neuroscience*, 88(3), 823-836.
- Sorrells, S.F., & Sapolsky, R.M. (2007). An inflammatory review of glucocorticoid actions in the CNS. *Brain Behav. Immun.* 21, 259-272.
- Southwick, S. M., Vythilingam, M. & Charney, D. S. (2005). The psychobiology of depression and resilience to stress: implications for prevention and treatment. *Annu Rev Clin Psychol*, 1, 255–291.
- Staikos, L., Malellari, L., & Chang, S.L. (2008). Lipopolysaccharide-induced pro-inflammatory cytokines in the brain of rats in the morphine-tolerant state. *J. Neuroimmune Pharmacol.* 3, 236-240.
- Stark, J. L., Avitsur, R., Padgett, D. A., Campbell, K. A., Beck, F. M., & Sheridan, J. F. (2001). Social stress induces glucocorticoid resistance in macrophages. *Am J Physiol Regul Integr Comp Physiol*, 280(6), R1799-1805.
- Stefanski, V. (2001). Social stress in laboratory rats: behaviour, immune function, and tumor metastasis. *Physio. Behav.* 73, 385-391.
- Stefanski, V. (2000). Social stress in laboratory rats: hormonal responses and immune cell distribution. *Psychoneuroendocrinology*, 25(4), 389-406.
- Stefanski, V., & Engler, H. (1999). Social stress, dominance and blood cellular immunity. *J Neuroimmunol*, 94(1-2), 144-152.

- Steptoe, A., Hamer, M., & Chida, Y. (2007). The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain Behav Immun*, 21(7), 901-912.
- Stirling, D. P., Koochesfahani, K. M., Steeves, J. D., & Tetzlaff, W. (2005). Minocycline as a neuroprotective agent. *Neuroscientist*, 11(4), 308-322.
- Stoll, G., Jander, S., & Schroeter, M. (2002). Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. *Adv Exp Med Biol*, 513, 87-113.
- Swiergiel, A. H., & Dunn, A. J. (2002). Distinct roles for cyclooxygenases 1 and 2 in interleukin-1-induced behavioral changes. *J Pharmacol Exp Ther*, 302(3), 1031-1036.
- Swiergiel, A. H., & Dunn, A. J. (2007). Effects of interleukin-1beta and lipopolysaccharide on behavior of mice in the elevated plus-maze and open field tests. *Pharmacol Biochem Behav*, 86(4), 651-659.
- Szeszko, P. R., Lipsky, R., Mentschel, C., Robinson, D., Gunduz-Bruce, H., Sevy, S., et al. (2005). Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. *Mol Psychiatry*, 10(7), 631-636.
- Taishi, P., Churchill, L., De, A., Obal, F., Jr., & Krueger, J. M. (2008). Cytokine mRNA induction by interleukin-1beta or tumor necrosis factor alpha in vitro and in vivo. *Brain Res*, 1226, 89-98.
- Tasker, J.G., & Herman, J.P. (2011). Mechanisms of rapid glucocorticoid feedback inhibition of the hypothalamic-pituitary-adrenal axis. *Stress*, 14(4), 398-406.

- Teeling, J. L., Cunningham, C., Newman, T. A., & Perry, V. H. (2009). The effect of non-steroidal anti-inflammatory agents on behavioural changes and cytokine production following systemic inflammation: Implications for a role of COX-1. *Brain Behav Immun*.
- Teeling, J. L., Felton, L. M., Deacon, R. M., Cunningham, C., Rawlins, J. N., & Perry, V. H. (2007). Sub-pyrogenic systemic inflammation impacts on brain and behavior, independent of cytokines. *Brain Behav Immun*, 21(6), 836-850.
- Town, T., Jeng, D., Alexopoulou, L., Tan, J., & Flavell, R.A. (2006). Microglia recognize double-stranded RNA via TLR3. *J. Immunol.* 176, 3804-3812.
- Turrin, N. P., Gayle, D., Ilyin, S. E., Flynn, M. C., Langhans, W., Schwartz, G. J., et al. (2001). Pro-inflammatory and anti-inflammatory cytokine mRNA induction in the periphery and brain following intraperitoneal administration of bacterial lipopolysaccharide. *Brain Res Bull*, 54(4), 443-453.
- Turrin, N. P., & Plata-Salaman, C. R. (2000). Cytokine-cytokine interactions and the brain. *Brain Res Bull*, 51(1), 3-9.
- Unlap, M.T., & Jope, R.S. (1997). Dexamethasone attenuates NF-kappa B DNA binding activity without inducing I kappa B levels in rat brain in vivo. *Brain Res Mol Brain Res.* 45, 83-89.
- Uzun, S., Kozumplik, O., Topic, R., & Jakovljevic, M. (2009). Depressive disorders and comorbidity: somatic illness vs. side effect. *Psychiatr Danub*, 21(3), 391-398.
- Verhagen, M., van der Meij, A., van Deurzen, P. A., Janzing, J. G., Arias-Vasquez, A., Buitelaar, J. K., et al. (2008). Meta-analysis of the BDNF Val66Met

- polymorphism in major depressive disorder: effects of gender and ethnicity. *Mol Psychiatry*.
- Verma, S., Nakaoke, R., Dohgu, S., & Banks, W.A. (2006). Release of cytokines by brain endothelial cells: a polarized response to lipopolysaccharide. *Brain Behav. Immun.* 20, 449-455.
- Vermeulen, L., Vanden Berghe, W., & Haegeman, G. (2006). Regulation of NF-kappaB transcriptional activity. *Cancer Treat Res*, 130, 89-102.
- Von Frijtag, J. C., Van den Bos, R., & Spruijt, B. M. (2002). Imipramine restores the long-term impairment of appetitive behavior in socially stressed rats. *Psychopharmacology (Berl)*, 162(3), 232-238.
- Wang, X., Zhu, S., Drozda, M., Zhang, W., Stavrovskaya, I. G., Cattaneo, E., et al. (2003). Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. *Proc Natl Acad Sci U S A*, 100(18), 10483-10487.
- Wang, J., & Campbell, I.L. (2002). Cytokine signalling in the brain: putting a SOCS in it? *J. Neurosci. Res.* 67, 423-427.
- Wann, B. P., Audet, M. C., Gibb, J., & Anisman, H. (2010). Anhedonia and altered cardiac atrial natriuretic peptide following chronic stressor and endotoxin treatment in mice. *Psychoneuroendocrinology*, 35, 233-240.
- Watanabe, H., Numata, K., Ito, T., Takagi, K., & Matsukawa, A. (2004). Innate immune response in Th1- and Th2-dominant mouse strains. *Shock*, 22(5), 460-466.
- Watanabe, Y., Gould, E., & McEwen, B. S. (1992). Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Res*, 588(2), 341-345.

- Wieczorek, M., Swiergiel, A. H., Pournajafi-Nazarloo, H., & Dunn, A. J. (2005). Physiological and behavioral responses to interleukin-1beta and LPS in vagotomized mice. *Physiol Behav*, 85(4), 500-511.
- Wilhelm, K., Mitchell, P. B., Niven, H., Finch, A., Wedgwood, L., Scimone, A., et al. (2006). Life events, first depression onset and the serotonin transporter gene. *Br J Psychiatry*, 188, 210-215.
- Wright, C. E., Strike, P. C., Brydon, L., & Steptoe, A. (2005). Acute inflammation and negative mood: mediation by cytokine activation. *Brain Behav Immun*, 19(4), 345-350.
- Wu, D. C., Jackson-Lewis, V., Vila, M., Tieu, K., Teismann, P., Vadseth, C., et al. (2002). Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci*, 22(5), 1763-1771.
- Yasukawa, H., Ohishi, M., Mori, H., Murakami, M., Chinen, T., Aki, D., Hanada, T., Takeda, K., Akira, S., Hoshijima, M., Hirano, T., Chien, K.R., Yoshimura, A., et al. (2003). IL-6 induces an anti-inflammatory response in the absence of SOCS3 in macrophages. *Nat. Immunol.* 4, 551-556.
- Yeager, M. P., Rassias, A. J., Pioli, P. A., Beach, M. L., Wardwell, K., Collins, J. E., et al. (2009). Pretreatment with stress cortisol enhances the human systemic inflammatory response to bacterial endotoxin. *Crit Care Med*, 37(10), 2727-2732.
- Yirmiya, R. (1996). Endotoxin produces a depressive-like episode in rats. *Brain Res*, 711(1-2), 163-174.

- Yirmiya, R., Weidenfeld, J., Pollak, Y., Morag, M., Morag, A., Avitsur, R., et al. (1999). Cytokines, "depression due to a general medical condition," and antidepressant drugs. *Adv Exp Med Biol*, 461, 283-316.
- Yokoyama, A., Kimura, Y., & Shigemura, J. (1996). Psychiatric side effects of interferon. *J Toxicol Sci*, 21(1), 93-96.
- Yrjänheikki, J., Tikka, T., Keinänen, R., Goldsteins, G., Chan, P.H., Koistinaho, J., et al. (1999). A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci U S A*. 96(23), 13496-13500.
- Zacharko, R. M., Gilmore, W., MacNeil, G., Kasian, M., & Anisman, H. (1990). Stressor induced variations of intracranial self-stimulation from the mesocortex in several strains of mice. *Brain Res*, 533(2), 353-357.
- Zalcman, S., Green-Johnson, J. M., Murray, L., Nance, D. M., Dyck, D., Anisman, H., et al. (1994). Cytokine-specific central monoamine alterations induced by interleukin-1, -2 and -6. *Brain Res*, 643(1-2), 40-49.
- Zhang, J., Terreni, L., De Simoni, M. G., & Dunn, A. J. (2001). Peripheral interleukin-6 administration increases extracellular concentrations of serotonin and the evoked release of serotonin in the rat striatum. *Neurochem Int*, 38(4), 303-308.
- Zhang, X., Beaulieu, J. M., Sotnikova, T. D., Gainetdinov, R. R., & Caron, M. G. (2004). Tryptophan hydroxylase-2 controls brain serotonin synthesis. *Science*, 305(5681), 217.

- Zhu, C. B., Blakely, R. D., & Hewlett, W. A. (2006). The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. *Neuropsychopharmacology*, 31(10), 2121-2131.
- Zhu, C. B., Carneiro, A. M., Dostmann, W. R., Hewlett, W. A., & Blakely, R. D. (2005). p38 MAPK activation elevates serotonin transport activity via a trafficking-independent, protein phosphatase 2A-dependent process. *J Biol Chem*, 280(16), 15649-15658.
- Zhu, S., Stavrovskaya, I. G., Drozda, M., Kim, B. Y., Ona, V., Li, M., et al. (2002). Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature*, 417(6884), 74-78.
- Zorrilla, E. P., Luborsky, L., McKay, J. R., Rosenthal, R., Houldin, A., Tax, A., et al. (2001). The relationship of depression and stressors to immunological assays: a meta-analytic review. *Brain Behav Immun*, 15(3), 199-226.