Behavioral, Immunological and Central Monoaminergic Alterations Induced by the Viral Imitator, Poly I:C: Augmentation by a Psychosocial Stressor.

Carleton University
Institute of Neuroscience
Dept. of Psychology
September 2006.

A Thesis presented to the department of Psychology in partial fulfillment of the requirements for the M.Sc. degree, Specialization: Behavioral Neuroscience.

Your file Votre référence
ISBN: 978-0-494-18362-5
Our file Notre référence
ISBN: 978-0-494-18362-5

Abstract

Considerable research has documented the effects of stressors and cytokines on behavioral, neuroendocrine, neurotransmitter processes. Given their similarities, it had been suggested that a combination of stressor and cytokine treatments may engender additive and/or synergistic effects on these physiological systems. Using an immune challenge comprising systemic injection of poly I:C (a synthetic double stranded RNA), we assessed the effects of this viral imitator in combination with a psychosocial stressor. The results indicated that poly I:C induced moderate signs of behavioral sickness, reduced locomotor activity, corticosterone elevations and alterations of monoamines (NE, DA and 5-HT) and their respective metabolites (MHPG, DOPAC and 5-HIAA) at hypothalamic and extra-hypothalamic sites in male CD-1 mice. Additionally, increased circulating levels of IL-6, TNF-α and IL-10 were evident. When the immune challenge was applied on a backdrop of a psychosocial stressor (comprising either isolated housing and regrouping or exposure to new residents) the effects of poly I:C on these systems were markedly augmented. Furthermore, mice that underwent a protracted period of chronic psychosocial stressors prior to the immune challenge displayed adaptations of behavioral and physiological functioning. The processes underlying these adaptations are uncertain, but the fact that it does occur might have clinical implications given that the response to cytokine immunotherapy may be adversely affected by distress.

Table of Contents

List of Figures

Introduction

Stressful experiences promote various physical and psychological disturbances. In this regard, affective and anxiety based disorders are thought to stem from the deleterious effects of stressors on neuroendocrine, neurochemical, and autonomic system functioning (Anisman et al., 2005; McEwen, 2003). Like traditional stressors, activation of the immune system and the release of pro-inflammatory cytokines (signalling molecules of the immune system) enhance the release of corticotropin releasing hormone (CRH) and increase the turnover of monoamines in brain regions thought to subserve affective illness (Brebner et al., 2000; Wichers and Maes, 2002; Schiepers et al., 2005). Indeed, the effects of cytokine challenges are strikingly similar to those triggered by psychogenic and neurogenic stressors (Merali et al., 2003).

Owing to the similar cascade of events triggered by both stressors and immune system activation, it had been proposed that the CNS may interpret pathogen presence in a fashion similar to that of how psychogenic stressors are perceived (Dunn 1995; Anisman and Merali, 1999). As such, stressors and cytokines could have additive and/ or synergistic effects, culminating in markedly pronounced deleterious consequences for physical health and mental well-being. Support for this contention comes from patients undergoing immunotherapy with either interleukin-2 (IL-2) or interferon-α (IFN-α) for certain types of cancer or for hepatitis C. Specifically, 30-50% of patients receiving IFN-α treatment experience depressive symptoms, often of sufficient

severity to warrant treatment discontinuation (Capuron et al., 2004). In view of the distress patients can be expected to be experiencing, the psychological repercussions observed may reflect the actions of cytokine exposure superimposed upon a background of severe distress.

Given the potential for stressor-cytokine synergy, the present investigation sought to assess the possibility that social stressors and cytokine treatments have additive and/or synergistic effects. To this end, we assessed the responses of systemic challenge with poly I:C (a synthetic double stranded RNA, and viral imitator), in conjunction with various psychosocial stressors (i.e., isolated housing followed by a regrouping; or a paradigm of chronic social stressors followed by exposure to novel mates) on behavioral, neuroendocrine, and central neurochemical alterations, some of which have been linked to depressive-like states in humans.

Influence of Stressors on Endocrine and Neurotransmitter Processes

Activation of the hypothalamic-pituitary-adrenal (HPA) axis occurs in response to psychogenic (psychological origin) or neurogenic (physical origin) stressors (Sapolsky et al., 2000; Wu Lu et al., 1999). Processive stressors (i.e., challenges that involve higher order sensory processing) instigate physiological and behavioural processes that serve to facilitate coping efforts, escape from the stressor, or minimize its impact (Anisman et al., 2001). Although these are adaptive responses to the challenge, stressors may have maladaptive consequences, particularly when they are prolonged, unpredictable, and severe.

The excessive and persistent neuroendocrine and neurotransmitter release may culminate in an allostatic overload (i.e., excessive wear and tear on the biological systems), rendering the organism vulnerable to physical illness and psychopathology (McEwen, 2000).

Corticotropin releasing hormone (CRH) neurons of the paraventricular nucleus (PVN) of the hypothalamus act as the initiator of a major stress pathway(s) of the brain, receiving converging inputs from internal and external sources (Dunn et al., 2004; Sawchenko et al., 1996). Efforts to maintain allostasis (i.e., homeostatic responses to stressors) involve increased synthesis and use of appropriate hormones and monoamines, endorphins, and other peptides (Sawchenko et al., 1996). One such hormone, whose release is stimulated by CRH, is corticosterone, which is rapidly released from the adrenal cortex in response to stressors. Glucocorticoids, such as corticosterone, play an important regulatory role. They initiate the mobilization of glucose and amino acids from storage to feed muscles and brain, concomitant with increases in blood pressure and heart rate, thereby transporting oxygen and nutrients to appropriate tissues (Smagin et al., 2001). Metabolism, digestion and certain aspects of immunity are also suppressed during this initial response, presumably to ensure that only the most stress-relevant systems are activated (Silverman et al., 2005). In order to maintain appropriate functioning, glucocorticoids are regulated through a negative feedback loop. These hormones bind to receptors largely concentrated in the hippocampus and hypothalamus, effectively

terminating the stress response by inhibiting further CRH release from the median eminence, pituitary adrenocorticotropic hormone (ACTH) and hence the release of adrenal glucocorticoids (Wilson, 2003).

Persistent stressor experiences result in excessive production and release of peptides such as CRH (Anisman and Merali, 2002), provoking marked and sustained elevations of HPA activity, reflected by enhanced c-Fos expression in CRH neurons (Kovacs, 1998). The increased CRH activity is accompanied by activation of arginine vasopressin (AVP), which is not regulated by the inhibitory effects of glucocorticoids, and with which CRH can synergistically trigger ACTH release from the pituitary (Jacobson, 2005). Indeed, chronic stressors can induce stable and long lasting changes in the coexpression of CRH and AVP within the median eminence, which enhances the neuroendocrine responses to future stressors (Tilders and Schmidt, 1998). Inasmuch as elevated CRH along with hypersecretions of glucocorticoids have been implicated in depression (Muller and Wurst, 2004; Stout et al., 2002; Wong et al., 2000; Merali et al., 2004), the data concerning the actions of stressor effects upon endocrine activity are consistent with their involvement in depressive illness.

In addition to the neuroendocrine changes, stressors have been shown to increase the utilization of monoamines in several brain areas, including hypothalamus and extra-hypothalamic sites (i.e., amygdala and pre-frontal cortex) that are involved in anxiety and fear (Hayley et al., 2003). In response to stressors of moderate severity increased monoamine release is accompanied by

increased amine synthesis thereby maintaining steady state transmitter levels (Anisman et al., 2001). However, if the stressor is sufficiently severe, and particularly if it is uncontrollable, then amine synthesis will exceed utilization, culminating in reduced amine availability. Under these conditions the organism is at increasing risk for the development of affective disturbances (Flugge et al., 2004). Beyond these immediate effects, stressors can engender the sensitization of HPA activity and neurotransmitter systems so that the response to later stressors is increased. Such effects have also been observed even when the two stressor sessions involve different types of challenges (Finlay et al., 1997).

As alluded to earlier, reciprocal communication may occur between the neuroendocrine and immune systems (Blalock, 1994). In this regard, various physiological systems share common ligands, such that neuropeptides can bind with immune cells and cytokines with endocrine glands. Indeed, the effects of stressors can stimulate immunity or act as immunosuppressants (Bailey et al., 2004). It may be that owing to the reciprocal communication, stressors may exert cross-sensitization effects with various cytokines such as interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α) and IL-1β, culminating in increased HPA activity and turnover upon later challenges (Hayley et al., 2003; Hayley et al., 2001).

Cytokine Effects on Endocrine and Neurotransmitter Processes

Cytokines, produced by lymphocytes, macrophages and T cells, regulate immune responses (Wichers and Maes, 2002). Additionally, as

indicated earlier, they may also serve as in a sensory capacity, informing the brain of viral or bacterial insults (Blalock, 1994; Merali et al., 2003). Peripheral cytokines are large hydrophilic molecules and as such do not readily pass the blood brain barrier. However, it has been suggested that they could affect the brain via activation of afferent vagal fibres, through neuroendocrine systems, by active transport into the brain, or by disturbing blood brain barrier permeability (Banks, 2005). In addition to peripheral events, IFN-α, IFN-γ, IL-2, IL-1, IL-6, and TNF-α are produced by astrocytes and microglia (McGeer and McGeer, 1995) within the hypothalamus, hippocampus, cerebellum, basal ganglia, forebrain regions and brainstem nuclei (Kronfol and Remick, 2000). As stressor exposure increases IL-1β levels in the brain (Lee et al., 2006) and the expression of IL-1β and TNF-α receptors have also been identified in stress-related areas such as the PVN and hippocampus (Alheim and Bartfai, 1998; Nadeau and Rivest, 1999), it would seem that stressors could affect behavioural outcomes through effects on central cytokine processes.

There is reason to believe that neuroendocrine and central neurotransmitter alterations implicated in depression can also be elicited by exposure to various cytokines. Like systemic challenges, central infusion of IL-1β enhanced the expression and release of CRH and AVP secretion from the PVN of the hypothalamus and consequently corticosterone from the adrenal cortex (Rivest and Rivier, 1994; Ericsson et al., 1994). Similarly, TNF-α, has been shown to increase CRH, ACTH and hence corticosterone secretion

(Turnbull et al., 1997). Similar to stressor-induced monoamine alterations, IL-1β increased *in vivo* NE, DA and 5-HT utilization at limbic sites, and increased turnover of 5-HT within the hypothalamus, prefrontal cortex and hippocampus in postmortem analyses (Brebner et al., 2000). Further to this point, similar effects have also been documented with TNF-α, specifically showing increased NE turnover within the PVN of the hypothalamus, amygdala, dorsal hippocampus, as well as 5-HT turnover within the prefrontal cortex, and central amygdala (Hayley et al., 1999).

As with stressors, cytokines engender sensitization of neurochemical functioning, and the combination of several proinflammatory cytokines may synergistically influence behaviour and neurochemical functioning. Systemic administration of TNF-α produced immediate elevations of plasma corticosterone and increased central NE, DA and 5-HT turnover. With regard to the latter, systemic administration of TNF-α combined with IL-1β elicited a substantial secretion of corticosterone. This copious corticosterone secretion was not evident when IL-1β was used in conjunction with IL-6 or when TNF-α and IL-6 were used together, implying specificity in terms of which cytokines have the ability to act synergistically (Anisman and Merali, 1999).

Poly I:C

Polyinosinic: polycytidylic acid (poly I:C), a synthetic double stranded RNA (ds RNA) and viral imitator, has been used to study the effects of immune system activation (Rouas et al., 2004; Fortier et al., 2004). A majority of viruses

lead to activation of the HPA axis and at some point during their replication cycle induce the synthesis of ds RNA, triggering a cellular response to this infection (Silverman et al., 2005). The identification of ds RNA initiates the activation of a number of enzymes such as protein kinase (PKR) and 2'5'oligodenylate synthetase (Jacobs and Langland, 1996). Both enzymes induce type I (α,β), and type II (γ), IFNs, pro-inflammatory cytokines which help protect neighboring cells by preventing further viral replication. In addition, IFNs also activate major histocompatibility complex class I expression (MHC-I) to enhance antigen presentation to CD8 + cytolytic cells (Loftis and Hauser, 2004). Complementary to the activation of IFNs, PKR also inhibits protein synthesis and phosphorylates NFκB, a transcription factor involved in regulating genes for the inception of inflammatory responses (Jacobs and Langland, 1996; Ueta et al., 2005).

Toll-like receptors (TLRs) are a family of innate recognition receptors that identify molecular patterns associated with micro-organisms such as bacteria, algae and fungi (Town et al., 2006). They have also been implicated in the response to viruses as mice deficient in PKR enzymes are still able to produce an immune response to poly I:C. Specifically, it has been documented that TLR-3 recognizes ds RNA and activation of this receptor initiates type I IFNs as well as NFκB responses (Alexopoulou et al., 2001). Concurrently, exogenous administration of poly I:C was reported to increase mRNA of TLR-3 (Diebold et al., 2003).

Data regarding behavioral, neuroendocrine, neurotransmitter and immunological alterations following exogenous exposure to poly I:C are rare compared with the abundance of literature regarding the effects of lipopolysaccharide (LPS), a product of the cell wall of gram negative bacteria and potent immune system activator. This is surprising given that poly I:C is a well known type I IFN inducer (Lien and Golenbock, 2003). Moreover, because viruses are more complex inflammatory reactants than bacteria (Silverman et al., 2005), poly I:C could have significant effects on endocrine, transmitter and subsequent affective states. In general, this view is supported by available data indicating that systemic poly I:C challenges activate both the central nervous and immune systems. When administered to rabbits, poly I:C vigorously activated the HPA axis and the release of corticosterone (Milton et al., 1992). Likewise, exposure to poly I:C resulted in a maximal fever, a five-fold increase of IL-6 and a four-fold increase of TNF-α, 2-3 hours after intraperitoneal (i.p.) administration. As well, markers of inflammation, such as IL-1β and cyclooxygenase-2 (COX-2) mRNA, were upregulated in the hypothalamus relative to controls (Fortier et al., 2004). Katafuchi et al., (2003) acclimated rats to a running wheel in their home cages for two weeks prior to poly I:C exposure. After treatment with poly I:C, daily running activity declined 40-60% from pre-study assessments, shortly thereafter returning to basal levels. Importantly, in this study IFN-α mRNA was also significantly increased in the cortex, hippocampus, and in many nuclei of the hypothalamus, including the PVN. In a follow up study, Katafuchi et al. (2005)

reported increases of IFN-α, and IL-1β mRNA in hypothalamic nuclei and in the cortex following immunological challenge of 3mg/kg of poly I:C. Moreover, they documented decreased extracellular concentrations of 5-HT in the PFC, an effect that was attenuated by administration of the SSRI, imipramine. At this juncture, however, it is premature to draw any substantial conclusions regarding causal relations of the processes subserving the behavioral and neurochemical effects of poly I:C.

To summarize, the evidence implicating cytokines as contributing to or maintaining affective illnesses has received considerable support, yet falls short of providing definitive evidence of an independent causal role in this regard. One important confounding factor is whether cytokine elevations are secondary to, as opposed to the contributing factor for depression (Schiepers et al., 2005; Anisman et al., 2005), and whether elevated cytokine levels is a sufficient condition for the emergence of depression (i.e., in the absence of concurrent stressor experiences).

Behavioral Effects of Cytokines

In humans, exogenous cytokine treatment elicits neurovegetative symptoms as well as moderate to severe depression, and in animals a constellation of physiological and psychological changes are provoked that are collectively referred to as "sickness behaviours" (O'Brien et al., 2004; Dantzer, 2001). The latter include lethargy, anorexia, anhedonia (an inability to experience pleasure for otherwise pleasurable activities, and a hallmark

symptom of all sub-types of human depression), fever, weakness, and drooping of the eyelids (Wichers et al., 2005). It is postulated that this behavioral profile helps initiate the organism's recovery by promoting actions necessary to fend off the illness (Capuron and Dantzer, 2003). As expected from a cytokine depression model, LPS or IL-1β treatment resulted in anhedonia, reflected by decreased consumption of a sweetened saccharine solution (Yirmiya, 1996), as well as reductions in responding for sucrose in a progressive ratio test (Merali et al., 1997), and in both instances the effects were attenuated by chronic antidepressant administration.

Commensurate with the animal studies, it was reported that relative to the controls, human participants given LPS treatment had higher levels of circulating cytokines, cortisol, and flu like symptoms and both anxiety and depression were elevated (Yirmaya et al., 2000). In a subsequent experiment, it was shown that the neurovegetative features were ameliorated as cytokine levels began to normalize (Pollak and Yirmiya, 2002). The human and animal studies, when considered together, certainly make a strong case for cytokine involvement in depression, but the possibility cannot be excluded that the effects were more a reflection of distress/depression associated with a medical condition than one linked to specific immune disturbances.

Synergistic Effects between Stressors and Cytokines

Given the similarities between stressor and cytokine-induced alterations on brain processes, it is possible that these two factors could combine to produce

additive or synergistic effects on the systems most vigorously activated during exposure to stressors or immune activation. Available data generally support this contention. Merali et al., (1997) assessed the *in vivo* variations of metabolites of monoamines (5-HT and DA) in response to systemically administered IL-1β, as well as to a neurogenic stressor (repeated air puffs). IL-1β increased 5-hydroxyindoleacetic acid (5-HIAA) in the nucleus accumbens and hippocampus, and this response was markedly augmented when administered in conjunction with a stressor. In addition, increased 3,4-dihydroxyphenylacetic acid (DOPAC) levels were reported in the PFC, an effect that was not apparent in the air puff group treated with saline. Song et al., (1999) used a similar procedure, and found that both IL-1 and IL-6 produced synergistic effects when used in conjunction with a series of air puffs. Specifically, they reported that significant increases in 5-HIAA were evident in the nucleus accumbens. The authors of these studies noted that although these data provide support for synergistic stimulation of central monoamines, it is likely that processive and systemic stressors induced these actions via different circuitry, thereby leading to diverse psychological outcomes.

Further evidence suggestive of synergistic effects between stressors and cytokines comes from individuals who have undergone immunotherapy using IFN-α for diseases including various types of cancers or hepatitis C (Raison et al., 2006). Despite the beneficial effects of anti-viral therapy, patients often develop severe depressive symptomology (Musselman et al., 2001). Capuron et

al., (2004) reported prevalence rates of depression as high as 30-50%, although it should be noted that a fair amount of variability has been reported by other researchers, possibly owing to the varying dosages used, duration of therapy, as well as different procedures used for diagnosis (Wichers et al., 2005). Capuron and Miller (2004) suggested that two reliable constellations of symptoms emerged in those undergoing immunotherapy: (a) a neurovegatative syndrome (that includes symptoms such as anorexia, fatigue, sleep disturbances, and memory deficits) which develops within two weeks and persists throughout and (b) a mood syndrome which appears after about two weeks and is associated with a depression and anxiety. Treatment with the specific serotonin re-uptake inhibitor (SSRI), paroxetine, can attenuate the mood syndrome, while leaving the neurovegatative syndrome unaffected. Interestingly, the best indicator of those who will become depressed as a result of undergoing IFN-α therapy are those who scored high on a basal pre-study depression scale (Capuron et al., 2002). Further, it was also observed that patients who later developed depression showed an exaggerated HPA response (i.e., ACTH and cortisol secretions) to initial injections of IFN-α (Capuron et al., 2003). Therefore, it is possible that chronic cytokine challenges superimposed upon a background of severe distress may set in motion neurochemical changes that favor the development of psychopathology.

Social Stressors and Immune Alterations

Social stressors markedly influence neuroendocrine and behavioural functioning (Carobrez et al., 2002), and because of the apparent ethological validity of such stressors, they have received increasingly greater attention. Indeed, social stressors have been viewed as particularly useful in the analysis of the evolution of human psychopathology (Bartolomucci et al., 2003). Animal models of social distress primarily comprise two paradigms, namely social isolation and social disruption stress (SDR).

Housing conditions have long been known to affect various attributes of animal well being. For instance, BALB/c mice housed individually for a period of 6-9 weeks produced significantly more IL-4 (a derivative of mature T-helper cells class II, which is required for humoral immunity, acting in an inhibitory capacity) than those of the same strain that were group housed. Similarly, isolated C57Bl/6 mice (6-9 weeks) produced more IL-2 (a derivative of TH-1, required for cell-mediated immunity) than their group housed counterparts (Karp et al., 1994). Bartolomucci et al., (2003) reported no changes in immuno-endocrine responses (i.e., lymphocyte proliferation of IL-2, IL-4, IL-10, IFN-γ or elevations in corticosterone productions) associated with individually housing mice for various periods of time (1, 7, 14, 21, or 42 days). However, in a second experiment, isolated housing for a period of 21 days followed by an acute stressor (exposure to a novel environment for 8 min.) resulted in a 300%

increase of basal corticosterone as well as a significant increase of IL-2 and IL-4, although, it is uncertain whether the increased physiological reactivity reflects increased vulnerability to illness. Additionally, social isolation has also been implicated in exacerbating the progression of autoimmune-based diseases. For instance, Childa et al., (2005) reported that psychosocial stressors exacerbated nephritis and lymphadenopathy using an animal model of autoimmunty.

In contrast to the social isolation experiments, SDR stress is based on the social defeat of rodents (mice or rats) in their home cages. It involves introducing a trained aggressive male into a cage of residents, thus disturbing the established social hierarchy. This disruption, and particularly, social confrontation, causes the secretion of "stress" hormones such as corticosterone, norepinephrine and epinephrine (Avitsur et al., 2001). In a typical paradigm this exposure occurs daily for two hours with the frequency of fighting, submissive posturing and exploratory behavior recorded. The animal is subjected to three consecutive days of distress, one day off, followed by three more days of distress (Engler et al., 2005). Using this procedure it was demonstrated that animals subjected to SDR display decreased sensitivity of their splenocytes to the otherwise inhibitory effects of corticosterone when stimulated with LPS *in vitro* (Avitsur et al., 2005; Engler et al., 2005). In addition, LPS stimulated splenocytes and monocytes secreted significantly greater levels of IL-6 (Avitsur et al., 2001) and TNF-α (Avitsur et al., 2005), relative to non-SDR animals. In order for this phenomenon to occur it appears as though direct contact was

necessary. Animals receiving SDR sensory contact (i.e., prevented from interacting with the aggressive intruders by a wire mesh partition) showed comparable responses to controls, with respect to the viability of LPS stimulated splenocytes in response to corticosterone (Bailey et al., 2004). Taken together, these data implicate social stress as a precipitant for the development of glucocorticoid resistance, and consequently to an over exposure of harmful inflammatory processes. Not surprisingly, Quan et al., (2001) reported that SDR mice are at an increased risk for mortality when subsequently challenged with LPS, in that, they showed higher expressions of IL-1β and TNF-α in the lungs, spleen, liver and brain in post-mortem analysis, following the systemic challenge. Moreover, animals most prone to these deleterious effects were those characterized as being submissive (Avitsur et al., 2001).

Commensurate with the findings that the SDR stressors elicit HPA axis and immune alterations, it has also been implicated as a precipitant for depressive illness. Von Frijtag et al. (2000) subjected male rats to a SDR procedure like that described earlier. Defeated rats subsequently housed individually for a period of three months showed a decline in anticipating a sucrose solution but not for food chow (i.e., a dissociation of anorexia and anhedonia) as well as reductions in open field exploration and in social interactions. Interestingly, however, returning the defeated animals to social housing counteracted these effects, including the anhedonia. In a follow-up study male rats were once again subjected to social defeat using SDR

procedures, after which they were individually housed. Those in the isolated group were either given daily injections of imipramine (an SSRI) or a vehicle treatment. Anticipatory responses for a sucrose solution assessed prior to and following 3-5 months of this chronic antidepressant treatment indicated that consumption of the desirable solution normalized, independent of other indices such as open field, body weight, or chow consumption, suggestive of a restoration of hedonic processes (Von Frijtag et al., 2002).

Thus, it appears as though social stressors can elicit comparable effects on endocrine and immune systems, akin to that observed with other processive stressors.

The Proposed Experiments

In summary, the data presented here seem to suggest that stressors and cytokines may elicit comparable behavioral, neuroendocrine, and neurochemical effects. Given these similarities, it has been proposed that the CNS may interpret pathogen presence in a fashion analogous to that of how it perceives a stressor (Anisman and Merali, 1999; Hayley et al., 1999). Consequently, together, the two treatments could elicit additive or synergistic effects, and may thus render an organism at increased risk for physical illnesses and psychopathology.

In contrast to bacterial endotoxins, only a limited number of studies have attempted to delineate the specific cytokines involved in response to poly I:C. In addition, there is limited information available concerning the HPA, central monoamine and behavioral effects of this viral imitator. It has been suggested

that relative to endotoxins, viral infections are a more complex inflammatory reactant, which can more accurately capture natural neuro-immune interrelations (Silverman et al., 2005), and, although poly I:C does not replicate, the potential alterations on stress and/or immune systems are still of great interest. Thus, the present investigation was undertaken to elucidate (a) the HPA axis, corticosterone, central monoamine and behavioral responses induced by systemic challenges of poly I:C at varying dosages. Given the well documented ability of bacterial and viral infections to trigger stressor and/or immunological responses in the host organism, it is postulated that (b) whether elevations of stress hormones, neurochemical substrates and plasma cytokines will be evident (c) psychosocial stressors and cytokines will have additive or synergistic properties using poly I:C as an immune challenge. In this regard, it is anticipated that the combination of treatments will result in elevated levels of corticosterone, and increased utilization of monoamines (5-HT, DA, NE) in hypothalamic (PVN) and extra-hypothalamic sites (median eminence, locus coeruleus, hippocampus, central nucleus of the amygdala (CeA), medial pre-frontal cortex (mPFC) and nucleus accumbens), possibly leading to reduced levels of the amines. Likewise, it is expected that increased overall sickness behaviors will be evident in mice subjected to social stressors concomitant with poly I:C exposure. Finally, it is hypothesized that (d) chronic psychosocial stressors will have particularly marked deleterious consequences comparable to those elicited by traditional stressors of a non-social nature.

Materials and Methods

Animals

CD-1 male mice of approximately six weeks of age were obtained from Charles River Canada (St. Constant, PQ) and were allowed 2 weeks to become accustomed to the vivarium surroundings prior to the beginning of any experiments. During this time they were housed in groups of four in standard (27 x 21 x 14 cm) polypropylene cages. All mice were kept on a 12 hour light-dark cycle (light 0700-1900) in a temperature controlled vivarium (21° C) and had free access to tap water and chow (Ralston Purina, St. Louis, MO). Precautions were taken to minimize any pain or discomfort towards the mice and the studies received approval from both the Canadian Council on Animal Care and the Carleton University Animal Care Committee. All experiments procedures were conducted between 0800-1200 to reduce variability associated with diurnal rhythms.

Experiment 1

Experiment 1 was conducted to ascertain some of the specific responses to the viral imitator, in the absence of a preceding stressor paradigm. A total of 45, male, naïve CD-1 mice approximately 60 days of age were randomly assigned (n=15) to undergo treatment with either vehicle (Fisher Scientific, Brockville, ON), 1mg/kg or 2 mg/kg of poly I:C (Sigma, St. Louis, M.O) . Throughout the study, mice continued to be housed in standard polypropylene cages, and their body weights were obtained the night before treatment administration. On test

day mice in each group received their treatment by an intraperitoneal (i.p.) injection, following which, locomotor activity and behavioral sickness were assessed for a 90 min period. Subsequently, mice were decapitated and free flowing trunk blood was collected for the analysis of plasma corticosterone and cytokines. Brains were then removed from the skull and various frontal slices were cut in order to obtain tissue samples for monoamine and metabolite determinations using HPLC procedures. Additionally, plasma cytokines were assayed to assess any increases of these substances.

Experiment 2

This study was conducted to determine whether psychosocial stressors and cytokines have additive and/or synergistic effects with respect to elevations in corticosterone, alterations of monoamines, increases in cytokine levels and in behavioural sickness. Mice were randomly assigned to one of eight groups (n=8/group). Half the mice continued to be housed in groups, whereas the remaining mice were separated into novel individual cages for a 2 week period. On test day, half the mice of both conditions continued to be housed in the group or isolated condition, while the remaining mice were transferred from either the group to an isolated condition, or from isolation to a grouped condition (i.e., reunited with their previously separated cagemates; 4 per cage). This regrouping period allowed for full sensory and physical contact with previous mates. For each manipulation a standard cage with fresh bedding was used. Each manipulation lasted 1 hr, after which mice were challenged with i.p. injection of either 2 mg/kg of poly I:C, or an equivalent volume of saline. Mice were then

returned to their original housing condition where sickness behaviors were monitored as described in Experiment 1, after which mice were decapitated and brains and blood collected for hormonal, neurochemical and cytokine levels.

Experiment 3

In order to assess whether prolonged social stressors act additively or synergistically with poly I:C, mice were randomly assigned to one of 8 groups (n=8/group). Control mice were group housed and left undisturbed, with the exception of briefly being handled twice a week to change their cages. Animals assigned to the chronic social stressor condition also lived in groups of four in a separate room from the control mice. For a period of three weeks these animals were exposed to different daily social stressors, between the hours of 0800 and 1500. On each stressor day the procedure was applied on two separate occasions (with some noted exceptions as described below). The timing of exposure was randomized daily and the mice were returned to their home cages with their original cagemates at the end of the day (i.e., after 1500). The chronically stressed mice received the following treatments:

1. Animals were isolated for the day in standard polypropylene cages containing approximately 80 ml of soiled bedding from a cage that housed a breeding male.

2. Animals were placed into a novel standard polypropylene cage (with fresh bedding) divided in half by a wire mesh screen (10x 10 cm). Mice were introduced to an aggressive CD-1 male for a duration of five minutes, during which contact between them was prevented by the screen. During the second

daily repetition of this procedure, the bedding was replaced and the mice were exposed to a novel aggressor.

3. Residents of one cage were placed into the home cage(s) of others and vice versa. This "swap" lasted 30 minutes, and the repetition occurred with a novel cage of chronic mice.

4. A rat cage (26 x 47 ½ x 20 cm) was divided into four equal quadrants using wire mesh partitions (24 x 42 cm), preventing physical contact. Mice from different home cages were each placed into one of the four areas, such that each had full sensory exposure to a non-cagemate for 15 minutes. Again, fresh bedding was introduced during the repetition.

5: All animals (including control mice) were placed in a novel cage and motor activity measured over a 10 min period using a Micromax system (Columbus, OH).

6. At approximately 1600, mice were deprived of Ralston Purina chow, and it was returned the next morning at 0800.

Chronically stressed mice received no experimental manipulations on one day each week, and were left in their cages undisturbed.

On test day, mice in both the control and chronic stress conditions were grouped for 1 hour with either novel cage mates (i.e., placed into a new cage with 3 new residents) or remained with their original cage mates. Thereafter, animals were either challenged with either 2 mg/kg of poly I:C or an equivalent dose of saline (based on body weight), and returned to their original housing

conditions for locomotor and sickness recordings followed by decapitation for collection of brain and blood. The assays performed were the same as those described in Experiment 1.

Locomotor Assessment

Immediately following treatment on the test day, mice in Experiments 1 and 3, were individually placed into standard sized mouse cages containing a handful (approximately 80 ml) of bedding obtained from their original home cage, thereby reducing distress that might otherwise occur with exposure to a novel environment. Photosensor activity detection units, surrounded each cage and motor activity was measured and recorded using a Micromax system (AccuScan Instruments, Inc., Columbus, OH). Locomotor activity was defined by breaks of an infra-red beam. In each of the experiments, locomotion was recorded over either 90 min (Experiment 1) or 60 min (Experiment 3), with activity being pooled over 10 min bins. Note that no assessment of locomotor activity was taken for Experiment 2.

Behavioral Measurements: Sickness Ratings

In each of the experiments, sickness behaviour of mice was rated over 6 (Experiment 1), and 4 (Experiment 2-3), consecutive 15 min intervals commencing 15 min after injection. The experimenter rated the overall appearance of each animal to assess degree of sickness experienced. Sickness measurements were scored on a four-point scale (1=no symptom, 2=one

symptoms present, 3=two symptoms presents, 4=all symptoms). Symptoms comprised decreased exploration and locomotion, curled body posturing, ptosis, ragged fur, lethargy, piloerection, drooping of the eyelids, and overall non-responsiveness. This procedure was previously found to yield better than 90% agreement between 2 raters blind to the treatment mice received.

Plasma Corticosterone Determinations

One hour following injections (i.e., following sickness scoring), animals were decapitated and free flowing plasma trunk blood was collected in tubes containing 10ml with 10μg of EDTA. Blood samples were centrifuged for 15 minutes at 3600 RPM, and the supernatant stored at -80^{o} C. Plasma corticosterone was later determined using a commercially available radioimmunoassay (RIA) kit (ICN Biomedicals, CA). The intra-assay variability was less than 10%.

Brain Dissection Technique

Immediately following decapitation and plasma corticosterone collection, the brains were removed and placed on a stainless steel metal brain matrix (1 x 1.5 x .75 inches) situated on top of a block of ice, with the slots (spaced approximately 500 μm apart) serving as guides for razor blades, which were then inserted into the matrix to cut various frontal sections. Areas of interest to assess monoamines pertinent to these studies (NE, DA and 5-HT) included the PVN of the hypothalamus, median eminence, locus coeruleus, hippocampus, central nucleus of the amygdala, mPFC, and the nucleus

accumbens. Using the mouse brain atlas of Franklin and Paxinos (1997) areas of interest were micro-punched using a hollow 20-gauge microdissection needle, with the exception of the median eminence that required an 18-gauge needle and was punched accordingly.

High Performance Liquid Chromatography (HPLC) Assay

Levels of NE, DA and 5-HT, and their respective metabolites, 3-methoxy,4-hydroxoyphenylethyleneglycol(MHPG), 3,4-dihydrooxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA), were determined by HPLC. Tissue punches were sonicated in a homogenizing solution comprising 14.17 g monochloroacetic acid, 0.0186 g disodium ethylenediamine tetraacetate (EDTA), 5.0 ml methanol and 500 ml H_2O. Following centrifugation, the supernatants were used for the HPLC analysis. Using an Agilent (Mississauga, ON) pump, guard column, radial compression column (5m, C18 reverse phase, 8mm x 10cm), and coulometric electrochemical detector (ESA Model 5100, A) 40 µl of the supernatant was passed through the system at a flow rate of 1.5 ml/min (1400-1600 PSI). Each liter of mobile phase consisted of sodium dihydrogen phosphate (90mM), 1-octase sulfonic acid (sodium sal) (1.7 mM), disodium ethylenediamine tetraacetate (EDTA) (50mM), citric acid (50 mM), potassium chloride (5 mM) and 10% acetonitrile. The mobile phase was then filtered (0.22 mm filter paper) and degassed. The area and height of the peaks were determined using an Agilent integrator. Protein content of each sample was determined using bicinchoninic acid with a protein analysis kit (Pierce

Scientific, Brockville, ON) and a Fluorostar colorimeter (BMG, Durham, NC). The lower limit of detection for the monoamines and metabolites was approximately 1.0 ρg.

Plasma Cytokine Analysis: Multiplex Bead-Based Immunoassay

The cytokine assay kit used for plasma samples was obtained from Upstate Cell Signaling Solutions (Lake Placid, NY). Initially, the Multi-Cytokine Standard 2 containing 5000 ρg of each of: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α, IFN-γ and granulocyte macrophage colony stimulating factor (GM-CSF) were resuspended in 1.0 ml of Standard Serum Diluent (SSD), followed by it being vortexed briefly for 15 seconds and then being placed on ice for 5 minutes. 25 μl of Beadlyte Cytokine Assay Buffer was placed in each well and vortexed briefly in order to wet the filter plate. Excessive liquid was removed by applying a vacuum manifold to the bottom of the filter plate and in addition, it was further blotted on paper towel to remove any remaining liquids. 50 μl of SSD was added to each well and the filter plate was incubated on a plate shaker for 20 minutes. The Beadlyte Anti-Mouse Multi-Cytokine Beads 2 was then vortexed at a medium speed for 15 seconds, and then sonicated for 15 seconds using a sonication bath. In each well, 25 μl of Bead Solution was added, and the plate was covered and vortexed on low speed. The plate was then incubated for 2 hrs in a dark room at room temperature. A vacuum manifold was applied to the bottom of the filter plate to remove excessive liquid, followed by it being resuspended in 50μl of Beadlyte Cytokine Assay Buffer. Thereafter it

was vortexed briefly and the vacuum was repeated. Following this, 25μl of Beadlyte Anti-Mouse Multi-Cytokine 2 Biotin was added to each well. The plate was then covered and mixed by vortexing it at low speeds. It was then incubated for 1.5 hrs in a dark room on a plate shaker. Prior to use of the Luminex 100, an alcohol flush was performed to ensure that all lines contained no air bubbles. The data collector on the Luminex 100 was then set up to accommodate a 50-region bead map. Precise cytokines corresponded to bead: #8 (IL-1β), # 21 (IL-2), # 52 (IL-4), # 9 (IL-5), # 38 (IL-6), # 36 (IL-10), # 50 (IL-12), # 06 (TNF-α), # 34 (IFN-γ), and finally # 54 (GM-CSF). The set sample size was 75 μl, 50-100 events were read and the gate was set to 8000-13500. To adjust the needle height, two disks and the same plate type were used. The Beadlyte Streptavidin-PE was diluted to a 1:25 ratio, slightly before the end of the incubation period. This dilution occurred in a Beadlyte Cytokine Assay Buffer using an empty mixing vial. 25 μl of diluted Beadlyte Streptavidin-PE was added to each well, covered and vortexed at low speed once the incubation period ended. Subsequently, the antibody was incubated for 30 minutes in a dark room again at room temperature and on a plate shaker. Thereafter, 25μl of Beadlyte Stop Solution was added to each well, vortexed and left for five minutes in the dark, at room temperature. The bottom of the filter plate was subjected to a vacuum manifold procedure and resuspended in 125 μl of sheath fluid. It was then vortexed on a low speed and placed on a shaker for 1 minute. The Luminex 100 instrument allowed for different bead sets to be distinguished based on internal

dyes, with the antibodies bound to the bead surface acting as targets. Likewise, reporters (tagged with a fluorescent label) were also bound to the target. The particular substrate being measured was unique to a specific bead set.

Statistical Analyses

Plasma corticosterone levels for Experiment 1-3 were analysed separately by between-group analysis of variance (ANOVA). Sickness measurements for each of the experiments were analysed independently using repeated mixed measures ANOVAs, as were the MMX data. Metabolites and monoamine levels for each study were analysed as independent between-group ANOVAs within each brain region. Likewise, the cytokine data were analysed independently for each type of cytokine of interest. Where appropriate, comparisons between significant main effects and/or means of simple main effects of significant interactions were performed by Bonferroni corrected t-tests (α=. 05). Over the course of these studies several samples were lost during brain dissection or in HPLC analyses. In addition, statistically deviant observations (>5 SD from group and overall mean) were treated as outliers and removed from their respective group means. As a result, the degrees of freedom varied across different types of dependent measures in each experiment.

Results

Experiment 1

Locomotor Activity

Movement by the animals, as depicted in Figure 1 (top), was found to vary as a function of the Treatment x Time Interval interaction, $F(10,200)=2.122$, $p<.05$. Follow-up comparisons of the means comprising the simple main effects for this interaction revealed that treatment with both doses of poly I:C reduced overall locomotor activity at each successive time point after the initial 30 minutes sampled contrasted with vehicle administration. Dose dependent differences between the 1 and 2 mg/kg treatments did not emerge over the course of the 90 min sampling period.

Sickness Behaviours

Commensurate with the reductions in locomotor activity, as depicted in Figure 1 (bottom), poly I:C affected the overall appearance and well-being of the animals, as the Treatment x Sickness Rating interaction was significant, $F(4,82)=2.52$, $p<.05$. Analysis of the simple main effects did not reveal any differences between the effects of the two doses of the treatment, although, relative to vehicle injections, sickness behaviors were significantly increased in poly I:C treated mice from 30-90 min after treatment administration up until decapitation time.

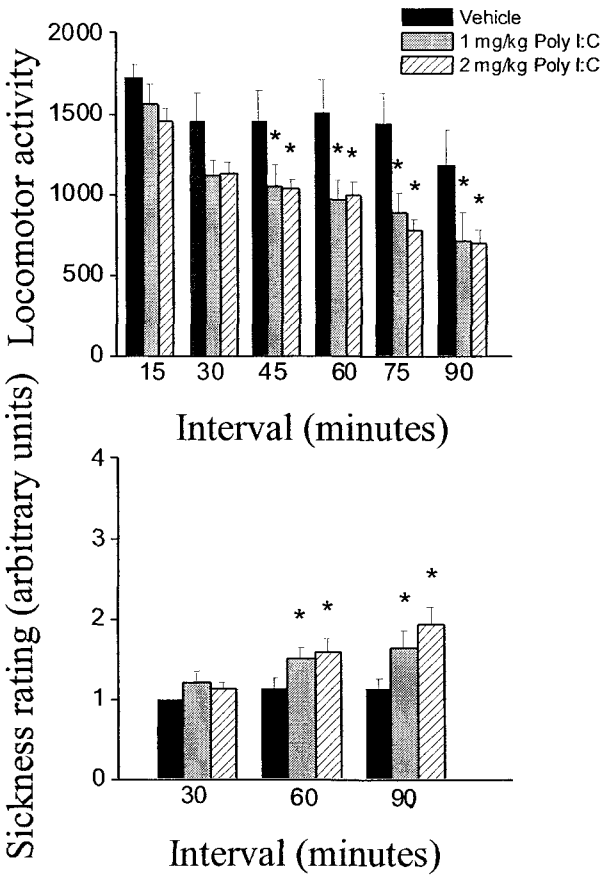

Figure 1.Mean (± S.E.M.) ratings of illness as reflected by locomotor activity (top) and sickness ratings (bottom) in mice treated with vehicle, 1 or 2 mg/kg of poly I:C. MMX and behavioral sickness were assessed over a 90 min. period. * P<.05 relative to saline treated mice.

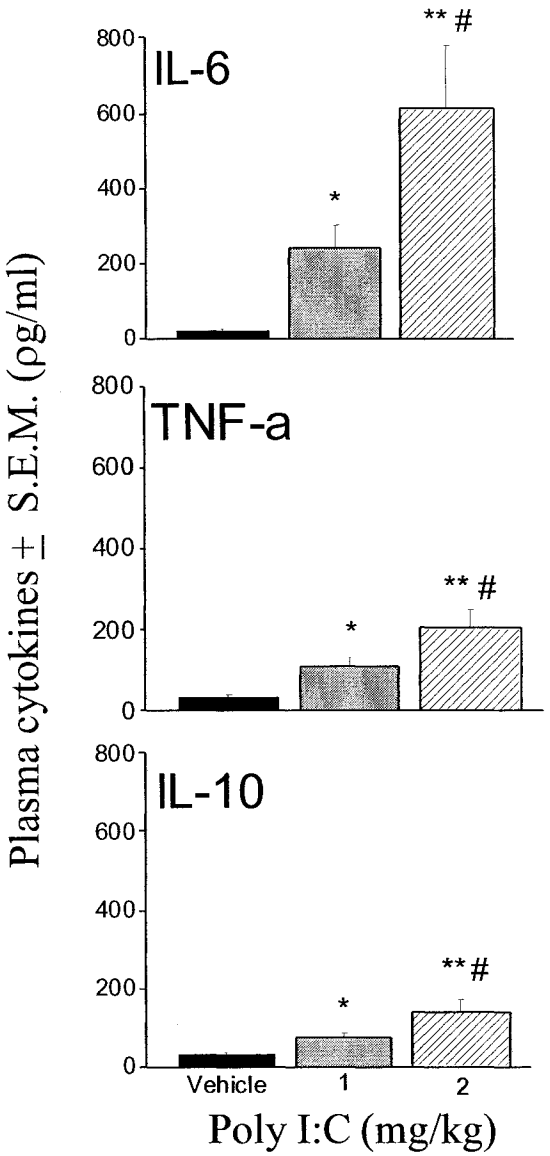

Figure 2. Mean (± S.E.M.) levels of circulating plasma cytokines, IL-6 (top), TNF-α (middle), and IL-10 (bottom), following administration of vehicle, 1 mg/kg or 2 mg/kg intraperitonal (i.p.) injection to group housed mice. Plasma cytokine levels were determined 90 min following challenge. * $P < 0.05$ relative to saline treated mice, ** $P < 0.01$ relative to saline treated mice, # $P < 0.05$ relative to mice given systemic challenge of 1 mg/kg poly I:C.

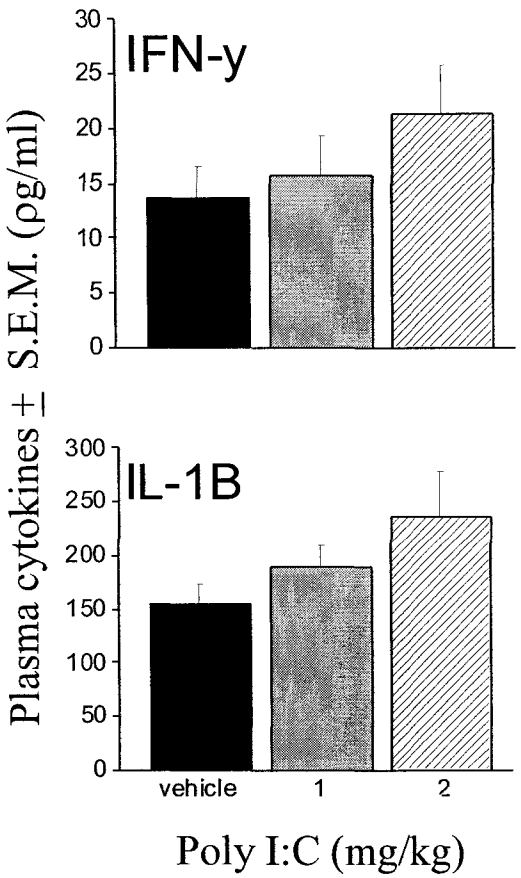

Figure 3. Mean (± S.E.M.) levels of IFN-γ (top) and IL-1β (bottom) following systemic challenge of either vehicle, 1 mg/kg or 2 mg/kg poly I:C to group housed mice. Plasma cytokine levels were determined 90 min following challenge.

Plasma Cytokines

Treatment with poly I:C influenced the levels of several circulating cytokine levels. As shown in Figure 2, poly I:C dose-dependently increased both IL-6 and TNF-α levels, $F(2, 40) = 8.50, 9.07$, p 's $< .01$, as was as that of the cytokine inhibitor, IL-10, $F(2, 40) = 7.26$, $p < .01$. Among the remaining cytokines there was a modest trend toward poly I:C increasing IFN-γ and IL-1β (Figure 3), but these effects did not reach an acceptable level of significance. There was no indication of the inhibitory transmitter, IL-4, being affected by the poly I:C treatment.

Plasma Corticosterone

Systemic challenge with poly I:C influenced plasma corticosterone levels, $F(2,40)=8.92$, $p<.001$. Follow-up tests indicated that corticosterone levels were elevated among mice that received both the 1 and 2 mg/kg doses of poly I:C relative to vehicle treated mice. Further comparisons between the 1 and 2 mg/kg doses of poly I:C revealed that the treatment effects approached, but did not reach statistical significance ($p=.08$).

Monoamines and Metabolites

Data regarding monoamine utilization and levels are organized and presented within each brain region, a selection are visually depicted in Figure 4. As *a priori* hypothesis had been made regarding the concentrations and levels of central neurochemicals in those groups exposed to varying dosages of poly I:C, comparisons were conducted irrespective of the significance of F-tests.

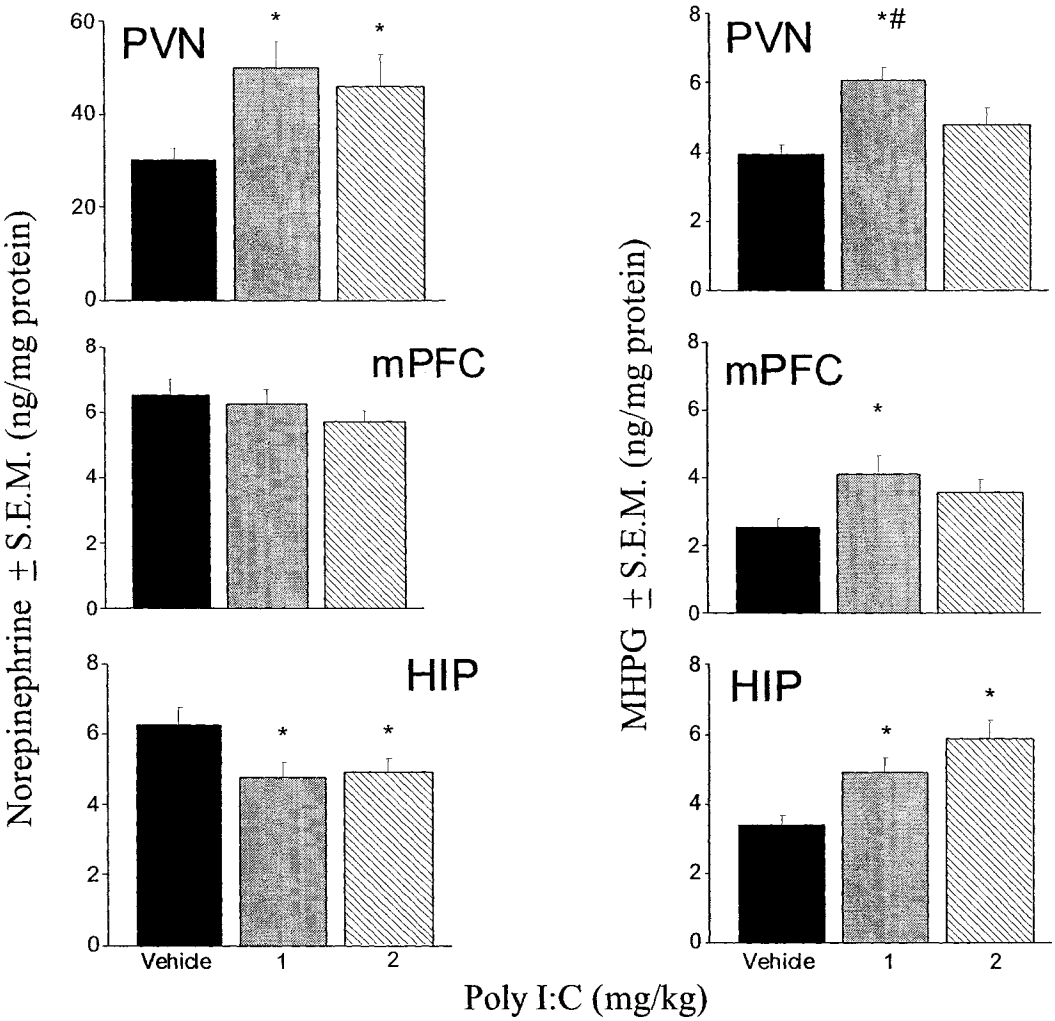

Figure 4. Mean (± S.E.M.) levels of NE and MHPG concentrations in the PVN (top), mPFC (middle), and hippocampus (bottom) following systemic challenge with either vehicle, 1 mg/kg or 2 mg/kg of poly I:C. Monoamine levels and concentrations were determined 90 min following the treatments. * $P < 0.05$ relative to saline treated mice, # $P < 0.05$ relative to mice challenged with 2 mg/kg of poly I:C

Paraventricular Nucleus

The effects of poly I:C on MHPG concentrations were pronounced, $F(2, 42)=8.34$, $p=.0009$. Follow-up comparisons revealed that relative to the vehicle or 2 mg/kg doses, MHPG concentrations were substantially elevated among mice that received the lower dose of poly I:C. Levels of NE were also influenced by exposure to the viral imitator, $F(2, 38)= 3.33$, $p<.05$, although, in this instance, follow-up tests indicated that both doses increased NE levels. With respect to DA, levels were also significantly altered, $F(2,40)=3.39$, $p<.05$, with the increases reflecting the effects of the higher dose, while the elevations in DA as a result of exposure to the 1mg/kg dose compared with the vehicle approached significance ($p=.09$). Examination of DA metabolites, as reflected by DOPAC concentrations, indicated an alteration in utilization, $F (2, 38) =5.07$, $p<.05$, with the follow up tests revealing that the concentrations of DOPAC were elevated only in those mice who received the 1 mg/kg exposure. Finally, neither serotonin levels nor 5-HIAA accumulations within the PVN were significantly affected by the treatments, $F(2, 42; 2, 40) =.58$ and 1.85, $p>.05$, respectively.

Median Eminence

Paralleling the variations within the PVN, MHPG concentrations within the median eminence were influenced by the systemic challenge, $F (2,41)=3.28$, $p<.05$. Bonferroni corrected t tests indicated that relative to the vehicle, the increases were attributed to the 2 mg/kg dose. Although variations of NE as well as DA were absent, ($F's< 1$), DOPAC values were influenced by the treatment,

$F(2, 40)=3.34$, $p<.05$. This effect was due to an elevation of the metabolite in the 2 mg/kg group relative to vehicle or 1 mg/kg treated mice. The accumulation of 5-HIAA increased by about 25-30% following the poly I:C treatment, but this increase did not reach statistical significance. Nevertheless, 5-HT levels were affected by the treatment, $F(2,40)=3.48$, $p<.05$, with the follow up tests indicating that mice given 2 mg/kg of poly I:C showed a reduction in serotonin levels compared with the other groups.

Locus coeruleus

Analysis of variance indicated no effects of the treatment upon MHPG concentrations, $F(2, 41)=2.01$, $p>.05$, although NE levels were affected, $F(2, 38)= 4.96$, $p<.05$. Specifically, follow up comparisons displayed that this effect was due to a reduction of NE levels of those mice exposed to the 2 mg/kg treatment.

Hippocampus

Hippocampal monoaminergic activity appeared to be relatively sensitive to the effects of poly I:C. The accumulations of MHPG were significantly influenced by the treatment, $F(2, 39)=8.25$, $p<.01$, with this effect attributable to an elevation of the metabolite in the 1 and 2 mg/kg groups. Although utilization was increased by both the doses of poly I:C, follow up tests did not show a difference between the 1 mg/kg and 2 mg/kg groups. Associated with increased MHPG concentrations was an alteration of NE levels, $F(2, 38)=3.67$, $p<.05$, with the follow-up tests indicating that reductions occurred with both doses of poly

I:C. While 5-HT levels were not influenced by the systemic challenge, $F(2,42)=1.06$, $p>.05$, 5-HIAA metabolites varied as a function of the treatment, $F(2, 40)=3.067$, $p=.05$. Follow-up tests indicated that 5-HIAA concentrations were elevated in response to the 2 mg/kg dosage.

Central Nucleus of the Amygdala

Given the sensitivity of the amygdala to stressor experiences, it was surprising that the administration of either the low or high dose of poly I:C failed to significantly affect any of the metabolites or monoamines within this region.

Prefrontal Cortex

Within the mPFC, MHPG metabolites were moderately affected by exposure to the treatment, $F(2,38)=3.360$, $p<.05$. The follow-up comparisons indicated that the elevations in utilization occurred in the 1 mg/kg group. However, these moderate increases of MHPG were not accompanied by a corresponding change of NE levels, ($F< 1$). Examination of DOPAC concentrations indicated that the treatment had an effect upon the metabolite's accumulation, $F(2,37)=3.473$, $p<.05$, with the follow-up tests confirming DOPAC accumulation was increased by both doses of poly I:C. Further examinations of the groups receiving poly I:C indicated that this accumulation was greatest in mice receiving the 1 mg/kg treatment. Other monoamines and metabolites alterations of DA, 5-HT, and 5-HIAA were absent, $F's < 1$.

Nucleus Accumbens

Despite the presumed sensitivity of the nucleus accumbens, the treatments did not influence DOPAC or DA concentrations within this site.

Experiment 2

Sickness Behaviors

Sickness behavior, was found to vary as a function of the Stressor x Test day housing x Poly I:C treatment interaction, $F(1,56) = 5.51$, $p < .05$. Figure 5 (top) depicts the sickness responses at the 60 min interval. The follow up comparisons, at 60 min following treatment indicated that among mice that had been continuously grouped housed or housed in isolation, or those that had been transferred from group housing to isolation, sickness behaviors were significantly greater following poly I:C than in saline treated mice. Among those mice that had been isolated, and then transferred to groups, the poly I:C treatment resulted in significantly greater sickness than among mice that had been isolated and then regrouped than among the other poly I:C treated groups.

Plasma Cytokines

Consistent with the data reported in Experiment 1, the poly I:C treatment increased the circulating levels of similar cytokines. Moreover, it was also found that the poly I:C treatment interacted, in certain regards, with the various social stressor conditions. For instance, as depicted in Figure 6, TNF-α, as well as IL-10 levels varied as a function of the Stressor x Test day housing x Poly I:C treatment interactions, $F(1, 52)=13.64$; 4.63, p's<.001, and <.05. In regard to the

former interaction, the follow-up tests indicated that poly I:C treated mice that were isolated and regrouped displayed the greatest elevations of this cytokine. Further, mice treated with the viral imitator that were either isolated for 2 weeks and remained so on the test day or were switched from a grouped to isolated condition also resulted in elevations of TNF-α. Likewise, the greatest increases of IL-10 in the latter interaction occurred in isolated and regrouped mice administered the ds RNA inducer. As expected, the effects of the vehicle injection had minimal effects on the proliferation of both these cytokines. Additionally, the poly I:C challenge increased the levels of IFN-γ, as well as that of IL-4, $F(1,50;1;53) = 10.47; 4.56$ p's$<.01$, and $<.05$. Despite a non-significant interaction 3 way interaction, the follow-up tests of the means of this interaction revealed that, mice isolated and regrouped concomitant with an immune challenge on the test day displayed elevated levels of these cytokines relative to vehicle treated mice that underwent the same paradigm of social stressors. Although the elevations of IL-6 were robustly increased by the viral imitator, $F(1,51)=33.65$, $p<.0001$, this finding should be interpreted with reservation as a number of samples were removed due to extreme variability in some instances (i.e., >1000 ρg/ml).

Corticosterone

Concentrations of plasma corticosterone varied as a function of the Stressor x Test day housing, and the Test day housing x Poly I:C treatment interactions, $F(1, 54) = 5.85$,

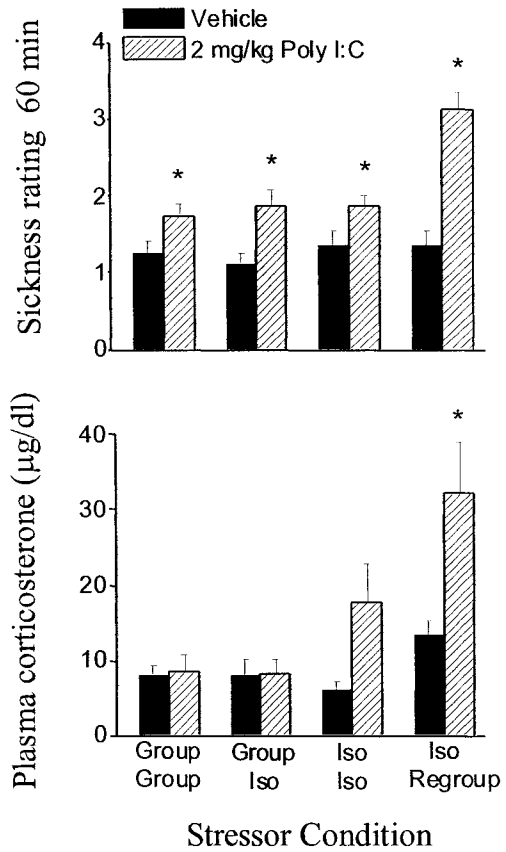

Figure 5. Mean (± S.E.M.) overall appearance (top) and plasma corticosterone (bottom) variations over a 60 min period as a function of the stressor and poly I:C treatments. Mice were either grouped housed or isolated for 2 weeks. On the test day half of the mice from each condition continued to be housed as such, whereas the remaining mice in each condition were transferred from either a grouped to an isolated or an isolated to grouped condition for 1 hour. Groups were further subdivided and received either vehicle or 2 mg/kg poly I:C injection. * P<.05 relative to vehicle treated mice (top). * P <0.05 relative to similarly stressed mice administered saline, as well as to continually group housed mice and group housed/isolated mice given either treatment (bottom).

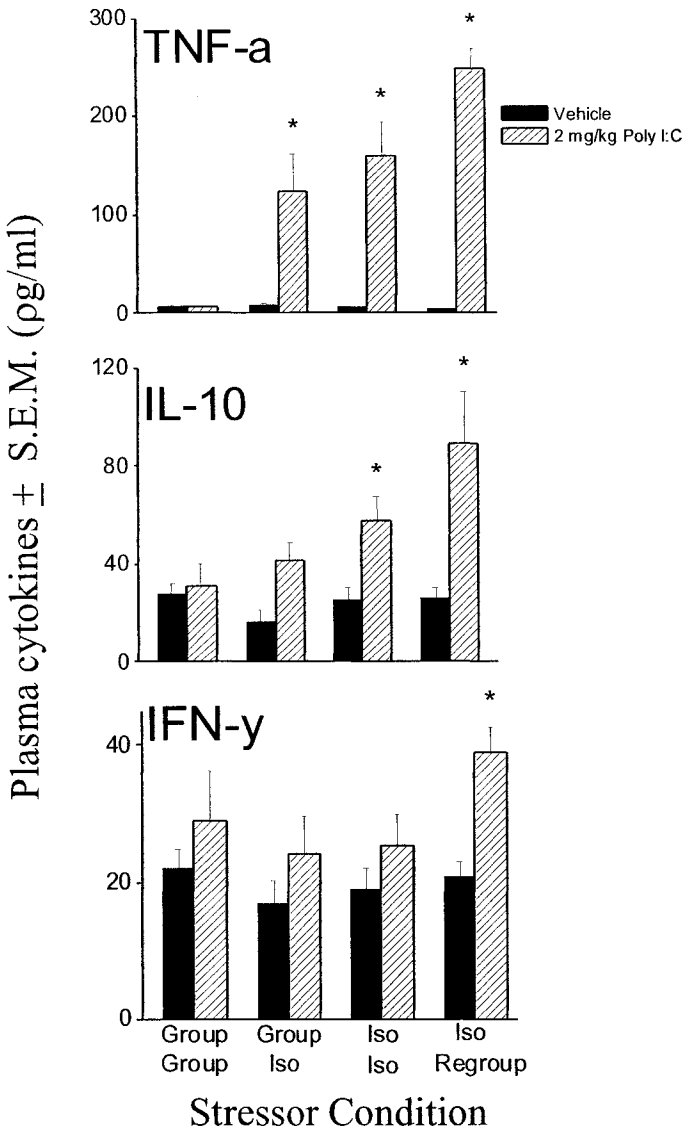

Figure 6. Plasma cytokine levels, mean (± S.E.M.), of TNF-α (top), IL-10 (middle), and IFN-γ (bottom), in mice either continually group housed, grouped and then isolated, isolated and remained as such or isolated and grouped on test day prior to treatment with either vehicle or poly I:C (2 mg/kg). * $P < 0.05$ relative to similarly stressed mice given vehicle injection.

10.86, p's < .05 and .01, respectively. The follow-up tests indicated that corticosterone levels among mice housed in isolation for 2 weeks and then regrouped were higher than among the remaining conditions. Moreover, among mice that were housed in isolation for 2 weeks and then challenged with poly I:C, corticosterone levels were higher than among mice that had been challenged with poly I:C following group housing. As depicted in Figure 5 (bottom), the elevated corticosterone levels among mice that had been isolated for two weeks and then given poly I:C following a brief period of regrouping was particularly high, although the 3-way interaction of the Stressor x Test day housing x Poly I:C treatment did not reach statistical significance.

Monoamines and Metabolites

Paraventicular Nucleus

The concentrations of MHPG within the PVN were not altered as a function of the Stressor x Test day housing x Poly I:C treatment interaction. However, metabolites were moderately elevated as a result of singly housing mice for 2 weeks, $F(1,52)=3.72$, $p=.05$, as well as in response to regrouping mice with their previous cage mates, $F(1,52)=10.28$, $p<.01$. Moreover, elevated MHPG accumulation was evident following poly I:C treatment, $F(1,52)=4.50$, $p<.05$. As predicted, examination of the means comprising the higher-order interaction revealed that mice isolated and regrouped prior to poly I:C treatment displayed additive increases of MHPG, contrasted with all other groups, with the exception of those mice group housed and administered the viral imitator (see

Figure 7). In regard to levels of this neurotransmitter, hypothalamic NE varied as a function of the Test day housing x Poly I:C treatment interaction, $F(1,54)=9.57$, $p < .01$. The follow-up tests indicated that regrouped mice challenged with poly I:C displayed lower levels of NE relative to similarly treated mice administered saline injections, or to those mice that remained isolated on test day and were injected with either substance. In view of the poly I:C induced alterations, $F(1, 54)=4.12$, $p<.05$, as well as our *a priori* predictions, we examined the means constituting the higher-order interaction. Indeed, the follow-up tests indicated that the isolated and regrouped mice given an immune challenge displayed appreciably reduced levels of NE relative to their similarly treated counterparts not given an immune challenge. Thus, in view of the elevations of MHPG and the corresponding NE reductions engendered by the combinations of the psychosocial stressors and poly I:C treatments, it is possible that the NE alterations reflects the cumulative effects of the elevated utilization.

Alterations of DA levels were very much like the variations seen with respect to NE. Specifically, DA levels were influenced by the Test day housing x Poly I:C treatment interaction, $F(1,53)=4.46$, $p<.05$, with the follow-up comparisons revealing that regrouped mice administered poly I:C displayed reduced DA relative to mice reunited with previous cage mates prior to vehicle treatment. Furthermore, post-hoc comparisons of the means constituting the 3 factor interaction, indicated that the 2 week period of isolation combined with the regrouping stressor synergistically reduced DA levels in mice challenged

with poly I:C, as opposed to the vehicle treatment. Although the reductions of DA levels were profound, corresponding changes of DOPAC concentrations were not observed in this instance, ($F<1$).

Serotonergic activity was also influenced by the Stressor x Test day housing interaction as well as the Stressor x Poly I:C treatment interactions, $F(1,52)=8.55, 4.85$ p's<.01 and.05 respectively. In concert with the levels of other significantly altered neurotransmitters with the PVN, examination of the means of the 3 factor interactions indicated that 5-HT levels were synergistically diminished when the poly I:C challenge followed the isolation and regrouping conditions. Finally, due to extremely high variability within some groups, follow-up tests concerning 5-HIAA concentrations failed to reveal any significant differences in the utilization of this metabolite.

Hippocampus

Although alterations of MHPG concentrations and levels of NE did not occur within the hippocampus, serotonergic activity was markedly altered in this region. The accumulations of 5-HIAA varied as a function of the Stressor x Test day housing, $F(1, 52)=8.62$, $p<.05$. Follow-up tests revealed that increases of the metabolites occurred in mice that were regrouped following the 2 weeks of isolation, relative to similarly treated mice that remained in isolation on test day. Although the poly I:C treatment failed to interact with either the stressor or test day housing conditions, administration of the viral imitator increased this metabolite, $F(1,52)=4.80$, $p<.05$, and, owing to our *a priori* hypothesis, follow-

up tests were performed of the simple effects for the higher-order interaction. These tests indeed indicated that mice that were isolated for 2 weeks and regrouped prior to the poly I:C injection, displayed synergistic elevations of 5-HIAA metabolites relative to similarly treated mice given vehicle injections. Additionally, the levels of 5-HT varied as a function of the Stressor x Test day housing x Poly I:C treatment interaction, $F(1, 54)=5.10$, $p< .05$. Paralleling the variations seen with the metabolites, the analysis of the means comprising this interaction indicated that levels of 5-HT were synergistically elevated in mice isolated and regrouped succeeded by administration of poly I:C challenge.

Central Nucleus of the Amygdala

As depicted in Figure 7, the amine variations in the CeA were very much like those observed in the PVN. The concentrations of MHPG as well as the levels of NE, varied as a function of the Stressor x Test day housing x Poly I:C treatment interactions, $F(1, 54; 1, 53)=3.26$, and 3.84 $p=.07$, and .05 respectively. In both instances, the utilization and levels of NE were synergistically escalated only in poly I:C treated mice who underwent the individual housing condition concomitant with a regrouping of cage mates on test day. Thus, in view of the variations of NE utilization and levels within the CeA, and the lack of effect with respect to other transmitters, it appears as though selectivity of NE neuronal function was exerted by the treatments within the central amygdala.

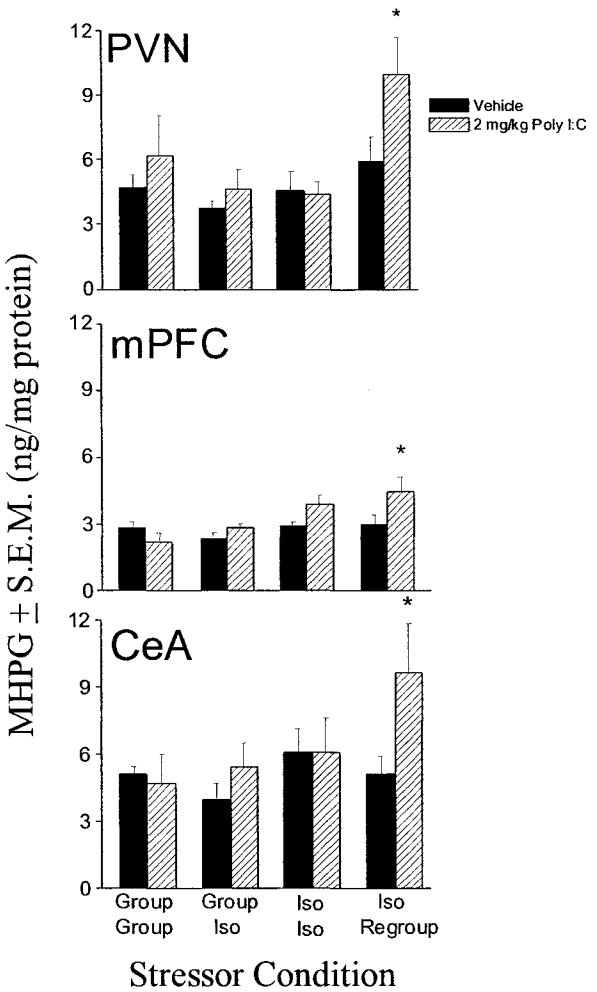

Figure 7. Mean (± S.E.M.) concentrations of MHPG in the PVN (top), mPFC (middle), and CeA (bottom) in mice that underwent conditions of: continual group housing, group housing followed by isolation, continual isolation housing or a period of 2 week isolation followed by a 1 hour grouping, prior to either vehicle or 2 mg/kg of poly I:C challenge. * $P < 0.05$ relative to similarly stressed mice administered vehicle treatment, as well as to continually group housed mice administered vehicle.

Prefrontal Cortex

With the exception of NE variations, the effects of the stressors or immune activation on neurotransmitter functioning were modest within the mPFC, an area which would otherwise be particularly responsive to psychosocial or cytokine exposures. The ANOVA indicated that levels of MHPG varied as a function of the Stressor x Poly I:C treatment interaction, $F(1, 55)=5.29$, $p<.05$. The follow up tests indicated that mice that were housed in isolation for a period of 2 weeks were particularly sensitive to the effects of poly I:C, as levels of MHPG were increased in this group contrasted with either similarly treated mice administered the vehicle, or to mice housed in groups for 2 weeks given either injection. Irrespective of the non-significance of the 3 factor interaction, upon examining the means comprising this interaction, the follow-up tests indicated that MHPG concentrations were additively increased in poly I:C challenged mice that underwent the isolated housing and regrouping conditions, relative to similarly treated animals administered the vehicle (see Figure 7). With respect to NE, the levels of this neurochemical were affected by the Stressor x Poly I:C treatment and to a limited extent the Test day housing x Poly I:C treatment interactions, $F(1,54)=5.13$; 2.92, p's $<.05$ and $=.09$. Commensurate with the variations of utilization, follow-up tests of the means of the higher order interaction indicated significant increases of NE in isolated and regrouped mice only when challenged with the viral imitator.

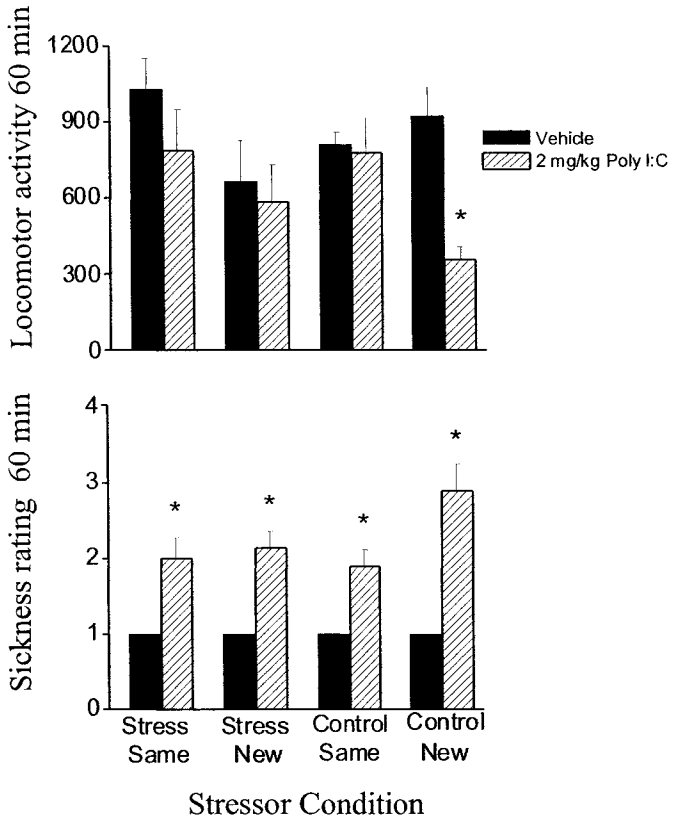

Figure 8. Mean (± S.E.M.) locomotor activity (top) and sickness ratings (bottom) in mice that were either chronically stressed for 3 weeks or remained group housed. On test day, these groups were further subdivided such that half of the mice of both conditions either remained with their same cage mates or were placed with new residents in a novel cage, prior to challenge with either vehicle or 2 mg/kg of poly I:C. * $P<0.05$ relative to similarly treated mice administered vehicle injections.

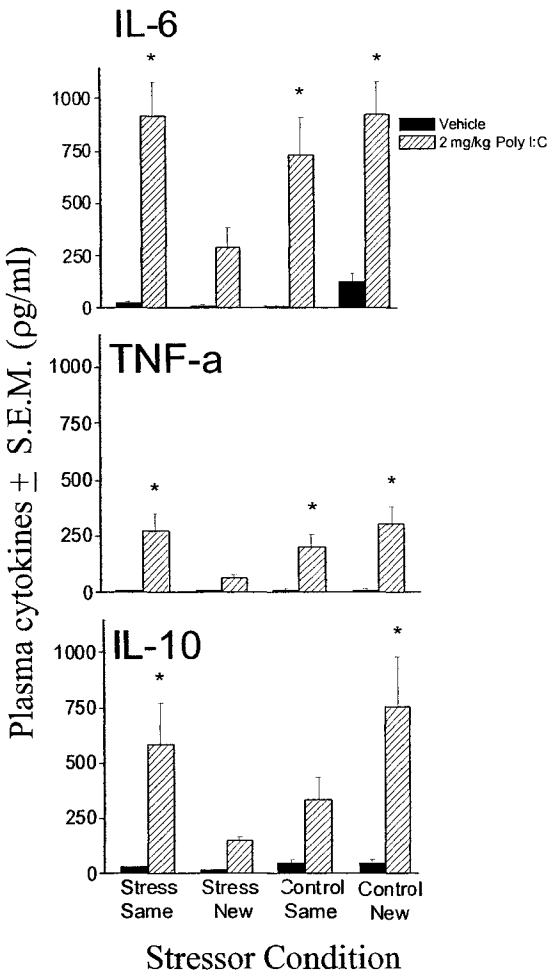

Figure 9. Plasma concentrations of circulating cytokines, IL-6 (top), TNF-α (middle) and IL-10 (bottom) as a function of the conditions and poly I:C treatments in mice that were either chronically stressed for 3 weeks or remained group housed. On test day, these groups were further subdivided such that half of the mice of both conditions either remained with their same cage mates or were placed with new residents in a novel cage for 1 hour, prior to challenge with either vehicle or poly I:C (2 mg/kg). * $P < 0.05$ relative to similarly treated mice given vehicle treatment.

Experiment 3

Locomotor Activity

Locomotor activity varied as a function of the Chronic social stressor x Acute housing x Poly I:C treatment interaction, $F(1, 56) = 3.77$, $p = .05$. Follow-up comparisons confirmed that among mice that had not been chronically stressed, but were introduced to novel cage mates for 1 hr, poly I:C provoked a marked reduction of motor activity relative to vehicle treated mice. In contrast, among control group housed mice that received poly I:C in the presence of their cage mates, locomotor activity was not affected. Mice that had been exposed to the 3 week chronic social stressors, followed by introduction to new cage mates, were later found to exhibit reduced motor activity relative to mice that remained with their cage mates. However, as depicted in Figure 8 (top), in the chronically stressed mice exposed to strangers, the poly I:C treatment did not produce a further reduction of locomotor activity. The figure depicts the peak effects (50-60 min) of poly I:C treatment on locomotor behaviors. As mixed measures ANOVA yielded the same outcomes regardless of whether the data were analysed across all time points or only at the time of the peak response, the figures were chosen for the sake of clarity.

Plasma Cytokines

Figure 9 shows the circulating cytokine levels as a function of the housing conditions and poly I:C treatments to which mice had been exposed. Analyses of variance indicated that poly I:C did not affect either IL-1, IL-2, or the inhibitory cytokine, IL-4. In contrast, the levels of IL-6 and TNF-α varied as a function of

the Chronic social stress x Acute housing x Poly I:C treatment interaction, $F(1, 52; 1,51) = 4.86, 6.39$ p's $< .05$ and .01 respectively. The follow-up tests indicated that in vehicle treated animals the levels of IL-6 and TNF-α were relatively low, but increased appreciably with poly I:C treatment. The magnitude of the increase, although fairly substantial, was least pronounced in mice that had been chronically stressed and then presented to new cage mates. A similar outcome was not evident by exposure to the chronic social stressor or simply presenting mice to strangers. The effects of the poly I:C and housing manipulations had very different effects with respect to other cytokines. Specifically, in the case of both IFN-γ and IL-1β it was observed that the Chronic social stressor interacted with the poly I:C treatments, $F(1,54) = 5.93, 2.86$, $p = .01$ and .07, respectively. The poly I:C treatment increased both IFN-γ and IL-1β, but these effects were only evident among mice that had endured the chronic social stressor.

Sickness Behaviours

The presence of sickness behaviors varied as function of the Acute housing x Poly I:C treatment interaction, $F(1, 56) = 4.26$, $p < .05$. The follow up tests confirmed that sickness behaviours elicited by poly I:C were more pronounced when administered following exposure to new cage mates than after exposure to original cage mates. Although the interactions with the chronic social stressor condition did not reach statistical significance ($p = .11$), as seen in Figure 8 (bottom) the effects of the poly I:C treatment on sickness tended to be

greater in mice that had not been exposed to the chronic stressor. The analyses yielded similar results whether the data was assessed across all time points or restricted to the peak effect. As such, the figure shows the effects of poly I:C treatment upon sickness behaviours only at the 60 min interval.

Corticosterone

Corticosterone levels varied as a function of the Chronic social stressor x Acute housing condition interaction, $F(1, 56) = 4.78$, $p < .05$. The follow-up tests indicated that mice that had not undergone the chronic stressor and were then exposed to strangers exhibited higher levels of this hormone than similarly treated mice that had been chronically stressed, or mice that had been chronically stressed but were maintained with their original cage mates. Although the ANOVA indicated that corticosterone increased as a function of the poly I:C treatment, $F(1, 56) = 6.72$, $p = .01$, this variable did not interact with either the housing or the chronic social stressor conditions. Nevertheless, the *a priori* hypothesis prompted follow-up tests of the means comprising the interaction. This analysis, indeed, indicated that poly I:C only provoked a significant rise of plasma corticosterone in mice that had not been exposed to the chronic social stressor but were subjected to 1 hr exposure to new mates.

Monoamines and Metabolites

Paraventicular Nucleus

As depicted in Figure 10, MHPG concentrations within the PVN varied as a function of the Chronic Social Stressor x Acute housing x Poly I:C

treatment interaction, F (1, 53)=3.89, p=.05. The follow-up planned comparisons revealed a marked increase in the level of MHPG in mice that had not been chronically stressed but had been exposed to strangers prior to poly I:C treatment. However, if mice had been exposed to a series of psychosocial stressors, then the effects of poly I:C were not enhanced by exposure to either an unfamiliar mice or to their previous cage mates. Levels of NE were somewhat increased among mice that had been chronically stressed and then exposed to poly I:C, but were not apparent among non-stressed mice, $F(1, 56) = 3.34$, $p = .07$. None of the remaining main effects or interactions reached statistical significance.

DOPAC concentrations were influenced by the Chronic social stressor x Poly I:C treatment and the Acute housing x Poly I:C treatment interactions, $F(1,54)=6.01; 4.49$, p's $<.05$. The follow up tests indicated that this effect was particularly pronounced for poly I:C treated mice who underwent the chronic social stressor condition and remained housed with their original cage mates. Levels of DA were found not to vary with the stressor or acute housing conditions, although they were increased by administration of poly I:C, $F(1, 54)=6.94$, $p=.01$.

As in the case of NE utilization, treatment with poly I:C increased the levels of 5-HT as well as 5-HT utilization, reflected by increased 5-HIAA accumulation , $F(1, 55) = 5.50, 5.04$, $p < .05$. However, this outcome did not

vary as a function of stressor history or whether mice had been introduced to strangers.

Locus coeruleus

Poly I:C and the chronic stress exposure interacted to influence MHPG accumulation within the locus coeruleus, $F(1,56)$ 4.82, $p < .05$. Placing mice in groups resulted in poly I:C later provoking a greater increase of MHPG provided that they had not been exposed to a chronic social stressor. Moreover, as depicted in Figure 10, this effect was particularly notable if the grouping manipulation involved unfamiliar mice. The variations of NE also varied as a function of this same interaction, $F(1,56)=3.97$, $p < .05$. In this instance NE levels, however, only increased significantly in response to poly I:C if mice had been placed together with unfamiliar mice and had not previously undergone the chronic social stress.

Hippocampus

The utilization of NE, as reflected by concentrations of MHPG, were moderately influenced by both the Chronic social stress x Acute housing and the Chronic social stress x Poly I:C treatment interactions, $F(1,53)=3.37, 2.97$, $p=.07$ and .09, respectively. However, due to a large amount of variability, follow up tests did not indicate any significant differences between these groups. While interactions amongst these factors were not evident with respect to NE levels, the chronic social stressor, acute housing, and poly I:C treatment conditions each influenced hippocampal NE levels, $F(1,56)=8.26, 5.9, 3.84$, $p<.05$. Follow-up

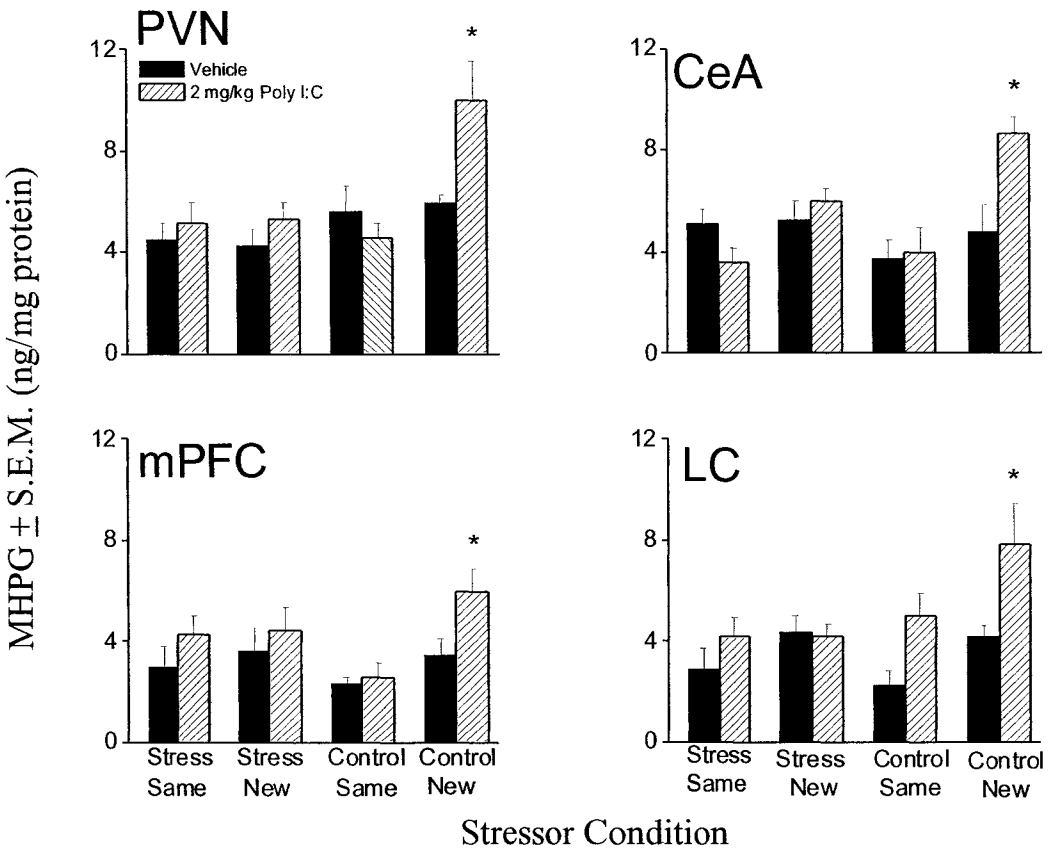

Figure 10. Mean (± S.E.M.) concentrations of MHPG in the PVN (top left), mPFC (bottom left), CeA (top right), and LC (bottom right) as a function of the conditions and poly I:C treatments in mice that either underwent a paradigm of chronic social stressors or were left group housed and undisturbed. On test day, half of the mice of each condition either remained with their same cage mates or were placed into a novel cage with 3 new residents for 1 hour. Thereafter, mice were challenged with either saline or 2 mg/kg of poly I:C. * $P < 0.05$ relative to similarly treated mice given vehicle treatment.

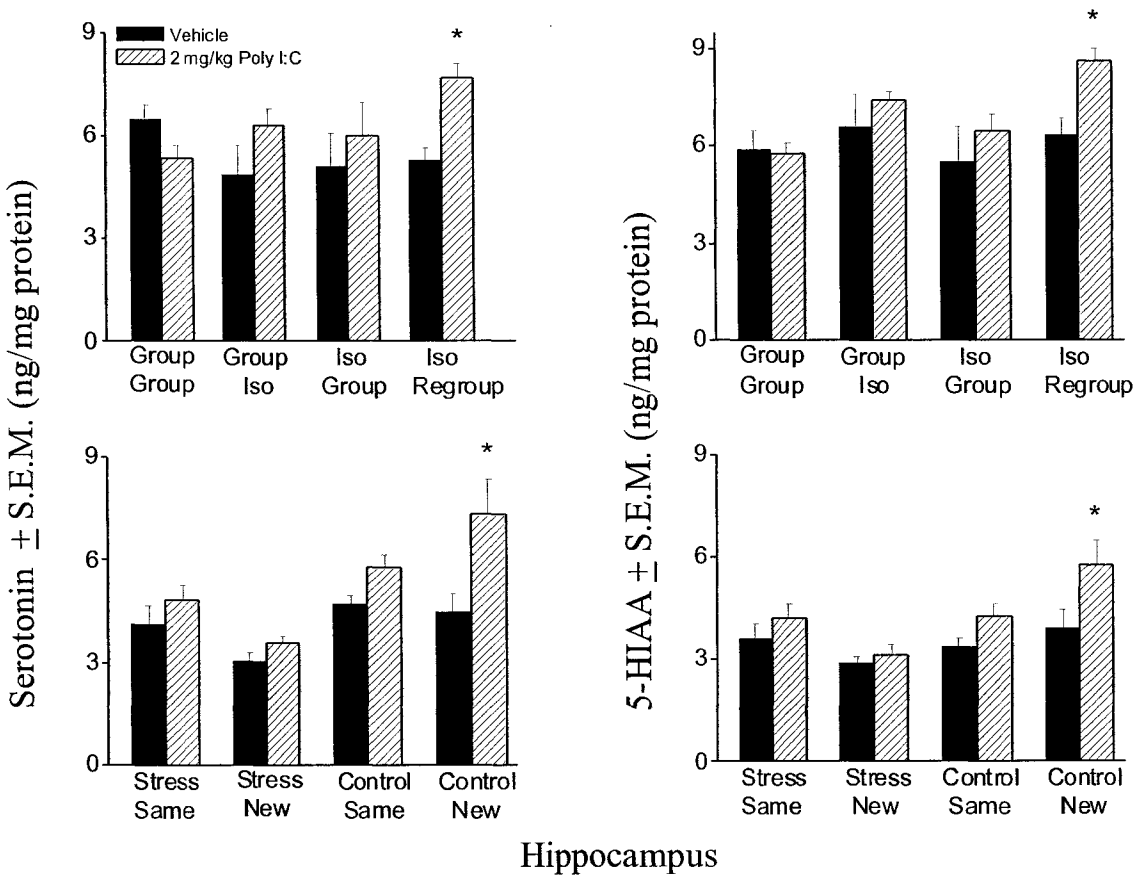

Figure 11. Levels of 5-HT and concentrations of 5-HIAA, mean (± S.E.M.), in Experiments 2 and 3, involving various types of psychosocial stressors prior to the test day injection of either vehicle or 2 mg/kg of poly I:C. * $P < 0.05$ relative to similarly treated mice given vehicle treatment.

tests indicated that 3 weeks of chronic social stress or exposure to new cage mates for 1hr resulted in reduced NE levels relative to control, non-stressed mice or mice that remained with their original cage mates, whereas treatment with poly I:C resulted in elevated levels of NE.

The variations of 5-HIAA (see Figure 11), much more so than those of MHPG concentrations, were more in line with the corticosterone changes. Specifically, 5-HIAA increased significantly among poly I:C animals that had not been exposed to the chronic social stress but had been housed with unfamiliar mice for a short period. A similar, albeit smaller increase of 5-HIAA was evident among mice that had been placed in a novel cage with their their previous cage mates. Comparable effects of poly I:C were not evident in mice that had received the chronic social stress. The interaction between the chronic social stress and acute housing condition was statistically significant, $F(1,54) = 11.01$, $p < .001$, as was the main effect for poly I:C treatment, $F(1,54) = 9.79$, $p < .01$. The changes of 5-HT concentrations roughly paralleled those of 5-HIAA showing that poly I:C increased 5-HT levels, $F(1, 54) = 21.98$, $p < .001$, but this effect varied with the chronic social housing condition, being evident only if mice had not been exposed to the chronic stressor before the acute rehousing condition, $F(1,54) = 6.22$, $p < .05$.

Central Nucleus of the Amygdala

Variations of MHPG in the CeA were similar to those observed in other areas of the brain. Specifically, the ANOVA indicated that metabolite levels

varied as a function of the Acute housing x Poly I:C treatment interaction, $F(1,53) = 5.67$, $p < .05$. The follow up tests indicated that poly I:C elicited a greater increase of MHPG if mice had been exposed to a stranger before the immune challenge. As depicted in Figure 10, however, this outcome was notable only among mice that had not undergone the chronic social stressor. There was also a trend towards increased DOPAC, as reflected by the housing of mice with new mates for 1hr, $F(1,56)=3.08$, $p=.08$. In contrast, levels of other monoamines and metabolites were not influenced within the CeA.

Prefrontal Cortex

Analyses of monoamine levels and metabolites within the mPFC indicated that both the acute housing condition and the poly I:C treatments, increased MHPG accumulation, $F(1, 52) = 6.73$ and 5.68, $p < .05$. Although the interaction with chronic social stressor was not significant, planned pairwise comparisons indicated that the rise of MHPG following poly I:C treatment was only significant (relative to similarly treated mice that received saline) if mice had not been exposed to the chronic stressor but exposed to strangers just prior to the poly I:C treatment (see Figure 10).

In regards to DA utilization and levels, the ANOVA indicated that both the DOPAC metabolites DA levels varied as a function of the Chronic social stressor x Acute housing x Poly I:C treatment interactions, $F(1,53;1,55)=13.14$ and 8.23 p's<.01. Comparisons of the means comprising the higher order interactions indicated that the poly I:C treated, non-stressed mice that were

exposed to strangers, displayed the greatest increases of concentrations and levels of DA relative to their similarly treated non-stressed counterparts administered saline, as well as to poly I:C treated mice that were chronically stressed and placed in a strangers cage (See Figure 12).

As in the case of MHPG, the accumulation of 5-HIAA within the mPFC varied as a function of the Chronic social stress x Acute housing treatment x Poly I:C treatment mice received, $F(1, 52) = 4.78$, $p < .05$. The follow-up tests confirmed that poly I:C increased the level of 5-HIAA in mice that had not received the chronic stressor, but had been placed in the company of strangers prior to injection. If mice had been chronically stressed for 3 weeks, then the poly I:C was without effect regardless of the acute housing stressor.

Nucleus Accumbens

Treatment effects for DOPAC within this brain region increased as function of poly I:C exposure, $F(1,55)=8.52$, $p<.01$, while mice given 3 weeks of chronic social stress displayed reductions of this metabolite relative to group housed controls, $F(1,55)=9.43$, $p<.01$.

Discussion

Considerable research has documented the deleterious effects of cytokines and stressors on physiological and behavioral functioning (Maes and Meltzer, 1995; Paykel, 2001; Hayley et al., 2001; Turnbull and River, 1999; Day et al, 1999; Anisman et al., 2005; Pucak and Kaplin, 2005), and these treatments, in fact, induce several common outcomes (Anisman and Merali, 1999). In view

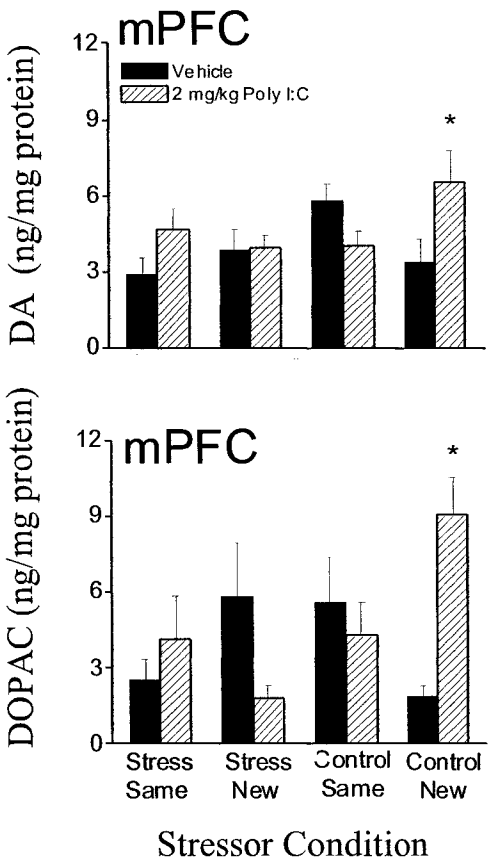

Figure 12. Levels of DA and concentrations of DOPAC, mean (± S.E.M.), as a function of the conditions and poly I:C treatment in mice that were either chronically stressed for 3 weeks or remained group housed. On test day, these groups were further subdivided such that half of the mice of both conditions either remained with their same cage mates or were placed with new residents in a novel cage for 1 hour, prior to challenge with either vehicle or poly I:C (2 mg/kg). * $P < 0.05$ relative to similarly treated mice given vehicle treatment as well as to chronically stressed mice exposed to new residents prior to poly I:C challenge.

of these similarities, it had been proposed that the CNS may be interpreting immune activation in a similar fashion to that of how it perceives a stressor (Anisman and Merali, 1999). Social stressors represent a common and unavoidable threat in mammalian species, and as such, are likely to contribute to shaping the stress response (Blanchard et al., 2001). Furthermore, social stressors embody a more ethologically relevant factor for animals than most laboratory stressors, and are also likely fundamental in the progression of stress related human diseases, given their frequency of occurrence and evolutionary importance in a social ordered species (Bartolomucci et al., 2003). As such, we assessed the potential for synergistic and/or additive responses using an immune challenge comprising poly I:C in conjunction with a social stressors. The findings from these studies indicated that neuroendocrine, central neurotransmitter and immunological alterations, similar to those observed in response to other immune system activators, also occurred in response to the viral imitator. Moreover, it appeared that poly I:C had a greater impact on these systems after a period of exposure to a psychosocial stressor comprising either a regrouping of cage mates or the introduction of mice to unfamiliar residents.

Behavioral and Neuroendocrine Variations

Ordinarily cytokines and endotoxins elicit neurovegetative symptoms such as lethargy, anorexia, ptosis, piloerection, fever, and weakness, collectively referred to as sickness behaviors (Dantzer, 2001; Dunn et al., 2005; O'Brien et al., 2004). Many of these symptoms are similar to those experienced by cancer

patients that received cytokine immunotherapy (Kelley et al., 2003), and may also promote symptoms more closely aligned with depressive states (e.g., anhedonia, and signs of anxiety) (Anisman et al., 2005; Merali et al., 2003). With respect to poly I:C, this treatment has been shown to reduce daily amount of spontaneous running wheel activity as well as open field exploration in models of immunologically induced fatigue (Katafuchi et al., 2003; 2005). In the present investigation we demonstrated that the viral imitator also induced sickness effects similar to those of cytokines, although at the doses used the magnitude of the behavioural sickness was modest relative to that observed with either LPS or IL-1β treatments (Swiergiel, et al., 1997; Dantzer et al., 2001). As predicted, when the poly I:C treatment was preceded by a psychosocial stressor comprising either the regrouping of cage mates following a period of isolation or the introduction of mice to strangers, the magnitude of the sickness effects (and reduced motor activity) was appreciably exaggerated. Thus, from the behavioral perspective, the sickness elicited by poly I:C was not simply a consequence of the cytokine changes engendered by the treatment, but were intricately dependent on other psychosocial stressor events.

The systemic poly I:C challenge, like other immune system activators, increased plasma corticosterone concentrations. The effects on HPA axis activity were modest as observed in other studies using the viral imitator (Miller et al., 1997; Ruzek et al., 1997). Relative to other pro-inflammatory cytokines and endotoxins (i.e., IL-1β, TNF-α and LPS), which at moderate doses induce

plasma corticosterone levels of approximately 40 $\pm$ 5 μg/dL (Swiergiel and Dunn, 2006; Chen et al., 2005; Fleshner et al., 1997), the elevations associated with either the treatment alone or the combination of the treatment with the social stressor were substantially less in the present investigation. Thus, poly I:C can be characterized as a moderate stimulator of endocrine activity, even in the presence of an additive effect.

With regard to the saline treated mice, the corticosterone levels were higher than usually observed, likely reflecting the fact that mice were placed in a novel environment (for behavioral tests) after immune activation, rather than being returned to their home cages. As such, the ultimate level of corticosterone stemming from the poly I:C challenge likely reflected the summed effects of the immune activation and the mild stressor (novel environment). Counterintuitively, however, if mice had experienced a chronic social stressor, as was the case in Experiment 3, poly I:C injection failed to provoke an increase of corticosterone levels that would ordinarily take place when immune activation occurred following exposure to a social stressor (Avitsur et al., 2005; Quan et al., 2001). These results were unexpected, especially given the well documented sensitization that occurs in response to stressors and cytokine challenges (Hayley et al., 2003; Hayley et al., 2000; Brebner et al., 1999). However, it has been shown that the initial elevations of CRH mRNA levels within the PVN of the hypothalamus, (in rodents subjected to chronic stressors), were attenuated following an additional acute exposure to a psychogenic, but not a systemic

stressor (Ostrander et al., 2006). Commensurate with this report, the findings in the present investigation are suggestive of an adaptation of endocrine responses following repeated exposure to unfamiliar mice, environments and aggressive conspecifics encountered over the course of the chronic social stressor condition. It is likely that desensitization may occur within psychogenic sensitive, limbic system neural circuitry or, alternatively, that activation of other as yet unidentified systems may counteract the effects that would otherwise occur.

Central Neurochemical and Plasma Cytokine Variations

As indicated earlier, accounts of poly I:C induced alterations of central monoaminergic activity have been relatively infrequent. However, there have been several reports indicating that poly I:C increased hypothalamic 5-HIAA/5-HT ratios, as well as serotonin transporter (5-HTT) mRNA, leading to reduced levels of 5-HT within the mPFC (Dunn and Vickers, 1994; Katafuchi et al., 2005); however, none examined the effects of the viral imitator at extra-hypothalamic sites beyond the mPFC, or the potential for synergy with stressor encounters. In the present investigation it was confirmed that poly I:C influenced central neurotransmitter activity, and not unexpectedly, such effects varied across brain regions. In contrast to studies suggesting that the endocrine and neurochemical responses to poly I:C lacked a noradrenergic component (Dunn and Vickers, 1994), it was observed that poly I:C induced alterations of NE and/or its metabolite, MHPG, in several regions, including the PVN, median eminence, hippocampus and mPFC. The noradrenergic system is believed to be

an important stress modulatory system, activated by both systemic and processive stressors in response to allostatic (homeostatic) challenges (Morilak et al., 2005; Aston-Jones, 2002; Standford, 1995). In view of the MHPG and NE alterations elicited across multiple brain regions following the treatment, it clearly appears that that poly I:C is capable of activating this system, albeit, in a manner that is less pronounced than the effects of traditional threats to homeostasis.

With respect to other amine alterations, levels of DA and DOPAC were increased within the PVN, and additionally, DOPAC concentrations were elevated in the mPFC. Further, 5-HIAA concentrations were increased in the hippocampus (Experiment 1-3), PVN (Experiment 2), whereas levels of 5-HT were elevated in the hippocampus, PVN and mPFC (Experiments 2-3). Paralleling the NE and MHPG alterations, the changes observed with other neurotransmitters and metabolites were consistent with the view that poly I:C may exert effects similar as that of other immune system activators, such as IL-β, TNF-α and LPS, although as already indicated, these actions were relatively modest (Dunn, 2001; Lacosta et al., 1998;1999; Hayley et al., 1999). Moreover, inasmuch as the treatment increased the levels 5-HT and DA as well as the concentrations of 5-HIAA and DOPAC, these findings may possibly reflect the increased synthesis (in addition to utilization) of these neurotransmitters, in stress and cytokines sensitive areas of the brain.

Beyond examining the responses to systemic challenge with intraperitoneal injection, an additional goal of the present investigation was to determine how a protracted social stressor might moderate or influence the effects of administering the synthetic double stranded RNA, with a particular focus on central neurochemical substrates believed to be aligned with depressive states (Urani et al., 2005). Commensurate with the behavioural and plasma corticosterone variations, the data of these experiments indicated both synergistic and additive effects with respect to central monoamine activity in response to the combination of immune activation and the psychosocial stressors. These findings are consistent with other documented reports of synergisms and additive effects between cytokines and stressors (i.e., Merali et al., 1997; Song et al., 1999); however, to our knowledge, these data represent the first such accounts using stressors that are of a social nature. The data presented here lend credence to the view that the CNS is interpreting cytokine challenges in a manner similar to processive insults (Dunn, 1995; Anisman and Merali, 1999). Further, in the present investigation, it also appeared that central neurochemical substrates are markedly elevated under such conditions, and might thus increase the probability for adverse outcomes, such as allostatic overload (McEwen, 2000).

It appeared as though hippocampal serotonergic activity was particularly sensitive to the joint effects of the poly I:C treatment and the stressor, as 5-HT levels and 5-HIAA concentrations were shown to be synergistically increased.

The pronounced alterations of these neurotransmitters and metabolites within this area are noteworthy, as the hippocampus is rich in serotonergic terminal projections from the dorsal raphe nuclei and is commonly activated in response to variety of stressors (McKittrick et al., 1995; Filipenko et al., 2002), as well as being the target for various exogenous cytokine challenges (Brebner et al., 2000; Cho et al., 1999). Indeed, it was shown here that the majority of serotonin alterations were confined to this brain region. This is not to suggest that other amine variations that occurred were irrelevant, particularly as the noradrenergic and dopaminergic alterations observed here are also consistent with the proposition that cytokines can induced stress-like neurochemical changes that are believed to mediate, in part, the etiology of affective disorders (Hayley et al., 2003).

At this juncture, however, it is important to note that there were discrepant results between the dose response study and the studies involving the poly I:C treatment coupled with the social stressor conditions. For instance, some of the amine alterations observed in certain areas of the brain (i.e., median eminence) in the dose response study were without effect in the 2nd and 3rd Experiments. Moreover, it will be recalled that some of the most profound neurotransmitter changes occurred in response to the *lower* dose of the viral imitator. Although the sources for these between-experiment differences are uncertain, in the present investigation, the dosages of poly I:C used were relatively modest and the effects of the viral imitator were assessed only at two time points (i.e., 1-1 ½

hours following i.p. injection). In view of the fact that the neurotransmitters responses to this substance remain largely unknown, it may have been advantageous to examine the consequences of the treatment at multiple time points. Further to this point, it ought to be considered that different types of social stressors (i.e., regrouping mice *versus* exposure to three new cage mates) might differentially influence monoamine utilization. Moreover, as opposed to assessing the monoamines and metabolites in post mortem brain tissue, as was the case in the studies presented here, an *in vivo* assessment of the amine alterations would have provided a more accurate representation of the variations of neuronal functioning in response to the conjoint effects of both the social stressors and poly I:C.

The findings presented here also revealed that systemic poly I:C challenge dose dependently increased circulating levels of IL-6 and TNF-α, consistent with reports of other studies using poly I:C (Fortier et al., 2004). Interestingly, in Experiment 3, elevations of IL-1β and IFN-γ were also evident; however, this only occurred in mice that underwent the chronic social stressor prior to the immune activation. Additionally, in Experiment 2, the conjoint effects of social stressors and cytokine treatment resulted in pronounced effects upon both IFN-γ and TNF-α in those mice that were isolated and then regrouped. It is particularly relevant that poly I:C increased the circulation of IL-1β, IL-6 and TNF-α, as these cytokines are believed to mediate many of the symptoms of sickness behavior (Swiergiel et al., 1997), and also stimulate the HPA axis (Silverman et

al., 2005; Chrousos, 1995). Furthermore, amongst other immune alterations, patients suffering from depression display elevations of IL-6, TNF-α, IL-1β, as well as IFN-γ (Lanquillon et al., 2000; Levine et al., 1999; Schiepers et al., 2005). As treatment with LPS also influenced plasma concentrations of these cytokines and affected mood states (Chen et al., 2005, Reichenberg et al., 2001), it is possible that poly I:C would have similar effects on psychological processes.

The similarities between the effects of LPS and poly I:C should not be misinterpreted as implying that immune system activation in response to these two agents is identical or that the same underlying processes are responsible for the comparable outcomes. In fact, the Toll-Like receptors activated by ds RNA are different from that of LPS (Alexopoulou et al., 2001), and, although viral and bacterial infections may share similar end effects, the specific plasma cytokine involvement, as well as other processes, for these actions need to be further elucidated. It is significant, however, that IL-6 knockouts treated with poly I:C displayed a blunted corticosterone response relative to wild type controls, or to IL-6 knockouts treated with LPS, suggesting that poly I:C glucocorticoid responses are, in part, IL-6 dependant (Ruzek et al., 1997).

In addition to poly I:C induced elavtions of proinflammatory cytokines, it was observed that the viral imitator increased circulation levels of IL-10, an inhibitory cytokine. Moreover, levels of this cytokine were further increased in mice who underwent the isolation and regrouping conditions prior to challenge

with poly I:C. Although it may appear counterintuitive that an anti-inflammatory cytokine would be substantially elevated in response to an immune activator, delicate balances between pro- and anti-inflammaotry factors may be ideal to maintain optimum functioning. Further to this point, although, this cytokine has been reported to inhibit the production of IL-1α, IL-1β, IL-8, and TNF-α, (de Vries, 1995), as well as to enhance antibody production, some have suggested that it may act as an intermediary to both negatively and positively regulate glucocorticoid production (Smith et al., 1999).

Conclusions

It appears clear, from the present experiments, that the administration of a synthetic ds RNA, such as poly I:C, can induce behavioral, neuroendocrine, neurochemical and circulating cytokine alterations, in a fashion similar to pro-inflammatory cytokines and endotoxins. Moreover, when immune system activation occurred on a backdrop of psychosocial stress, these alterations became markedly enhanced, resulting, in synergistic or additive responses, thus, placing exceeding greater demands upon the physiological systems.

The PVN of the hypothalamus receives and summates excitatory and inhibitory sensory information regarding threats to homeostasis (Herman et al., 2003). It has been suggested that direct physiological challenges, or systemic stressors, are relayed directly to the PVN, possibly by brainstem catecholaminergic afferents, while stressors which necessitate higher order processing (processive stressors) are first processed by limbic system circuitry

(i.e., hippocampus, central nucleus of the amygdala and medial prefrontal cortex) prior to being relayed to the PVN (Herman & Cullinan, 1997). Furthermore, while these circuits sparsely innervate the PVN, they show extensive overlap in regions such as the bed nucleus of the stria terminalis, hypothalamus and brainstem, and as such, may be integrated at subcortical sites preceding the processing within the PVN (Herman et al., 2005). Thus, forebrain influences on the PVN appear to be polysynaptic and relay through structures that are activated in response to processive stressors, while also overlapping with structures that are activated in response to systemic stressors (Herman et al., 2003). Although speculative, it is possible that the synergistic and/or additive responses involve these overlapping neural circuitry and thus project this combined information to the PVN, culminating in a more pronounced endocrine and neurotransmitter response.

Poly I:C treatment has been shown to produce widespread increases of IFN-α mRNA expression in the brain (Katafuchi et al., 2003; 2005; Alsharifi et al., 2005) via TLR-3 activation (Alexopoulou et al., 2001). Further, microglia are believed to be an important intermediary between the CNS and immune systems, and are particularly sensitive to TLR-3 stimulation releasing type I IFN's following poly I:C treatment (Town et al., 2006). Inasmuch as stressor exposure also increased cytokine expression in the brain (Grippo et al., 2005), it is possible that the synergies are mediated, in part, by the augmented IFN-α increase. For example, immune activation following the period of social

stressors may have altered the otherwise strict regulatory actions of autoreceptors, culminating in pronounced neurochemical secretions, or, alternatively, endogenous cytokines may be in excess, acting to vigorously stimulate the HPA activity.

In summary, although a thorough understanding of the processes governing the synergies between cytokines and stressors have yet to be offered, the findings of the present investigation are consistent with the view that the brain and immune systems interact (Maier, 2003). Moreover, it seems that immune activation may have particularly marked consequences on physiological and behavioral functioning when preceding a psychosocial stressor, but adaptation may occur if animals have a history of chronic social stressful experiences. What processes may underlie this adaptation are uncertain, but the fact that such an adaptation does occur might have clinical implications given that the response to cytokine immunotherapy may be adversely affected by distress.

Acknowledgements

This research was supported by a grant from the Canadian Institutes of Health Research. Special thanks to Marzena Sieczkos, Natalie Lukenbill, Dr. Jerzy Kulczycki (for their expert technical assistance), and finally, to Dr. Hymie Anisman for patiently supervising the thesis.

References

Alexopoulou, L., Holt, A.C., Medzhitov, R. & Flavell, R.A. (2001). Recognition of double-stranded rna and activation of nf-κb by toll-like receptor 3. Nature, 413, 732-738.

Alheim, K. & Bartfai, T. (1998). The interleukin-1 system: Receptors, ligands, and ice in the brain and their involvement in the fever response. Annals of the New York Academy of Sciences, 840, 51-58.

Alsharifi, M., Lobigs, M., Regner, M., Lee, E., Koskinen, A., & Mullerbacher, A. (2005).Type I interferons trigger systemic, partial lymphocyte activation in response to viral infection. Journal of Immunology, 175, 4635-4640.

Anisman, H. & Merali, Z. (2002). Cytokines, stress, and depressive Illness. Brain, Behavior, and Immunity,16, 513-524.

Anisman, H. & Merali, Z. (1999). Anhedonic and anxiogenic effects of cytokine exposure. Advances in Experimental Medicine and Biology, 461, 199-233.

Anisman, H., Hayley, S. & Merali, Z. (2001). Behavioral and central neurochemical consequences of cytokine challenge. In J. Bienenstock, R.

Gorzynski and I. Berczi (Eds.) Neuroimmune Biology: New Foundation of Biology. Elsevier Science, 141-162.

Anisman, H., Merali, Z., Poulter, M.O., & Hayley, S. (2005). Cytokines as a precipitant of depressive illness: Animal and human studies. Current Pharmaceutical Design, 11, 963-972.

Aston-Jones, G. (2002). Norepinephrine. In: Davis K.L., Charney, D., Coyle J.T., Nermeroff, C. (Eds.) Neuropsychopharmacology. The Fifth Generation of Progress. Lippincott Williams and Wilkins, Philadelphia, p.p. 47-57.

Avitsur, R., Kavelaars, A., Heynen, C. & Sheridan, J.F. (2005). Social stress and the regulation of tumour necrosis factor-α secretion. Brain, Behavior, and Immunity, 19, 311-317.

Avitsur, R., Stark., J.L., & Sheridan, J.F. (2001). Social stress induces glucocorticoid resistance in subordinate animals. Hormones and Behavior, 39, 247-257.

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, Behavior, and Immunity, 18, 416-424.

Banks, W.A. (2005). Blood-brain barrier transport of cytokines: A mechanism for neuropathology. Current Pharmaceutical Design,11, 973-84.

Bartolomucci, A., Palanza, P., Sacerdote, P., Ceresini, G., Chirieleison, A., Panerai, A.E., & Parmelia, S. (2003). Individual housing induces altered immuno-endocrine responses to psychological stress in male mice. Psychoneuroendocrinology, 28, 540-558.

Blalock, J.E. (1994). Shared ligands and receptors as a molecular mechanism for communication between the immune and neuroendocrine systems. Annals of the New York Academy of Sciences, 741, 292-298.

Blanchard, R.J., McKittrick, C.R., & Blanchard, D.C. (2001). Animal models of social stress: Effects on behavior and brain neurochemical systems. Physiology and Behavior, 73, 261-271.

Brebner, K., Hayley, S., Merali, Z., & Anisman, H. (2000). Synergistic effects of interleukin-1β, interleukin-6 and tumor necrosis factor-α: Central monoamine, corticosterone and behavioral variations. Neuropsychopharmacology, 22, 566-580.

Capuron, L. & Dantzer, R. (2003). Cytokines and depression: The need for a new paradigm. Brain, Behavior, and Immunity, 1, 119-24.

Capuron, L., & Miller, A.H. (2004). Cytokines and psychopathology: Lessons from interferon-α. Biological Psychiatry, 56, 819-824.

Capuron, L., Ravaud, A., Miller, A.H., & Dantzeer, R. (2004). Baseline mood and psychosocial characteristics of patients developing depressive symptoms during il-2 and or ifn-α cancer therapy. Brain, Behavior, and Immunity, 18, 205-213.

Capuron, L., Raison, C.L., Musselman, D.L., Lawson, D.H., Reemsynder, A., & Nemeroff, C.B. (2002). Neurobehavioral effects of interferon-α in cancer patients: Phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacology, 26, 643-652.

Capuron, L., Raison, C.L., Musselman, D.L., Lawson, D.H., Nermeroff, C.B. & Miller, A.H. (2003). Association of exaggerated hpa axis response to the initial injection of interferon-α with development of depression during interferon-alpha therapy. American Journal of Psychiatry, 160, 1342-1345.

Carobrez, S.G., Gasparotto, O.C., Buwalda, B., & Bohus, B. (2002). Long-term consequences of social stress on corticosterone and il-1β levels in endotoxin-challenged rats. Physiology & Behaviour, 76, 99-105.

Childa, Y., Sudo. N. & Kubo., C. (2005). Social isolation stress exacerbates autoimmune disease in mrl mice. Journal of Neuroimmunology, 11, 18-38.

Chen, R., Zhou, H., Beltran, J., Malellari, L., & Chang, S.L. (2005). Differential expressions of cytokines in the brain and serum during endotoxin tolerance. Journal of Neuroimmunology, 163, 53-72.

Cho, L., Tsunoda, M., & Sharma, R.P. (1999). Effects of endotoxin and tumor necrosis factor-α on regional brain neurotransmitters in mice. Natural Toxins, 7, 187-195.

Dantzer, R. (2001). Cytokine-induced sickness behavior: Where do we stand? Brain, Behavior, and Immunity, 15, 7-24.

Day H.E., Curran E.J., Watson, S.J., & 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-1β. Journal of Comparative Neurology, 413, 113-128.

de Vries, J.E. (1995). Immunosuppressive and anti-inflammatory properties of IL-10.Annals of Medicine, 27, 537-541.

Diebold, S.S., Montoya, M., Unger, H., Alexopoulou, L., Roy, P., Haswell, L.E.,

Shamkhani, A.A., Flavell, R., Borrow, P. & Reis e Sousa, C. (2003). Viral infection switches non-plasmacytoid dendritic cells into high interferon producers. Nature, 424, 324-328.

Dunn, A.J. (2001). Effects of cytokines and infections on brain neurochemistry. In:Adler, R., Felten, D.L. & Cohen, N. (Eds.). Psychoneuroimmunology. New York: Academic Press, 649-666.

Dunn, A.J. (1995). Interactions between the nervous system and immune system. In F.E. Bloom & D.J. Kupfer (Eds.) Psychopharmacology: The Fourth Generation of Progress, 719-731. New York: Raven Press.

Dunn, A.J., Swiergiel, A.H., & de Beaurepaire R.(2005). Cytokines as mediators of depression: what can we learn from animal studies? Neuroscience and BioBehavioral Reviews, 29, 891-909.

Dunn, A.J., Swiergiel, A.H. & Palamarchouk, V. (2004). Brain circuits involved in corticotrophin-releasing factor-norepinephrine interactions during stress. Annals of the New York Academy of Sciences, 1018, 25-34.

Dunn, A.J., & Vickers, S.L. (1994). Neurochemical and neuroendocrine responses to newcastle disease virus administration in mice. Brain Research, 645, 103-112.

Ericsson, A., Kovacs, K.J., & Sawcheno, P.E. (1994). A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. Journal of Neurochemistry, 14, 89-91.

Engler, H., Engler, A., Bailey, M.T., & Sheridan, J.F. (2005). Tissue-specific alterations in glucocorticoid sensitivity of immune cells following repeated social defeat in mice. Journal of Neuroimmunology, 163, 110-119.

Filipenko, M.L., Beilina, A.G., Alekseyenko, OV., Dolgov, V.V., Kudryavtseva N.N. (2002). Repeated social defeats increases serotonin transporter and monoamine oxidase A mrna levels in raphe nuclei of male mice. Neuroscience Letters, 321, 25-28.

Finlay, J.M., Jedema, H.P., Rabinovic, A.D., Mana, M.J., Zigmond, M.J. & Sved, R.(1997). Impact of corticotrophin-releasing hormone on extracellular norepinephrine in prefrontal cortex after chronic cold stress. Journal of Neurochemistry, 69, 144-150.

Fleshner, M., Silbert, L., Deak, T., Goehler, L.E., Martin, D., Watkins, L.R., & Maier, S.F. (1997). Tnf-α-induced corticosterone elevation but not serum protein or corticosteroid binding globulin reduction is vagally mediated. Brain Research Bulletin, 44, 701-706.

Flugge, G., van Kampen, M. & Janneke Mijnster, M. (2004). Perturbations in brain monoamine systems during stress. Cell Tissue Research, 315, 1-14.

Fortier, M.E., Kent, S., Ashdown, H., Poole, S., Boksa, P. & Luheshi, G.N. (2004). The viral mimic polyinosinic: polycytidylic acid (poly i:c), induces fever in rats via an interleukin-1 dependant mechanism. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology, 287, 59-66.

Franklin, K.B.J. & Paxinos, G. (1997). A Stereotaxic Atlas of the Mouse Brain. New York: Academic Press.

Fujimoto, C., Nakagawa, Y., Ohara, K. & Takahashi, H. (2004). Polyriboinosinic polyribocytidylic acid (poly i:c)/tlr 3 signaling allows class I processing of exogenous protein and induction of hiv-specific cd8+ cytotoxic t lymphocytes. International Immunology, 16, 55-63.

Grippo, A.J., Francis, J., Beltz, T.J., Felder, R.B., & Johnson, A.K. (2005). Neuroendocrine and cytokine profile of chronic mild stress-induced anhedonia. Physiology and Behavior, 84, 697-706.

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. Journal of Neuroscience, 19, 5654-5665.

Hayley, S., Lacosta, S., Merali, Z., van Rooijen, N. & Anisman, H. (2001).

Central monoamine and plasma corticosterone changes induced by a bacterial endotoxin: Sensitization and cross-sensitization effects. European Journal of Neuroscience, 13, 1155-1165.

Hayley, S., Merali, Z. & Anisman, H. (2003). Stress and cytokine-elicited neuroendocrine and neurotransmitter sensitization: Implications for depressive illness. Stress, 6, 19-32.

Hayley, S., Staines, W., Merali, Z., & Anisman, H. (2001). Central monoamine and plasma corticosterone changes induced by a bacterial endotoxin: Sensitization and cross-sensitization effects. European Journal of Neuroscience, 13, 1155-1165.

Herman, J.P., & Cullinan, W.E. (1997). Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. Trends in Neuroscience, 20 78-84.

Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., Choi, D.C., & Cullinan, W.E. (2003). Central mechanisms of stress integration: Hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Frontiers in Neuroendocrinology, 24, 151-180.

Herman, J.P., Ostrander, M.M., Mueller, N.K., & Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: Hypothalamo-pituitary-adrenocortical axis. Progress in Neuropsychopharmacology and Biological Psychiatry, 29,

1201-1213.

Jacobs, B.L. & Langland, J.O. (1996). When two strands are better than one: The mediators and modulators of the cellular responses to double-stranded rna. Virology, 219, 339-349.

Jacobson, L. (2005). Hypothalamic pituitary adrenal cortical axis regulation. Endocrinology and Metabolism Clinics of North America, 34, 271-292.

Karp, J.D., Cohen, N., & Moynihan, J.A. (1994). Quantitative differences in interleukin-2 and interleukin-4 production by antigen stimulated splenocytes from individually and group-housed mice. Life Sciences, 55, 789-795.

Katafuchi, T., Kondo, T., Yasaka, T., Kubo, K., Take, S. & Yoshimura, M. (2003). Prolonged effects of polyriboinosinic: polyribocytidylic acid on spontaneous running wheel activity and brain interferon-α mrna in rats: A model for immunologically induced fatigue. Neuroscience, 120, 837-845.

Katafuchi, T., Kondo, T., Take, S., & Yoshimura, M. (2005). Enhanced expression of brain interferon-α and serotonin transporter in immunologically induced fatigue in rats. European Journal of Neuroscience, 22, 2817-2826.

Kelley, K.W., Blute, R.M., Dantzer, R., Zhou, J.H., Shen, W.H., Johnson, R.W., & Broussard, S.R. (2003). Cytokine-induced sickness behavior. Brain, Behavior, and Immunity, 17, S112-S118.

Kovacs, K.J. (1998). Functional neuroanatomy of the paravocellular vasopressinergic system: Transcriptional responses to stress and glucocorticoid feedback. Progress in Brain Research, 119, 31-43.

Kovacks, K.J. (1996). The paraventricular nucleus of the hypothalamus and the functional neuroanatomy of visceromotor responses to stress. Progress in Brain Research, 107,201-222.

Kronfol, Z. & Remick, D.G. (2000). Cytokines and the brain: Implications for clinical psychiatry, American Journal of Psychiatry, 157, 683-694.

Lacosta, S, Merali, Z., & Anisman, H. (1998). Influence of interleukin-1beta on exploratory behaviors, plasma acth, corticosterone, and central biogenic amines in mice. Psychopharmacology,137, 351-361.

Lacosta, S., Merali, Z., and & Anisman, H. (1999). Behavioral and neurochemical consequences of lipopolysaccharide in mice: Anxigenic-like effects. Brain Research, 818, 291-303.

Lanquillon, S., Krieg, J.C., Bening-Abu-Shach U., & Vedder, H. (2000). Cytokine production and treatment response in major depressive disorder. Neuropsychopharmacology, 22, 370-379.

Lee,Y.J., Choi, B., Lee, E.H., Choi, K.S. & Sohn, S. (2006). Immobilization stress induces cell death through production of reactive oxygen species in the mouse cerebral cortex. Neuroscience Letters, 392, 27-31.

Levine, J., Barak, Y., Chengappa, K.N., Rapoport, A., Rebey, M., Barak, V. (1999). Cerebrospinal cytokine levels in patients with acute depression. Neuropsychobiology, 40, 171-176.

Lien, E. & Golenbock, T. (2003). Adjuvants and their signaling pathways: Beyond tlr's. Nature Immunology, 4, 1162-1164.

Loftis, J.M., & Hauser, P. (2004). The phenomenology and treatment of interferon-induced depression. Journal of Affective Disorders, 82, 175-190.

Maier, S.F. (2003). Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition. Brain, Behavior, and Immunity, 17, 69-85.

Maes, M., & Meltzer, H.Y. (1995). The serotonin hypothesis of major depression. In Bloom F.E., & Kupfer, D.J. (Eds.). Psychopharmacology: The Fourth Generation of Progress. (p.p. 933-944). New York: Raven Press.

McEwen, B.S. (2000). Allostasis and allostatic load: Implications for Neuropsychopharmacology. Neuropsychopharmacology, 22, 108-124.

McEwen, B.S. (2003). Mood disorders and allostatic load. Biological Psychiatry, 54, 200-207.

McGeer, P.L., & McGeer, G. (1995). The inflammatory response system of the brain: Implications for therapy of alzheimers and other neurodegenerative diseases. Brain Research Reviews, 21, 195-218.

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. Biological Psychiatry, 37, 383-393.

Merali, Z., Brennan, K., Brau, P., & Anisman, H. (2003). Dissociating anorexia and anhedonia elicited by interleukin-1β: Antidepressant and gender effects on responding for free chow and earned sucrose intake. Psychopharmacology, 165, 413-418.

Merali, Z., Lacosta, S. & Anisman, H. (1997). Effects of interleukin-1β and mild stress on alterations of norepinephrine, dopamine and serotonin

neurotransmission: A regional microdialysis study. Brain Research, 761, 225-235.

Merali, Z., Du, L., Hrdina, P., Palkovits, M., Faludi, G., Poulter, M. & Anisman, H. (2004). Dysregulation in the suicide brain: Mrna expression of corticotrophin releasing hormone receptors and gaba(a) receptor subunits in frontal cortical brain region. Journal of Neuroscience, 24, 1478-1485.

Miller, A.H., Spencer, R.L., Pearce, B.D., Pisell, T.L., Tanapat, P., Leung, J.J., Dhabhar, F.S., McEwen, B.S., & Biron, C.A. (1997). Effects of viral infection corticosterone secretion and glucocorticoid receptor binding in immune tissues. Psychoneuroendocrinology, 22, 455-474.

Milton, N.G., Hillhouse, E.W. & Milton, A.S. (1992). Activation of the hypothalamo- pituitary-adrenocortical axis in the conscious rabbit by the pyrogen polyinosinic:polycytidylic acid is dependant on cortiocotrophin-releasing factor-41. Journal of Endocrinology, 135, 69-75.

Morilak, D.A., Barrera, G., Echevarria, D.J., Garcia, A.S., Hernandez, A., Ma., S., & Petre, C.O. (2005). Role of brain norepinephrine in the behavioral response to stress. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 29, 1214-1224.

Muller, M.B. & Wurst, W. (2004). Getting closer to affective disorders: The role of crh receptor systems. Trends in Molecular Medicine, 10, 409-415.

Musselman, D.L., Lawson, D.H., Gumnick, J.F., Manatunga, A., Penna, S., Goodkin, R., Greiner, K., Nemeroff, C.B., & Miller, A.H. (2001).Paroxetine for the prevention of the depression and neurotoxicity induced by high dose interferon alpha. New England Journal of Medicine, 344, 961-966.

Nadeau, S. & Rivest, S. (1999). Effects of circulating tumor necrosis factor on the neuronal activity and expression of the genes encoding tumor necrosis factor receptors (p55 and p75) in the rat brain: A view from the blood-brain barrier. Neuroscience, 93, 1449-1464.

O'Brien, S.M., Scott, L.V., Dinan, T.G. (2004). Cytokines: Abnormalities in major depression and implications for pharmacological treatment. Human Psychopharmacology: Clinical and Experimental, 19, 397-403.

Ostrander, M.M., Ulrich-Lai, Y.M., Choi, D.C., Richtand, N.M., Herman, J.P. (2006). Hypoactivity of the hypothalamo-pituitary-adrenocortical axis during recovery from chronic variable stress. Endocrinology, 147, 2008-2017.

Paykel E.S. (2001). Stress and affective disorders in humans. Seminars in Clinical Neuropsychiatry, 6, 4-11.

Pollack, Y., & Yirmiya, R. (2002). Cytokine-induced changes in mood and behaviour: Implications for depression due to a general medical condition, immunotherapy and anti-depressant treatment. International Journal of Neuropsychopharmacology, 5, 389-399.

Pucak, M.L. & Kaplin, A.I. (2005). Unkind cytokines: Current evidence for the potential role of cytokines in immune-mediated depression. International Review of Psychiatry, 17, 477-83.

Quan, N., Avitsur, R., Stark, J.L., He., L., Shah, M., Caligiuri, M., Padgett, D.A., Marucha, P.T., & Sheridan, J.F. (2001). Social stress increases the susceptibility to endotoxic shock. Journal of Neuroimmunology, 115, 36-45.

Raison, C.L., Capuron L. & Miller, A.H. (2006).Cytokines sing the blues: Inflammation and the pathogenesis of depression. Trends in Immunology, 27, 24-31.

Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., & Morag, A. (2001). Cytokine-associated emotional and cognitive disturbances in humans. Archives in General Psychiatry, 58, 445-452.

Rivest, S., & Rivier, C. (1994). Stress and interleukin-1β-induced activation of c-fos, ngfi-b, and crf gene expression in the hypothalamic pvn-comparison

between sprague-dawley, fisher-344 and lewis rats. Journal of Neuroendocrinology, 6, 101-117.

Rouas, R., Lewalle, P., Ouriaghli, F.E., Nowak, B., Buvillier, H. & Martiat, P. (2004). Poly i:c used for human dendritic cell maturation preserves their ability to secondarily secrete bioactive il-12. International Immunology, 16, 767-773.

Ruzek, M.C., Miller, A.H., Opal, S.M., Pearce, B.D., Biron, C.A. (1997). Characterization of early cytokine responses and an interleukin (il)-6-dependent pathway of endogenous glucocorticoid induction during murine cytomegalovirus infection. Journal of Experimental Medicine, 185, 1185-1192.

Sapolsky, R.M., Romero, L.M. & Munck, A.U. (2000). How do glucocorticoids influence stress responses. Intergrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews, 21, 55-89.

Sawchenko, P.E., Brown, E.R., Chan, R.K., Ericsson, A., Li, H.Y., Roland, B.L., & Kovacs, K.J. (1996). The paraventricular nucleus of the hypothalamus and the functional neuroanatomy of visceromotor responses to stress. Progress in Brain Research, 107, 201-222.

Schiepers, O.J., Wichers, M.C., & Maes, M. (2005). Cytokines and major

depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29, 201-217.

Silverman, M.N., Pearce, B.D., Biron, C.A. & Miller, A.H. (2005). Immune modulation of the hypothalamic-pituitary-adrenal (hpa) axis during viral infection.Viral Immunology, 18, 41-78.

Smagin, G.N., Heinrichs, S.C. & Dunn, A.J. (2001). The role of crh in Behavioral responses to stress. Peptides, 22, 713-72.

Smith, E.M., Cadet, P., Stefano, G.B., Opp, M.R., & Hughes, Jr., T.K. (1999). IL-10 as mediator in the hpa axis and brain. Journal of Neuroimmunology, 100, 140-148.

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, 823-836.

Stanford, S.C. (1995). Central noradrenergic neurons. Pharmacology and Theraputics, 68,297-342.

Stout, S.C., Owen, M.J.& Nemeroff, C.B. (2002). Regulation of corticotropin-releasing factor neuronal systems and hypothalamic-pituitary-adrenal axis activity by stress and chronic antidepressant treatment. The Journal of

Pharmacology and Experimental Therapeutics, 300, 1085-1092.

Swiergiel, A.H., & Dunn, A.J. (2006). Feeding, exploratory, anxiety and depression related behaviors are not altered in interleukin-6-deficient male mice. Behavioral Brain Research, 171, 94-108.

Swiergiel, A.H., Smagin, G.N., Johnson, L.J., & Dunn, A.J. (1997). The role of cytokines in the behavioral responses to endotoxin and influenza virus infection in mice: Effects of acute and chronic administration of the interleukin-1-receptor antagonist (IL-1ra). Brain Research, 776, 96-104.

Tannenbaum, B., Tannenbaum, G. & Anisman, H. (2002). Neurochemical and behavioral alterations elicited by a chronic intermittent stressor regimen: Implications for allostatic load. Brain Research, 953, 82-92.

Tilders, F.J.H. & Schmidt, E.D. (1998). Interleukin-1 induced plasticity of hypothalamic crh neurons and long-term stress hyperresponsiveness. Annals of the New York Academy of Science, 840, 65-73.

Town, T., Jeng, D., Alexopoulou, L., Tan, J., & Flavell R.A. (2006). Microglia recognize double-stranded rna via tlr 3. Journal of Immunology, 176, 3804-3812.

Turnbull, A.V., & River, C.L. (1999). Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: Actions and mechanisms of action. Physiological Reviews, 79, 1-71.

Turnbull, A.V., Pitossi, F.J., Lebrun, J.J., Lee, S., Meltzer, J.C., Nance, D.M., del Rey, A., Besedovsky, H. & Rivier, C. (1997). Inhibition of tumor necrosis factor-α within the cns markedly reduces the plasma adrenocorticotropin response to peripheral local inflammation in rats. Journal of Neuroscience, 17, 3262-3273.

Ueta, M., Hamuro, J., Kiyono, H., Kinoshita, S. (2005). Triggering of tlr 3 by poly i:c in human corneal epithelial cells to induce inflammatory cytokines. Biochemical and Biophysical Research Communications, 331, 285-294.

Urani, A., Chourbaji, S., & Gass, P. (2005). Mutant mouse models of depression: Candidate genes and current mouse lines. Neuroscience and Biobehavioral Reviews, 29, 805-828.

Von Frijtag, J.C., Reumers, L.G., Van der Harst, J.E., Leus, I.E., Van der Bos, R., Spruijt, B.M. (2000). Defeat followed by individual housing results in long-term impaired reward and cognitive-related behaviors in rats. Behavioral Brain Research, 117, 137-146.

Von Frijtag, J.C., Van der Bos, R., & Spruijt, B.M. (2002). Imipramine restores the long-term impairment of appetitive behavior in socially stressed rats. Psychopharmacology, 162, 232-238.

Wichers, M.C., & Maes, M. (2002). The psychoneuroimmuno-pathophysiology of cytokine induced depression in humans. International Journal of Neuropsychopharmacology, 5, 375-388.

Wichers, M.C., Koek, G.H., Robaeys, G., Praamstra, A.J., & Maes, M. (2005). Early increase in vegetative symptoms predicts interferon-α induced cognitive-depressive changes. Psychological Medicine, 35, 433-41.

Wilson, J.F. (Eds.). (2003). Stress and the nervous system. Biological Foundations of Behaviour. Thompson Wadsworth: Toronto, p.p. 408-439.

Wong, M.L., Kling, M.A., Munson, P.J., Listwak., S., Licinio, J., Prolo, P., Karp, B.,

McCutcheon, I.E., Geracioti, T.D., DeBellis, M.D., Rice, K.C., Goldstein, D.S., Veldhuis, J.D., Chrousos, G.P., Oldfield, E.H., McCann, S.M., & Gold, P.W. (2000). Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: Relation to hypercortisolism and corticotrophin-releasing hormone. Proceedings of the National Academy of Sciences of the United States of America, 97, 325-330.

WuLu, Z., Hayley, S., Ravindran, A.V., Merali, Z. & Anisman, H. (1999).

Influence of psychosocial, psychogenic and neurogenic stressors on several aspects of immune functioning in mice. Stress, 3, 55-70.

Yirmiya, R. (1996). Endotoxin produces a depressive-like episode in rats. Brain Research, 711, 163-174.

Yirmiya, R., Pollack, Y., Morag, M., Reichenberg, A., Barak, O., Avitsur, R., Shavit, Y., Ovadia, H., Weidenfeld, J., Morag., A., Newman, M.E., & Pollmacker, T. (2000). Illness, cytokines and depression. Annals of New York Academy of Sciences, 917, 478-487.