The additive or synergistic actions of social stressors and endotoxin challenge: Behavioural and neurochemical effects

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© Julie Eva Gibb

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Abstract

Activation of the inflammatory immune system has been implicated as a possible instigator of depressive-like states. In this regard, it has been suggested that immune activation and the release of cytokines promote several brain neurochemical changes in stressor-sensitive brain regions, which then favour the evolution of depression. As these neurochemical effects are reminiscent of those elicited by traditional stressors, it was suggested that stressors and immune activation may have additive or synergistic effects. In the present investigation we demonstrate that the effects of lipopolysaccharide-induced immune activation on sickness behaviour, hypothalamic-pituitary-adrenal functioning, and brain monoamine activity are markedly increased when applied on a psychosocial stressor backdrop (e.g., regrouping mice for 1 hour following a prolonged period of isolation). These effects are not nearly as pronounced when the stressor is of a nonsocial nature (e.g., restraint, noise, or tail pinch), or when physical interaction is not permitted during the regrouping.

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Figure 28. Mean (±SEM) MHPG within the hippocampus among mice that were group housed, isolated for 14 days, isolated and exposed to novelty, isolated and regrouped for 1 hr without physical contact, or isolated and regrouped for 1 hr permitting physical contact. Mice were then treated with saline or LPS (5 or 10ug).

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1. Introduction

Activation of the inflammatory immune system has been implicated as a possible instigator of depressive-like states (Anisman, Hayley, Turrin, & Merali, 2002; Anisman, & Merali, 2003; Anisman, Merali, Poulter, & Hayley, 2005; Dunn, Swiergiel, & de Beaurepaire, 2005b; Raison, Capuron, & Miller, 2006; Schiepers, Wichers, & Maes, 2005; Simmons, & Broderick, 2005). Indeed, immune activation, and the subsequent release of proinflammatory cytokines, elicits behavioural, neuroendocrine and neurochemical responses comparable to those elicited by traditional stressors. In particular, like traditional stressors, these cytokines influence hypothalamic-pituitary-adrenal (HPA) functioning, effectively promoting the release of corticotropin releasing hormone (CRH) and hence glucocorticoids (Dunn, 2005a). As well, proinflammatory cytokines promote monoamine release in several stressor sensitive brain regions, including the paraventricular nucleus of the hypothalamus (PVN), locus coeruleus and prefrontal cortex (PFC) (Schiepers et al., 2005).

Elevated levels of proinflammatory cytokines are associated with a variety of stress-related behavioural disturbances (Raison et al., 2006). For instance, administration of the bacterial endotoxin lipopolysaccharide (LPS), or exogenously administered cytokines (immune signaling molecules), such as interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α), elicit a wide range of behavioural effects referred to as sickness behaviour (Anisman, & Merali, 1999; Dantzer, 2001; Kent, Bluthe, Kelley, & Dantzer, 1992). These behaviours, which include anhedonia, fever, pilo erection, ptosis, anorexia, reduced social and exploratory behaviours, and anhedonia, are reminiscent of symptoms that accompany depressive disorders. As both immune activation and stressors

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produce analogous behavioural and neurochemical effects, it was suggested that they might have additive or synergistic effects, which then favour the evolution of depression (Anisman et al., 2005). Thus, it was of particular interest in the present investigation to assess the potential additive or synergistic actions of immune activation and social stressors. In particular, we investigated the combined actions of a social stressor (induced by regrouping animals for 1 hour after a prolonged period of isolation), and immune challenge (induced by a high or low dose of LPS), on behavioural, neuroendocrine, neurotransmitter and cytokine functioning.

1.1. Depression and the Stress Response

Affective disorders comprise a spectrum of illnesses, including dysthymia, typical and atypical depression, unipolar and bipolar disorders, each with their own, but not mutually exclusive, set of symptoms and pathophysiology, and possibly diverse underlying processes (Anisman, & Matheson, 2005). Thus, in some respects it is difficult to provide valid animal models of "depression". This notwithstanding, the development of depression has been linked to neuroendocrine and neurotransmitter disturbances, many of which are elicited by stressors. The view has, indeed, been offered that depression can result from the dysregulation of HPA functioning provoked by stressors, leading to disruption of monoaminergic neurotransmitter system, particularly in prefrontal cortical regions, and hence the evolution of depression (Anisman, et al., 2005). This view was recently elaborated upon with the proposal that cytokines, like stressors, can lead to depression through multiple routes, including those that involve interactions between CRH, gamma-aminobutyric acid (GABA) and serotonin (5-HT), those that involve

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reduced brain derived neurotrophic factor (BDNF), and those that might be related to still other processes that favor neuronal degeneration (Hayley, Poulter, Merali, & Anisman, 2005; Pizarro, Lumley, Medina, Robison, Chang, Alagappan, et al., 2004).

The term 'stressor' can be defined as a negatively appraised situation or event that elicits a biological response (stress response) in an effort to adapt or accommodate environmental or psychological challenges (McEwan, 2000). Herman and Cullinan (1997) characterize stressors as being either 'processive' (those involving higher-order sensory processing) or 'systemic' (those involving physical insults resulting in circulatory, respiratory, hemodynamic or immune alterations). The former stressors can be further characterized as being either psychogenic or neurogenic; psychogenic stressors are those that are purely psychological in nature (e.g. restraint or predator exposure), whereas, neurogenic stressors are those that involve a physical stimulus (e.g. foot shock). It has been suggested that processive and systemic stressors may promote neuroendocrine activity through different neuronal circuits (Herman, & Cullinan, 1997). For instance, processive stressors are processed by higher brain structures, mainly the limbic system, which send afferent projections to the PVN in order to activate the HPA axis. Systemic stressors, however, bypass the limbic system and directly innervate the PVN. Regardless of the neuronal circuits involved, both processive and systemic stressors elicit the same neuroendocrine response. Specifically, they activate select neurons in the PVN, which secrete both CRH and arginine-vasopressin (AVP). The co-expression of CRH and AVP then trigger the release of adrenocorticotropic hormone (ACTH) from the pituitary gland, which stimulates the adrenal cortex to release glucocorticoids, such as corticosterone (cortisol, in humans) (Sapolsky, Romero, & Munck, 2000).

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In addition to neuroendocrine effects, processive and systemic stressors also provoke a cascade of neurotransmitter alterations, including monoamine (norepinephrine, dopamine and serotonin) release at hypothalamic and extrahypothalamic sites, such as the PFC, nucleus accumbens, and several amygdala nuclei (Anisman, Zalcman, & Zacharko, 1993). The stress response is ordinarily thought to have adaptive value, preparing the organism to deal with environmental insults. However, prolonged stress can become maladaptive as individual resources may become overly taxed. This situation, termed allostatic overload, can lead to the development of physiological and psychological disorders (McEwen, 2000). In this regard, it is thought that stressor provoked reductions of monoamine levels in several brain regions, stemming from excessive and prolonged utilization, may result in behavioural pathologies such as depression (Anisman, & Merali, 1999).

1.2. Social Stressor Induced Behavioural, Neurochemical and Cytokine Alterations

Although the effects of a wide variety of psychogenic and neurogenic stressors have been investigated, increasing attention has been devoted to assessing the impact of "ethologically relevant" stressors, notably social stressors. Ordinarily, rodents develop social hierarchies, and efforts have been focused on assessing the impact of *Social Disruption (SDR) Stress* or *Social Reorganization (SRO).* Typically, experimental paradigms have been based on the natural behaviour of male rodents acquiring and defending a territory (for a review see Bartolomucci, 2005). Rodents that are group-housed develop a social hierarchy, becoming either dominant or subordinate in relation to other animals (Sheridan, Stark, Avitsur, & Padgett, 2000). In SDR paradigms the rodents

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status is characterized (based on the animal's original housing condition) as being either resident dominant, intruder dominant, resident subordinate and intruder subordinate (Bartolomucci, 2005; Bartolomucci, Palanza, Gaspani, Limiroli, Panerai, Ceresini et al., 2001).

Other social stress models have focused primarily on the aggressive nature of rodents. Often termed *Social Defeat Stress*, this paradigm focuses on the fighting behaviour of rodents, and investigates the behavioural and neurochemical effects associated with winning or defeat (Martinez, Calvo-Torrent, & Pico-Alfonso, 1998; Merlot, Moze, Dantzer, & Neveu, 2003; Pizarro, et al., 2004; Stefanski, 2000; Stefanski, & Engler, 1999). Finally, as rodents are highly social, some studies have investigated the impact of social isolation stress on subsequent behavioural and physiological states (Bartolomucci, Palanza, Sacerdote, Ceresini, Chirieleison, Panerai et al., 2003; Chida, Sudo, & Kubo, 2005; D'Arbe, Einstein, & Lavidis, 2002). This recent surge of ethologically relevant paradigms has permitted the analysis of social stressors on behavioural, neurochemical and immune systems in relation to depressive-like states.

Social stressors consistently elicit behavioural changes in rats and mice including decreased locomotor, grooming, and exploratory activities, reduced food and water intake, as well as increased anxiety-like behaviour (Kudryavtseva, Bakshtanovskaya, & Koryakina, 1991; Martinez et al., 1998). These behaviours, which to some extent mimic the depressive symptoms seen in humans (Bartolomucci, 2005), are often accompanied by physiological changes such as increased blood pressure, heart rate and temperature (Tornatzky, & Miczek, 1993) and reduced body weight (Kudryavtseva et al., 1991). In addition, social stressors prompt neuroendocrinological changes, and particularly

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increased secretion of glucocorticoids (Blanchard, Sakai, McEwan, Weiss, & Blanchard, 1993). The chronic stimulation of HPA functioning, through repeated exposure to social stressors, can result in relative hypertrophy of the adrenal gland and thymic involution (Bartolomucci, 2005; Engler, Engler, Bailey, & Sheridan, 2005). Beyond the HPA changes, social stressors increase release of 5-HT in the preoptic nucleus, amygdala, and hippocampus, of the release of norepinephrine (NE) in the locus coeruleus, hypothalamus and PFC, and that of dopamine (DA) in the prefrontal cortex and nucleus accumbens (Blanchard, McKittrick, & Blanchard, 2001; Blanchard et al., 1993). It will be appreciated that some of these same neurochemical disturbances have been implicated in human depressive disorder.

In addition to the neuroendocrine and neurotransmitter alterations, social stressors have immunosuppressant actions, reflected by reduced lymphocyte proliferation, natural killer cell cytotoxicity and central cytokine production in animals (Bartolomucci, 2005; Bartolomucci et al., 2001; Stefanski, & Engler, 1999). Moreover, social stressors provoked glucocorticoid resistance in immune cells (Avitsur, Kavelaars, Heijnen, & Sheridan, 2005; Avitsur, Stark, & Sheridan, 2001; Engler et al., 2005; Sheridan et al., 2000; Stefanski, 2000). Glucocorticoids are immunosuppressant, reducing levels of circulating lymphocytes, inhibiting the proliferation and activation of lymphocytes, suppressing the activity of natural killer cells, reducing the circulating levels of monocytes and inhibiting the secretion of IL-1β, IL-6 and TNF-α (O'Connor, O'Halloran, & Shanahan, 2000). It has been shown that chronic social stressors, but not physical stressors, increase systemic glucocorticoid levels that can lead to changes in the responsiveness of the immune cells to the anti-inflammatory properties of this hormone

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(Stefanski, 2000). This, in turn, can provoke enhanced production of proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, and can subsequently lead to immunopathology (Engler et al., 2005).

1.3. Cytokine Induced Behavioural and Neurochemical Changes

Cytokines are primary signaling molecules of the immune system produced by immunocompetent cells, such as lymphocytes and macrophages, in order to regulate the immune response. There are two distinct categories of cytokines. Proinflammatory cytokines, including IL-1, IL-6, IL-8, TNF-α, interferon (INF)- α and INF-γ, are responsible for the activation of the inflammatory immune response, synthesis of acute phase proteins, and activation of neuroendocrine systems (Turrin, & Plata-Salaman, 2000). In contrast, anti-inflammatory cytokines, such as IL-4, IL-10, and IL-1 receptor antagonist, are responsible for dampening the immune response by inhibiting the actions of their proinflammatory counterparts (Turrin, & Plata-Salaman, 2000). It appears that maintaining health and well-being depends on the delicate balance of these two types of cytokines.

Cytokines are relatively large molecules and are not able to penetrate the blood-brain barrier (BBB) readily. However, there are physiological conditions wherein the BBB is compromised, allowing peripherally released cytokines to gain access to the central nervous system (CNS). For instance, the integrity of the BBB may be impaired by trauma or certain pathological illnesses (e.g. multiple sclerosis; Merrill, & Murphy, 1997), and cytokines themselves can impair the BBB (Chandler, Miller, Clements, Lury, Corkill, Anthony et al., 1997). In addition, there are certain brain regions where the BBB

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is naturally less restrictive (e.g. the median eminence), and cytokines may be passively transported from the blood into the brain parenchyma (Watkins, Maier, & Goehler, 1995). It has also been suggested that peripheral cytokines may gain access to the CNS by active transport, by which specific carrier proteins transfer cytokines across the BBB (Banks, Farr, & Morley, 2003). Finally, it has recently been demonstrated that cytokine signals may be influencing the brain through activation of vagal afferent fibers, originating in the abdominal cavity, which transmit these signals to the nucleus of the solitary tract (NST) in the brain stem (Raison et al., 2006). The NST then serves as a hub, relaying the signals to other brain regions such as the PVN. In addition to infiltration from the periphery, cytokines (including IFN-α, IFN-γ, IL-1, IL-2, IL-6 and TNF-α) may be produced in the CNS itself, mainly by astrocytes and microglia (McGeer, & McGeer, 1995). Cytokine production has been found to occur in several brain sites, including the hypothalamus, hippocampus, cerebellum, forebrain regions, basal ganglia, and brainstem nuclei (Kronfol, & Remick, 2000).

Like stressors, cytokine challenges can elicit a wide spectrum of behavioural effects in laboratory animals. For instance, systemically administered IL-1β typically provokes sickness behaviour, as described earlier (Kent et al., 1992), and IL-1 antagonists can attenuate these effects (Bluthe, Dantzer, & Kelley, 1997). In addition, animals treated with IL-1β and TNF-α demonstrated anhedonia, as measured by the reduced consumption of a palatable snack (chocolate milk) that returned to basal levels 24 hours later (Brebner, Hayley, Zacharko, Merali, & Anisman, 2000). The fact that the behavioural symptoms elicited by cytokine administration cease relatively soon after the

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treatment supports a causal role for cytokines in the mediation of this condition (Schiepers et al., 2005).

Like stressors, systemic and central administration of IL-1β increase the expression and secretion of CRH and AVP from the PVN of the hypothalamus and the median eminence, leading to increased ACTH release from the pituitary and corticosterone from the adrenal glands (Watanobe, & Takebe, 1993). Furthermore, as will be discussed later, systemic IL-1β administration may result in a long-lasting increase in the co-production of AVP in hypothalamic CRH neurons, leading to an exaggerated HPA response to subsequent immune or stressor challenges (Tilders, & Schmidt, 1998). Increased HPA activation was also reported with the systemic administration of TNF-α, which increased CRH in the median eminence, resulting in increased circulating levels of ACTH and corticosterone (Hayley, & Anisman, 2005; Hayley, Brebner, Lacosta, Merali, & Anisman, 1999; Hayley, Merali, & Anisman, 2002). Furthermore, co-administration of IL-β and TNF-α induced circulating corticosterone elevations that were greater than those elicited by the additive effects of the individual treatments (Brebner et al., 2000).

The administration of proinflammatory cytokines also induces a wide variety of neurochemical alterations. For instance, systemic IL- 1β administration increased the utilization of NE in the median eminence, PVN, locus coeruleus and PFC, of DA in the median eminence and PFC and of the 5-HT precursor tryptophan in the brainstem, hypothalamus, hippocampus and PFC (Lacosta, Merali, & Anisman, 1998; Zalcman, Green-Johnson, Murray, Nance, Dyck, Anisman et al., 1994). Although similar in some respects, the neurochemical effects of TNF-α are distinguishable from the effects of IL-

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1β. TNF- α significantly reduced NE concentration in the hippocampus and locus coeruleus, and increased NE within the amygdala. Furthermore, 5-HT levels were reduced in the PVN, but were increased in the PFC and amygdala (Hayley et al., 1999). Finally, in contrast to the lack of effect of IL-1β on DA levels in the nucleus accumbens, TNF-α increased the utilization of DA within the nucleus accumbens (Hayley et al., 1999). Together, these studies illustrate that the systemic administration of proinflammatory cytokines mimic the effects elicited by traditional stressors, and may thus influence the development of stress-related behavioural pathology.

Clinical studies also support the claim that immune activation may influence the development of depression. For instance, depressed patients have been shown to have elevated levels of circulating blood lymphocytes and monocytes, increased production of cytokines (such as IL-1, IL-2, IL-6 and INF-γ) in mitogen-stimulated cells, and elevated plasma concentrations of IL-1β, soluble IL-2 receptors (sIL-2R), IL-1Ra, IL-6, soluble IL-6 receptors (sIL-6R) and INF-γ (Maes, Smith, & Scharpe, 1995). Furthermore, depressed patients also exhibit increased plasma concentrations of complement proteins, C3 and C4, and IgM, as well as positive acute phase proteins haptoglobin, α1-antitrypsin, α1 and α2 macroglobulin, coupled with reduced levels of negative acute phase proteins (Maes, Sharpe, Bosmans, Vandewoude, Suy, Uyttenbroeck, et al., 1992). It has also been reported that during depression, the levels of proinflammatory cytokines continue to increase despite the hypersecretion of glucocorticoids, suggesting that the glucocorticoid receptors on the immune cells are hypofunctional in patients with depression and therefore that they fail to suppress the production of these cytokines (Leonard, 2001). As

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mentioned earlier, functional glucocorticoid resistance was also observed in animals in response to social stress.

It is difficult to know whether the immune alterations observed in depressed patients are secondary to the illness or one of the causes of its development. However, recent studies assessing the behavioural pathologies associated with interferon-α (IFN-α) immunotherapy have been consistent with the view that an association exists between cytokines and depression.

INF-α is a proinflammatory cytokine commonly used to treat chronic conditions such as liver diseases, like hepatitis C, and certain types of cancer. Chronic treatment over several months is associated with adverse effects, including depression, anorexia, fatigue, lethargy, clumsiness and social and cognitive impairment (Menkes, & MacDonald, 2000). Additionally, treatment with INF-α is associated with high rates of attempted suicide, which necessitate the discontinuation of the treatment (Yokoyama, Kimura, & Shigemura, 1996). It has been suggested that the depressive symptoms observed in patients taking interferon therapy could be related to the actions of the cytokines on monoamine metabolism (Capuron, Ravaud, Neveu, Miller, Maes, & Dantzer, 2002). For instance, animal and clinical studies show that INF-α suppresses tryptophan availability, which in turn decreases 5-HT synthesis (Capuron et al., 2002; Menkes, & MacDonald, 2000). Capuron et al. (2002) demonstrated that the occurrence of depressive symptoms (as assessed by the Montgomery-Asberg Depression Rating Scale) induced by cytokine treatment was negatively related with decreases in serum tryptophan levels, and that this relationship was dose-dependent. In addition to affecting tryptophan availability, IFN-α also increases 5-HT reuptake, which subsequently

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decreases synaptic 5-HT neurotransmission that could lead to depressive-like states (Turner & Blackwell, 2005). Interestingly, the depressive symptoms induced by the IFN therapy can often be attenuated with the use of antidepressants, and more specifically with selective serotonin reuptake inhibitors (SSRIs) (Musselman, Lawson, Gumnick, Manatunga, Penna, Goodkin et al., 2001).

Like other proinflammatory cytokines, IFN-α can have profound effects on the metabolism of monoamines, such as 5-HT, NE and DA in laboratory animals. In a study on the effects of centrally administered INF-α in rats, Kamata et al. (2000) observed a dose-dependent reduction of 5-HT levels and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the PFC, striatum, hypothalamus and midbrain. Additionally, decreased levels of NE were found in the frontal cortex, which could be responsible for the occurrence of lethargy and fatigue seen in human patients (Kamata, Higuchi, Yoshimoto, Yoshida, & Shimizu, 2000). In addition, it was reported that chronic systemic injections of INF-α in mice significantly decreased levels of DA and its metabolite, DOPAC (Shuto, Kataoka, Horikawa, Fujihara, & Oishi, 1997). It appears that INF-α decreases the neurotransmission of monoamines thought to play a role in the development of major depressive disorder. Moreover, these findings suggest that INF-α induces effects like those of stressors and may share common neuronal circuits.

1.4. Sensitization and Cross-Sensitization Effects of Stressors and Cytokines

In addition to their immediate effects, cytokines may provoke the sensitization of neuroendocrine functioning in response to stressors, cytokine and endotoxin administration (Hayley, & Anisman, 2005; Hayley et al., 1999; Hayley, Lacosta, Merali,

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van Rooijen, & Anisman, 2001; Hayley, Merali, & Anisman, 2003; Tilders, & Schmidt, 1998; Tilders, Schmidt, & de Goeij, 1993). In this regard, prolonged stressor and cytokine exposure can elicit substantial increases in the co-production, co-storage and co-secretion of AVP in the hypothalamic CRH neurons, provoke elevated expression of CRH and AVP mRNA in the PVN, and enhance co-expression of CRH and AVP in the external lamina of the median eminence, leading to the hypersensitivity of the HPA axis following subsequent challenges (Hayley et al., 1999; Hayley et al., 2003; Tilders, & Schmidt, 1998; Tilders et al, 1993). Sensitization resulting from stressor or cytokine exposure is also evident in regard to behavioural changes. For instance, although acute treatment with TNF-α does not produce marked sickness behaviour in animals, TNF-α did provoke a time-dependent sensitization wherein re-exposure to the cytokine provoked profound sickness behaviour along with elevated plasma corticosterone (Hayley et al., 1999; Hayley et al., 2003).

In addition to behavioural and neuroendocrine effects, stressor and cytokine exposure can also induce sensitization of monoamine variations. For instance, stressor-induced sensitization effects were observed in monoamine activity in several brain regions, including increased NE release in the hypothalamus, hippocampus and amygdala, as well as increased DA release in the PFC (Anisman et al., 1993). Furthermore, administration of TNF-α induced a sensitization of NE in the PVN and 5-HT in the central amygdala and PFC (Hayley et al. 1999). Taken together, these findings suggest that both stressors and cytokines can induce sensitization effects that may prime neuronal systems so that an enhanced response is elicited by later exposure to the same challenges.

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Stressors and cytokines may also produce cross-sensitization, whereby one type of challenge elicits an augmented response to the subsequent exposure to a different challenge. For instance, cross-sensitization effects were found between the administration of IL-1β and subsequent stressors, such as footshock, as demonstrated by pronounced increases of ACTH and corticosterone responses in pre-treated rats (Schmidt, Binnekade, Janszen, & Tilders, 1996; Schmidt, Janszen, Wouterlood, & Tilders, 1995). Immune challenge, induced by the administration of LPS, also produced cross-sensitization effects when followed by cytokine administration or a restraint stressor (Hayley et al., 2001). Exposure to TNF-α influenced 5-HT release within the central amygdala, as well as DA release within the PFC in LPS pretreated mice (Hayley et al., 2001). Stressor exposure, by means of inescapable tail shock, was also found to induce cross-sensitization effects to later exposure to endotoxin challenge (Johnson, O'Connor, Deak, Stark, Watkins, & Maier, 2002; Johnson, O'Connor, Watkins, & Maier, 2004). Johnson et al. (2002), reported that inescapable tail shock produced marked elevations of plasma corticosterone, ACTH and IL-6, as well as central IL-1β expression in the hypothalamus, hippocampus, cortex and pituitary upon subsequent administration of LPS. Moreover, treatment with IL-1ra attenuated the enhancement of central IL-1β expression, and plasma IL-6, but did not affect corticosterone response after exposure to LPS (Johnson et al., 2004), suggesting that some of the cross-sensitization effects observed between stressor and endotoxin exposure are mediated by central IL-1β. Inasmuch as stressors and cytokines elicit their own independent effects, their cross-sensitization effects are reminiscent of the additive and synergistic effects observed on neurochemical and immunological functioning.

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1.5 The Combined Effects of LPS and Social Stressors

The bacterial endotoxin LPS, the active fragment of endotoxin from gram-negative bacteria, is a potent activator of the HPA axis, and also elicits depressive-like sickness behaviour in otherwise healthy animals (Dantzer, 2001; Dantzer, Bluthe, Laye, Bret-Dibat, Pernet, & Kelley, 1998; Hayley et al., 2001; Yirmiya, 1996). The behavioural and physiological effects of LPS have been attributed to the peripheral release of proinflammatory cytokines, such as IL-1β, IL-6 and TNF-α, which then act on the brain (Dantzer, 2001). Furthermore, LPS has been reported to induce changes of monoamine transmission in several brain regions, as indicated by increased NE utilization in the PVN, median eminence, hippocampus and locus coeruleus, increased 5-HT utilization within the PVN, median eminence, and the hippocampus, and increased DA utilization in the PVN and median eminence (Lacosta, Merali, & Anisman, 1999). Likewise, the behavioural and physiological effects of a bacterial endotoxin are not only produced in laboratory animals, but can also be seen in humans. For instance, a low dose of endotoxin in humans induced a mild elevation in core body temperature, increased levels of depression and anxiety, provoked cognitive impairment and elevated circulating levels of TNF-α, IL-6, IL-1ra and cortisol (Reichenberg, Yirmiya, Shuld, Kraus, Haack, Morag et al., 2001).

Systemic administration of LPS in laboratory animals not only induces the production of proinflammatory cytokines in the periphery, but also stimulates cytokine expression in the brain, as reflected in the increased IL-1α and IL-1β mRNA in the pituitary, hippocampus, striatum and hypothalamus following peripheral administration of LPS (Ban, Haour, & Lenstra, 1992; Goujon, Parnet, Laye, Combe, Kelley, & Dantzer,

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1995). Interestingly, it has been shown that exposure to a mild stressor, such as 15 min restraint, can downregulate the LPS induced expression of cytokines in these brain regions (Goujon, et al., 1995), further emphasizing the possible interaction between stressors and immune activation.

Moreover, beyond the sickness inducing effects, LPS has also been reported to induce anhedonia in laboratory animals (Borowski, Kokkinidis, Merali, & Anisman, 1998; Yirmiya, 1996). Yirmiya (1996) reported that rats administered LPS showed decreased consumption of and preference for saccharin solution as well as decreased sexual behaviour. Interestingly, these behaviours were attenuated with the chronic treatment using the tricyclic anti-depressant imipramine. Although it's difficult to separate the anhedonic and anorexic effects of LPS, Borowski et al. (1998) reported that LPS reduced the rewarding value of hypothalamic brain stimulation, an effect not compromised by potential anorexic effects of the endotoxin (Borowski et al., 1998). Taken together, these findings suggest that LPS produces anhedonia reminiscent of that seen in human depressive disorder, but it can be excluded that this outcome may actually be a result of the sickness induced in the animal rather than a product of the cytokines on HPA functioning and monoamine transmission.

Possibly due to glucocorticoid resistance of immune cells or cross-sensitization effects, socially stressed animals are more susceptible to LPS induced endotoxic shock (Avitsur et al., 2005; Avitsur et al. 2001; Carobrez, Gasparotto, Buwalda, & Bohus, 2002; Engler et al., 2005; Quan, Avitsur, Stark, He, Shah, Caligiuri et al., 2001). Quan et al. (2001) demonstrated that a social stressor (SDR), but not physical restraint stress, increased mortality in mice when subsequently challenged by a septic dose of LPS. The

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socially stressed animals exhibited higher IL-1β and TNF-α expression in the spleen compared to controls and physically stressed animals (Quan et al., 2001). As secretion of glucocorticoids normally provides defense against endotoxic shock by inhibiting the production and action of cytokines, these findings suggest that social stressors can cause functional glucocorticoid resistance, resulting in the failure to inhibit the production of proinflammatory cytokines, rendering animals susceptible to endotoxic shock (Quan et al., 2001). These findings highlight the importance of further investigating the interaction between the inflammatory immune response and psychosocial stressors.

1.6. The Proposed Research

In light of the potential interaction between immune activation and social stressors, in the present investigation, we assessed the additive and/or synergistic actions of LPS and social stressors in four independent experiments. The first study assessed the behavioural, neuroendocrine, neurotransmitter and immunological effects of a social stressor (induced by a 1 hr episode of regrouping following a 14 day period of isolation), in combination with an immune challenge (induced by LPS). The second study evaluated different periods of social isolation (1, 3, 7 and 14 day isolation) to see which would elicit an additive or synergistic effect following the 1 hr reunion and LPS challenge. The third study compared the effects of social stressors (by regrouping) to non-social stressors such as restraint, startle and tail pinch, in order to determine whether other psychogenic and neurogenic stressors also act additively or synergistically with immune challenges, or if the effect is unique to social stressors. The final study determined whether the physical

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contact of being reunited, or the social aspect of the reunion, elicited the observed behavioural and neurochemical changes.

Individual housing has been reported to induce physiological changes in rodents, such as inducing HPA hyperactivity, accelerating autoimmune disease progression in specific strains of mice (Chida et al., 2005; Weiss, Pryce, Jongen-Relo, Nanz-Bahr, & Feldon, 2004), increasing the sympathetic release of neurotransmitters (D'Arbe et al., 2002), reducing LPS-stimulated cell proliferation and macrophage activity (Lu, Hayley, Ravindran, Merali, & Anisman, 1999), as well as increasing emotional reactivity to subsequent stressors (Bartolomucci et al., 2003; Weiss et al., 2004). Furthermore, in preliminary studies we observed that reuniting mice after being isolated for a period of two weeks elicits elevated corticosterone levels, and induces greater sickness behaviour in LPS challenged mice, than those with stable housing conditions. In light of these findings, it was expected that the effects of immune challenge, induced by systemic injection of LPS, on behavioural, neuroendocrine and neurotransmitter functioning would be markedly increased when applied on a psychosocial backdrop. More specifically, it was hypothesized that (a) animals subjected to a 1 hr regrouping session following a 2 week isolation period, as opposed to those in stable housing environments, would demonstrate greater sickness behaviour, elevated plasma corticosterone and cytokine levels and increased monoamine secretion in stressor-sensitive brain regions (b) a longer period of isolation (7 and 14 days) before the regrouping would be necessary to produce the additive or synergistic behavioural and neurochemical effects, compared to shorter periods (1 and 3 days) (c) the behavioural and neurochemical effects observed in the socially stressed animals would not be nearly as pronounced when the stressor was of a

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non-social nature, namely physical restraint, startle and tail pinch (d) the distress of the social reunion was the primary cause of the increase in sickness scores, plasma corticosterone and monoamine alterations, as opposed to the physical contact.

2. Materials and Methods

2.1. Subjects

Naïve, male CD-1 strain mice obtained from Charles River (St. Constance, Quebec) at 35-40 days of age, were permitted approximately two weeks to acclimatize to the laboratory prior to serving as experimental subjects. Mice were housed four per cage, and maintained on a 12-h light/dark cycle in a temperature (21°C) controlled room with food and water available *ad libitum*. All experiments complied with the current guidelines set by the Canadian Council on Animal Care and were approved by the Carleton University Animal Care Committee.

2.2. Experiment 1

Mice were randomly assigned to 3 separate conditions, namely isolation followed by regrouping of cage mates, isolated controls and group-housed controls. Originally housed in groups of 4, experimental mice were isolated for 14 days before being reunited with former cage mates and allowed to physically interact for 1 hr in a novel cage. Isolated and group-housed animals served as control groups, and remained undisturbed in their home cages. After 1 hr of regrouping, or equivalent time for the control groups, animals were intraperitoneally (i.p.) challenged with saline (Abbott Laboratories, sodium chloride 9 μg/mL) or 1, 5 or 10μg of the bacterial endotoxin LPS (Sigma L-3755 from *Escherichia coli* serotype O26:B6). Animals were monitored for sickness behaviour, as

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will be described in the general methods, every 15 mins for 90 mins post-injection, and were then decapitated. Trunk blood was collected to determine plasma levels of corticosterone and cytokines. The brain was removed, sliced and micropunched to extract tissue for analyses of monoamines and their respective metabolites from specific brain regions, namely the PVN, locus coeruleus, PFC, central amygdala and hippocampus.

2.3. Experiment 2

In order to assess the importance of length of social isolation prior to regrouping on subsequent behavioural and neurochemical responses, mice were isolated at specific times prior to the experiment (1, 3, 7 or 14 days), according to their randomly assigned experimental group, with the isolated control animals isolated for 14 days. To ensure that mice were being reunited with former cage mates, all 4 animals per cage were assigned to the same isolation period. On the day of the study, experimental mice from each period of isolation were reunited with their original cage mates and allowed to physically interact for 1 hr. As in the previous experiment, isolated and group-housed animals served as control conditions, and remained undisturbed in their home cages. After the 1 hr regrouping session, or equivalent time for control groups, animals were challenged i.p. with saline or 10μg of LPS. Animals were monitored for sickness behaviour every 15 mins for 90 mins post-injection, and were then decapitated. Trunk blood was collected to analyze plasma levels of corticosterone. The brain was removed, sliced and micropunched as described in Experiment 1.

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2.4. Experiment 3

To determine whether the effects observed in previous experiments were unique to a social stressor, two experiments were conducted. Experiment 3a compared the effects of the 1-hr regrouping with those of a 5 min. restraint and remaining undisturbed in an isolated or group-housed environment. Experiment 3b compared the effects of the 1-hr regrouping with those of a 5 min tail pinch, 6 min startle, isolation and group-housing. The social stressor in both experiments was the 1 hr regrouping of former cage mates following a 14 day period of isolation, as described in previous experiments. Restraint stress (5-min) involved placing mice in a transparent, triangular shaped polyethylene bag, made from Fisherbrand specimen bags (01-816B), with a small hole at the end for air. The bag was snug fitting and restricted movement. Startle involved placing mice in an acrylic cylinder tube (inside diameter of 3.2cm, and overall length between 7.5cm and 10cm) which was placed in an insulated startle chamber (Med Associates, Vermont). After an acclimatization period of 2.5 mins, mice were exposed to bursts of noise of various intensities (65-105dB) every 30 seconds for 10 trials. The tail pinch stressor involved placing mice in a novel cage with a small foldback binder clip (Grand & Toy, #99817) placed at the base of the tail for a period of 5 min. A gauze pad was placed between the tail and the clip to avoid damage. Immediately following their respective treatments, or at random times for the isolated and group-housed controls, animals were i.p. challenged with saline or 5 μg or 10 μg of LPS in both experiments. Animals were monitored for sickness behaviour for 90 mins post-injection, and were then decapitated. Trunk blood and brain tissue was collected for further analyses, as described earlier.

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2.5. Experiment 4

The fourth study was conducted to determine whether physical contact upon regrouping was a necessary condition for stressor synergy with LPS to occur. Mice were randomly assigned to 5 separate conditions; namely, (a) animals were regrouped and allowed physical contact, (b) animals were regrouped but separated by a metal partition, (c) animals remained isolated but were placed behind the metal partition to control for the novelty of the metal grid and surroundings, (d) isolated controls, and (e) group-housed controls. As in the other experiments, mice in the physical contact group were reunited with former cage mates following a 14 day isolation period and allowed to interact physically for 1 hr in a novel cage. Mice in the second condition were regrouped with former cage mages for 1 hr following an identical isolation period, although no physical contact was permitted. The cage was divided into quadrants using a perforated metal partition grid (10mm x 10mm, Home Depot) to divide the cage. Mice were therefore allowed olfactory, visual and auditory stimulation from one another, but were unable to physically interact or to develop any social hierarchies. Mice in the third condition were similarly placed in the quadrant cage divided by the metal partition grid, yet no additional mice were added to the cage. Isolated and group-housed controls remained undisturbed in their home cages. Following the 1 hr regrouping session, or equivalent time for the control groups, mice were injected with either saline or 5 μg or 10 μg of LPS and monitored for sickness behaviour for 90 mins prior to being decapitated for corticosterone and monoamine analyses.

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2.6. Sickness Behaviour

Sickness behaviour was scored for 10 sec at 15 min intervals following injection over a 90 min period. Animals were closely monitored for signs of illness, and were evaluated on the following symptoms: lethargy (demonstrated by increased sleep and diminished locomotor and exploratory activities), ptosis (demonstrated by drooping eyelids), and pilo erection (ruffled and greasy fur, typically at the neck). Animals were rated on a 4-point scale (1 = no sickness symptoms, 2 = 1 symptom present, 3 = 2 symptoms present, 4 = all three symptoms present). This procedure was found to provide less than 10% variability between raters blind to the treatment mice received (Hayley et al., 1999)

2.7. Plasma Corticosterone Analysis

Blood plasma was analyzed to determine corticosterone levels in response to the social housing and LPS treatments. Following decapitation, the plasma was collected and stored at -80°C until assayed. Plasma levels were quantified using a commercial radioimmunoassay RIA kit (CORT H3 kit, MP Biomedics) according to the manufacturer's instructions. For each study corticosterone was determined in a single run to avoid inter-assay variability. Intra-assay variability was less than 10%.

2.8. Brain Dissection Technique

Following decapitation, brains were removed and placed on a stainless steel dissecting block (placed on dry ice) with slots spaced approximately 0.5 mm apart. Brains were sectioned into a series of coronal slices using razor blades. Brain sections

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were placed on glass slides resting on a bed of dry ice and, following the mouse brain atlas of Franklin and Paxinos (1997), the PVN of the hypothalamus, locus coeruleus, mPFC, central amygdala, and dorsal hippocampus were removed by micropunch using hollow 16 and 20 gauge needles with a beveled tip. The tissue punches were placed in 0.3 M monochloroacetic acid containing 10% methanol and internal standards, and stored at –80°C for monoamine analyses.

2.9. High Performance Liquid Chromatography (HPLC) assay

Levels of DA, NE and 5-HT, and their metabolites, DOPAC, MHPG and 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, Ontario) 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), 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, Ontario) and a Fluorostar colorimeter (BMG,

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Durham, NC). The lower limit of detection for the monoamines and metabolites was approximately 1.0 pg.

2.10. Plasma Cytokine Analysis with Luminex

The Luminex 100 is a suspension based bead array system that uses xMAP technology, and can detect up to 100 different analytes in a single 50µl sample. Sets of micro spheres (5.6 um beads) are internally dyed with different ratios of fluorophores, each conjugated to a different capture probe (cytokine specific antibody). Following incubation, a classification laser identifies the particular cytokine bound and a second reporter laser quantifies the signal present. In the present study, we used a custom-designed kit encompassing both pro-inflammatory (IFN-γ, TNF-α, IL-1β, IL-2, IL-5, IL-6, IL-12) and anti-inflammatory (IL-4, IL-10) cytokine detection (Upstate, Cell Signaling Solutions). The cytokines of interest were IFN-γ, TNF-α, IL-1β, IL-2, IL-6, IL-12, and IL-10.

In order to detect and quantify the presence of plasma cytokines, a mouse serum diluent kit (Cat # 43-007) was used to dilute plasma supernatants. A Beadlyte Mouse Multi-Cytokine Detection System 2 kit (Upstate, Cell Signaling, Cat # 48-004) was used in conjunction with the Luminex 100 system. First, a 5000pg Multi-Cytokine 2 standard was suspended in 1ml of Standard Serum Diluent (SSD), and vortexed on medium speed for 15 seconds. A series of serial dilutions were then performed to cover a range of standards (from 5000-6.9 and 0 pg/ml). Then, 25µl of Beadlyte Cytokine Assay Buffer was placed in each well and a gentle vacuum removed excessive liquid. Plates were blotted dry and 25µl of plasma diluent and 25µl of plasma added to each well. The filter plate was then incubated on a shaker for 20 minutes. Following incubation, samples were

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vortexed at medium speed for 15 seconds and then sonicated for an additional 15 seconds. To finalize the initial reaction, 25µl of bead solution was added to each well and the plate was covered and vortexed on low speed.

Plates were placed on a plate shaker and incubated overnight at 4°C in a dark room. Following removal of excessive liquid, plasma samples were resuspended in 75µl of Beadlyte Cytokine Assay Buffer, this step was performed twice. Next, following a short vortex and vacuum step, 25µl of Biotin conjugated Beadlyte Anti-Mouse Cytokine was added to each well. After 1.5 hours of incubation with the biotin antibodies in a dark room, 25µl of diluted beadlyte streptavidin-PE was added to each well, covered, and mixed by vortex at a low speed. The antibody was incubated for 30 minutes in the dark at room temperature on a plate shaker. Finally, 25µl of Beadlyte stop solution was added to each well to halt the reaction and samples were resuspended in 125µl of sheath fluid and read in the Luminex 100 instrument. Once the filter plate was placed in the Luminex, 100 different bead sets were distinguished based on its internal dye ratios. The antibodies bound to the bead surface act as targets for the substrates. Reporters, are also tagged with a fluorescent label, and also bind to the target. The substrate being measured is unique to particular bead set.

2.11. Statistical Analyses

Elevated sickness behaviour scores were not apparent until 45 min post-injection. As sickness scores were highly correlated over time, scores from 45 to 90mins were added together to create a total sickness score, which was analyzed using a two-factor (Stressor condition and LPS treatment) between-subjects analysis of variance (ANOVA).

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Monoamines (NE, DA and 5-HT) and their respective metabolites (MHPG, DOPAC and 5-HIAA) were analyzed independently in each brain region (PVN, locus coeruleus, PFC, central amygdala and hippocampus) using 2-factor ANOVAs, considering both LPS treatment and Stressor condition. Finally, plasma cytokines (INF-γ, IL-10, IL-12, IL-6, IL-1β, IL-2 and TNF-α) and corticosterone were analyzed independently using 2-factor between-subjects ANOVA, with Stressor condition and LPS treatment being the 2 factors. Following initial ANOVAs, Newman-Keuls test were used for analyses of means of significant effects of the treatments and their interactions.

3. Results

3.1. Experiment 1: Behavioural, neurochemical and immune effects of a social stressor and LPS

Sickness Behaviour: Total sickness behaviour was significantly influenced by the Stressor condition, $F_{(2,94)} = 4.32$; $p < .02$, and LPS treatment, $F_{(3,94)} = 20.15$; $p < .0001$. As seen in Figure 1, and confirmed by Newman-Keuls multiple comparisons, animals exposed to a 1-hr regrouping session following a period of isolation demonstrated significantly higher sickness scores than that evident in either isolated or group-housed controls. The latter groups did not differ from one another. Furthermore, LPS elicited dose-dependent behavioural variations; each of the doses of LPS induced greater sickness than did saline, and the 10μg of LPS provoked greater sickness than lower doses of LPS (i.e., 1 or 5μg).

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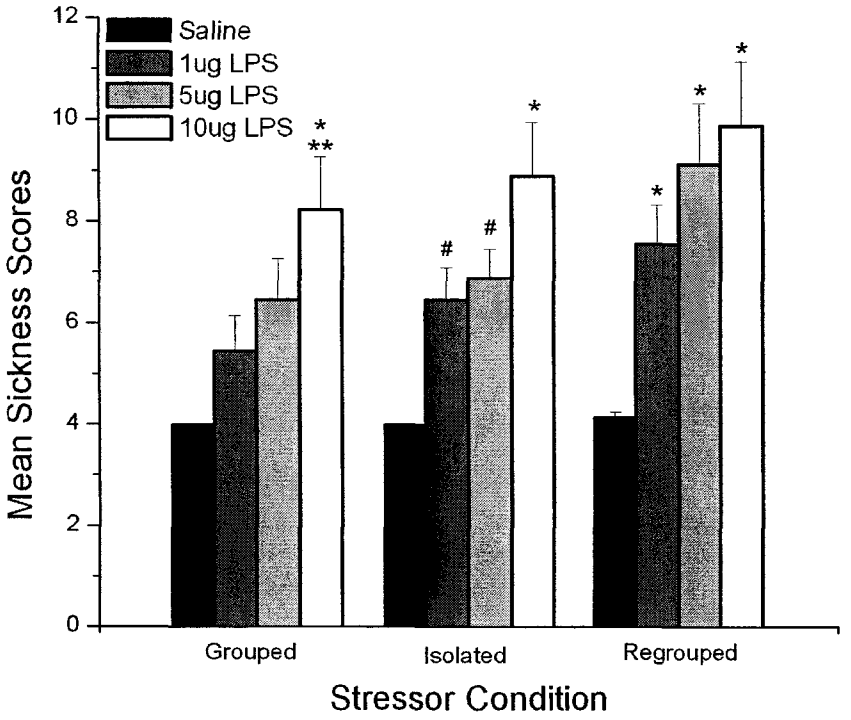

Figure 1. Mean sickness scores (±SEM) totaled over 4 ratings among mice that had been group-housed, isolated for 14 days, or isolated and regrouped for 1 hr. Mice were treated with saline or LPS (1, 5 or 10ug). * $p < .01$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with saline ** $p<.05$ relative to similarly treated mice injected with 1ug of LPS.

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Plasma Corticosterone: Levels of plasma corticosterone, shown in Figure 2, varied as a function of the Stressor condition, $F_{(2,94)} = 28.41$; $p < .0001$, and LPS treatment $F_{(3,94)} = 25.84$; $p < .0001$. Paralleling the behavioural changes, follow-up analyses revealed that socially stressed animals had higher circulating corticosterone levels relative to isolated and group-housed mice that did not receive the social reintegration. Moreover, animals treated with 10μg of LPS secreted higher corticosterone levels than those that received a lower dose of LPS or saline, and animals that received 5μg of LPS secreted higher corticosterone levels than those that received saline. In effect, the two treatments had additive effects so that levels of corticosterone in previously isolated animals treated with LPS following a period of regrouping were particularly elevated.

Insert Figure 2 about here

Monoamine Variations: In general, social stressors and LPS treatment influenced central monoamine neurotransmission in a region specific manner. Despite the sensitivity of the PVN neurons to stressors, significant differences of NE or DA, or their respective metabolites, MHPG or DOPAC, were not observed among treatment groups. However, 5-HIAA accumulation was significantly influenced by the LPS treatment, $F_{(3, 81)} = 3.06$; $p < .05$, data not shown. Newman-Keuls multiple comparisons revealed that the minimum dose of 1μg of LPS elicited higher 5-HIAA levels than did saline. No differences were observed with higher doses. Furthermore, no differences were observed in the parent amine, 5-HT.

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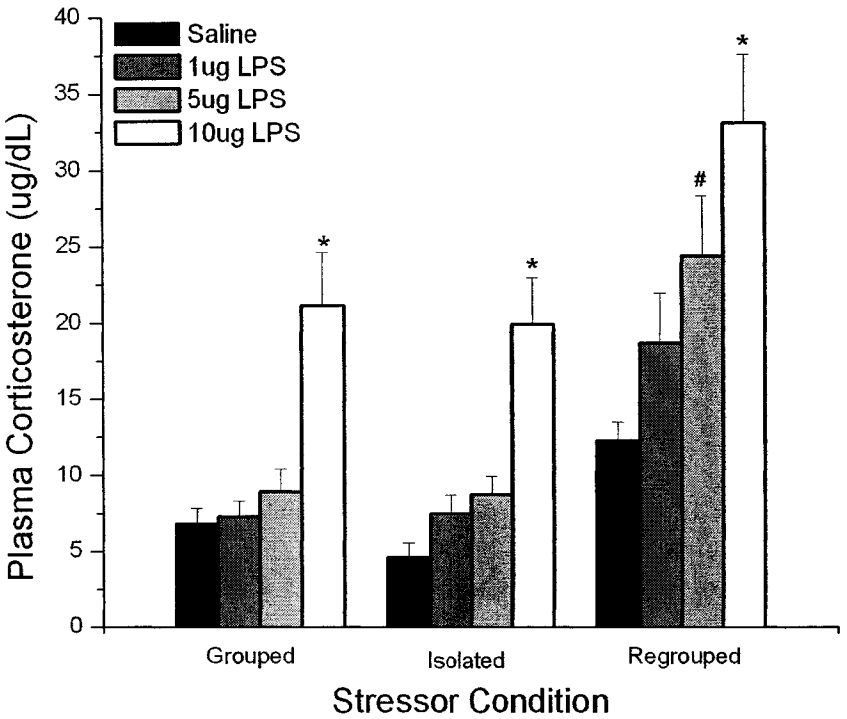

Figure 2. Mean plasma corticosterone (±SEM) among mice that had been group-housed, isolated for 14 days, or isolated and regrouped for 1 hr and then treated with saline or LPS (1, 5 or 10ug). * $p < .01$ relative to similarly treated mice injected with saline or a lower dose of LPS # $p<.01$ relative to similarly treated mice injected with saline.

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Within the locus coeruleus, MHPG was significantly influenced by LPS treatment, $F_{(3, 79)} = 3.10$; $p < .05$. Multiple comparisons indicated that 10μg of LPS elicited higher levels of MHPG than that evident in saline-treated animals. Unlike the MHPG accumulation, the levels of NE did not differ dramatically across groups.

In the PFC, the site of terminal regions of neurons originating in the locus coeruleus, MHPG levels varied as a function of the Social Stressor Condition, $F_{(2, 81)} = 10.45$; $p < .0001$, and the LPS treatment, $F_{(3, 81)} = 7.62$; $p < .001$. Subsequent analyses, as shown in Figure 3 (upper panel), revealed that the 1-hr regrouping elicited higher MHPG levels in the PFC than those evident in either isolated or group-housed controls. Furthermore, 10μg of LPS elicited higher MHPG levels than a lower dose (1 or 5μg) or saline. As seen in Figure 3 (lower panel), NE levels were also influenced by Social stressor condition, $F_{(2, 81)} = 7.20$; $p < .01$. The follow-up comparisons indicated that regrouped animals had higher NE levels than both isolated and group-housed controls. In addition to the NE changes, 5-HIAA levels were also significantly influenced by the Stressor condition, $F_{(2, 80)} = 7.02$; $p < .001$ within the PFC, with both regrouped animals and group-housed controls demonstrating elevated levels of 5-HIAA compared to isolated controls. No differences of 5-HT were found among treatment groups.

Insert Figure 3 about here

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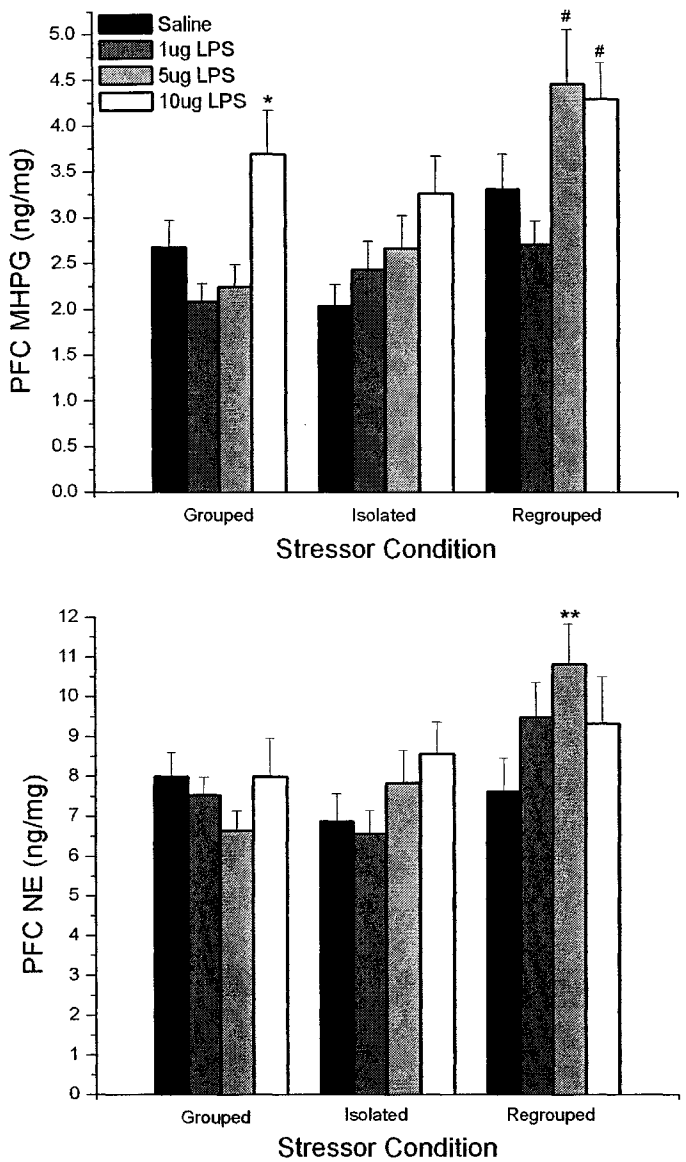

Figure 3. Mean (±SEM) MHPG (upper panel) and NE (lower panel) within the PFC among mice that had been group-housed, isolated for 14 days, or isolated and regrouped for 1 hr. Mice were treated with saline or LPS (1, 5 or 10ug). * $p < .05$ relative to similarly treated mice injected with saline or a lower dose of LPS ** $p < .05$ relative to similarly treated mice injected with saline # $p<.05$ relative to similarly treated mice injected with 1ug of LPS.

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Within the central amygdala, the accumulation of MHPG varied as a function of the Social stressor, $F_{(2, 81)} = 5.74$; $p < .05$, and LPS treatment, $F_{(2, 81)} = 6.85$; $p < .01$, whereas NE levels were unaffected by the treatments. As shown in Figure 4, and confirmed by multiple comparisons, MHPG levels were higher in animals that had been maintained in the group-housed condition than in mice that received the 1-hr regrouping or mice that remained isolated. Furthermore, 5μg of LPS elicited higher MHPG levels than 10μg, 1μg or saline. In contrast to the MHPG effects, no significant differences of DA, DOPAC, 5-HIAA or 5-HT were observed among treatment groups.

Insert Figure 4 about here

Within the hippocampus, both NE and its metabolite, MHPG, were influenced by LPS treatment, F's $_{(3, 82)} = 2.79$ and 3.20; $p < .05$, respectively (see Figure 5). In both instances, 10μg elicited significantly higher levels of MHPG or NE relative to the saline treatment. In addition to the NE effects, 5-HIAA was also significantly influenced by LPS treatment, $F_{(3, 82)} = 4.31$; $p < .01$. Subsequent analyses indicated that both 10μg and 1μg of LPS elicited higher 5-HIAA levels than that seen in saline-treated animals. Levels of 5-HT within the hippocampus were not affected by Social stressor condition or LPS treatment.

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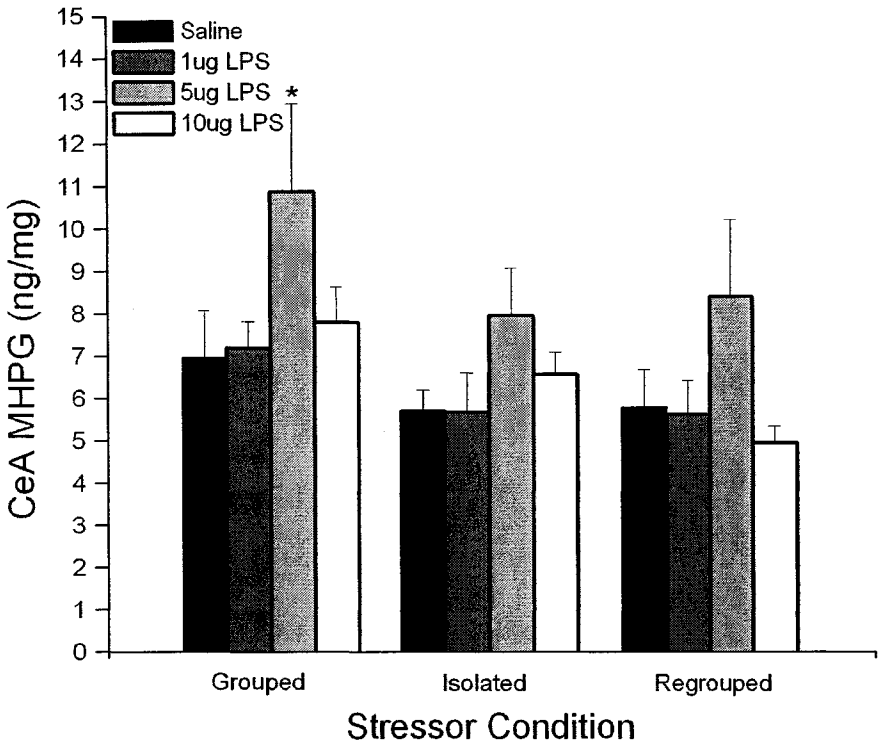

Figure 4. Mean (±SEM) MHPG within the central amygdala among mice that had been group-housed, isolated for 14 days, or isolated and regrouped for 1 hr. Mice were treated with saline or LPS (1, 5 or 10ug). * $p < .05$ relative to similarly treated mice injected saline, 1ug or 10ug of LPS.

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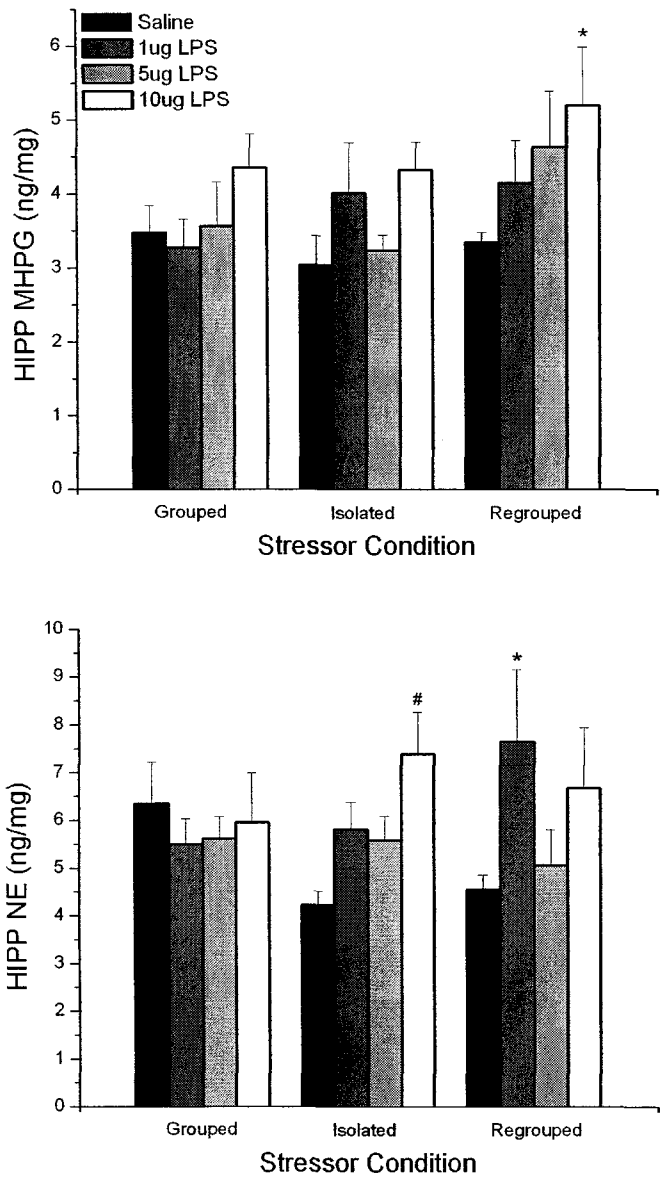

Figure 5. Mean (±SEM) MHPG (upper panel) and NE (lower panel) within the hippocampus among mice that had been group-housed, isolated for 14 days, or isolated and regrouped for 1 hr. Mice were treated with saline or LPS (1, 5 or 10ug). * $p < .01$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with saline

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Plasma Cytokine Analysis: Circulating TNF-α levels were significantly influenced by LPS condition, $F_{(3,94)} = 8.59$; $p < .0001$, as shown in Figure 6 (upper panel). Follow-up analyses revealed that 10μg of LPS elicited higher plasma TNF-α levels than did lower doses (1μ or 5μg of LPS) or saline. In addition to TNF-α effects, circulating IL-10 levels varied as a function of the interaction between the Social stressor condition and LPS treatment, $F_{(6, 105)} = 2.20$; $p < .05$. The follow-up comparisons indicated that among the grouped and individually housed animals LPS dose-dependently increased IL-10 levels, with significant elevations being only evident at the highest (10μg) dose of LPS. In contrast, among regrouped animals that had undergone a period of isolation, each of the doses increased IL-10 levels. Indeed, as seen in Figure 6 (lower panel), at the 5μg dose, which hardly affected IL-10 in the isolated and group-housed mice, a 10-fold increase of IL-10 was evident among mice following regrouping. No significant differences of circulating INF-γ, IL-1β, IL-2, or IL-6 or IL-12 were found among treatment groups.

Insert Figure 6 about here

3.2. Experiment 2: Influence of various periods of isolation prior to a social stressor on LPS-induced behavioural, neuroendocrine and monoamine variations

Sickness Behaviour: Total sickness behaviour scores varied as a function of the interaction between Social stressor condition and LPS treatment $F_{(5,83)} = 2.85$; $p < .05$. As seen in Figure 7, and confirmed by Newman-Keuls follow-up tests, the endotoxin challenge elicited higher sickness scores than did saline across all social stressor conditions. The sickness scores elicited by LPS increased with more prolonged periods of

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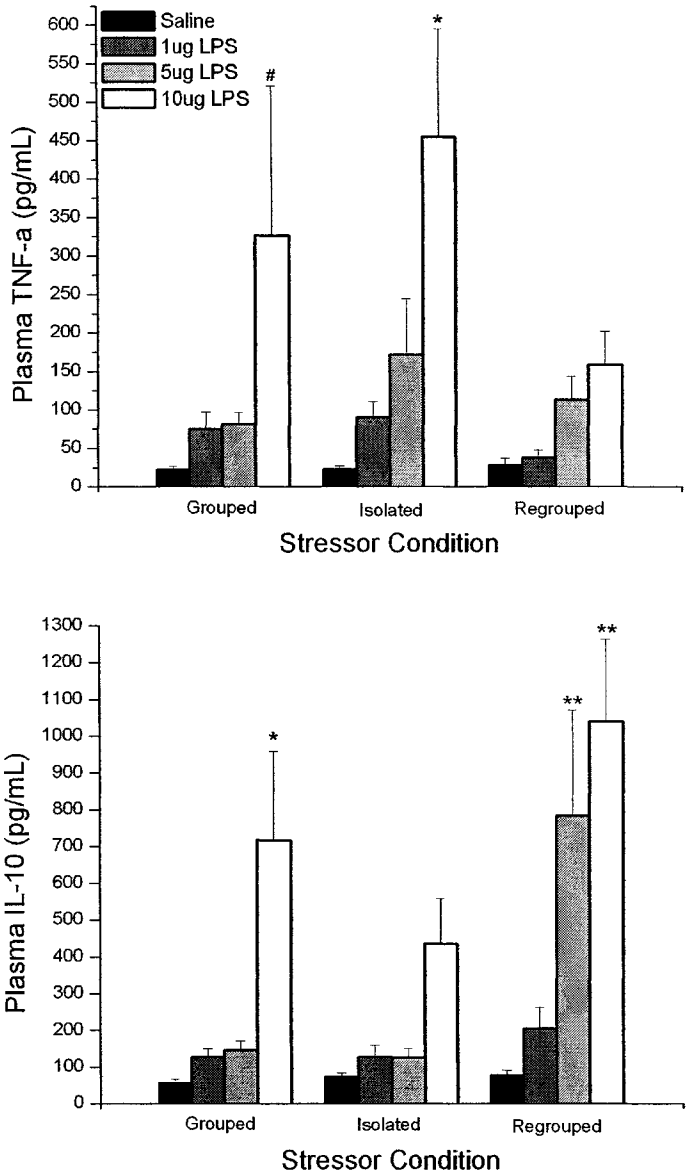

Figure 6. Mean (±SEM) plasma TNF-á (upper panel) and IL-10 (lower panel) among mice that had been group-housed, isolated for 14 days, or isolated and regrouped for 1 hr. Mice were treated with saline or LPS (1, 5 or 10ug). * $p < .01$ relative to similarly treated mice injected with saline or lower doses of LPS # $p < .05$ relative to similarly treated mice injected with saline or lower doses of LPS ** $p < .01$ relative to similarly treated mice injected with saline or 1ug of LPS

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isolation before regrouping. Although no significant differences were observed among the saline treatment group, upon LPS administration those mice that had been isolated for 7 or 14 days before regrouping exhibited greater sickness scores than the 1 and 3 day isolation conditions before regrouping, or mice that remained isolated or group-housed.

Insert Figure 7 about here

Plasma Corticosterone: Plasma corticosterone levels were significantly altered by the Social stressor condition, $F_{(5,82)} = 4.21$; $p < .002$, and LPS treatment, $F_{(1,82)} = 134.76$; $p < .0001$. Subsequent analyses revealed that a 7 or 14 day period of isolation followed by regrouping elicited higher plasma corticosterone levels than 1 day of isolation, or that evident in isolated or group-housed controls. Furthermore, 10μg of LPS elicited significantly higher levels of plasma corticosterone than did saline. Thus, as depicted in Figure 8, the differing social isolation conditions and the LPS treatments had additive effects on plasma corticosterone levels.

Insert Figure 8 about here

Monoamine Variations: Monoamines and their metabolites were analyzed within the PVN and PFC, and varied in a region specific manner. Within the PVN, MHPG was elevated among mice that received 10μg of LPS relative to saline treated mice, $F_{(1, 70)} = 10.13$; $p < .01$ (see Figure 9, upper panel). The housing condition did not affect MHPG, and likewise the interaction between LPS and housing condition was not significant. In addition to the NE effects, 5-HT varied as a function of the interaction between the Social stressor and LPS treatment, $F_{(5, 69)} = 2.44$; $p < .05$ (see Figure 9, lower panel).

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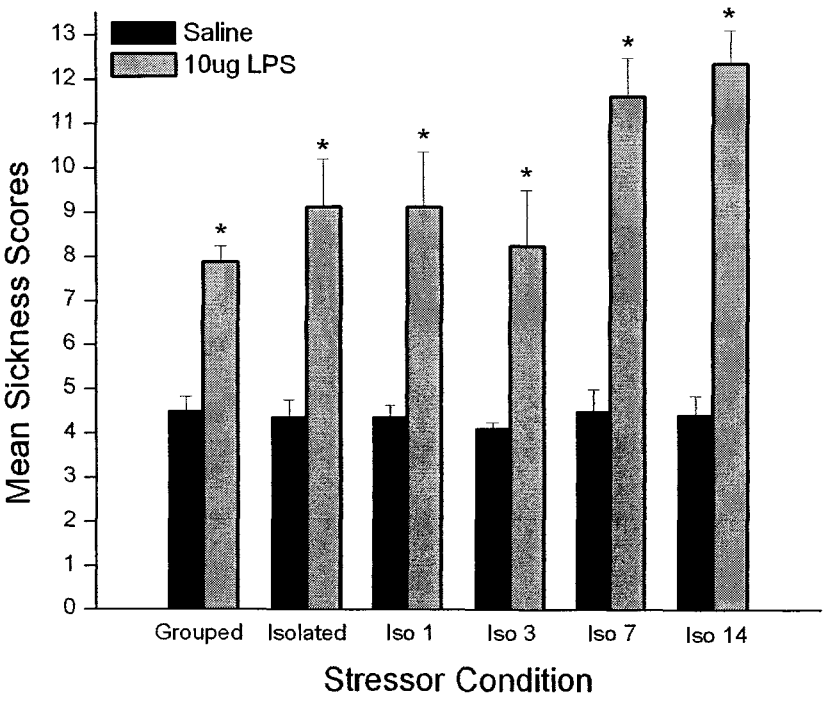

Figure 7. Mean sickness scores (± SEM) totaled over 4 ratings among mice that had been grouped housed, isolated for 14 days, or isolated for 1, 3, 7, or 14 days and regrouped for 1 hr. Mice were then treated with saline or 10ug of LPS. * $p < .01$ relative to similarly treated mice injected with saline.

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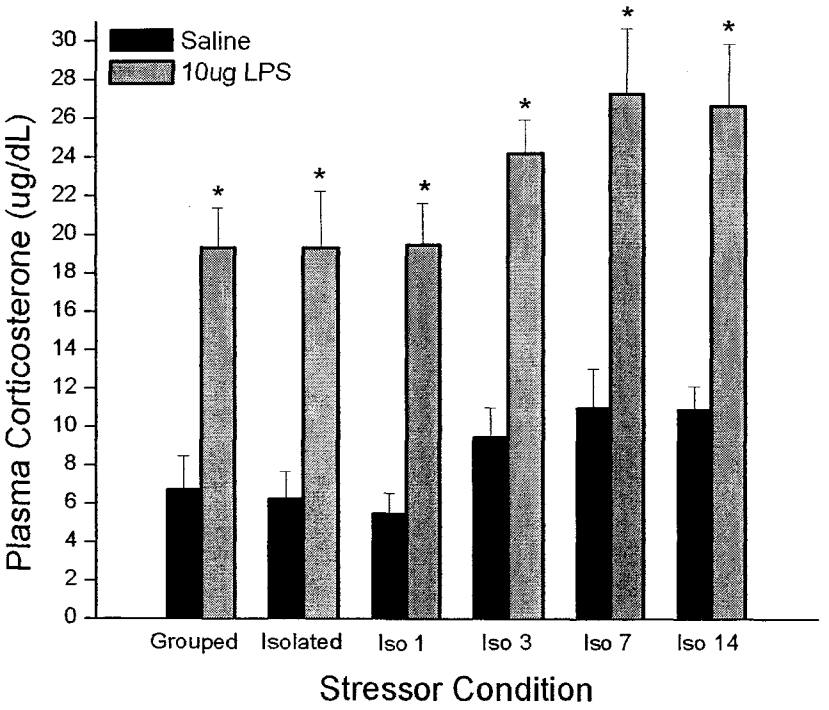

Figure 8. Mean plasma corticosterone (± SEM) among mice that had been grouped housed, isolated for 14 days, or isolated for 1, 3, 7, or 14 days and regrouped for 1 hr. Mice were then treated with saline or 10ug of LPS. * $p < .01$ relative to similarly treated mice injected with saline.

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The follow-up analyses indicated that LPS administration in mice that experienced 7 and 14 days of isolation before regrouping, exhibited higher 5-HT levels than did similarly housed mice that were injected with saline. Despite the 5-HT changes evident within the PVN, there was no indication of 5-HIAA varying with the treatments administered.

Insert Figure 9 about here

Within the PFC the accumulation of MHPG was elevated in the LPS-treated mice relative to the metabolite concentrations in animals that received saline, $F_{(1, 70)} = 4.29$, $p < .05$. As well, it was found that NE levels varied as a function of the Social stressor condition, $F_{(5, 70)} = 2.52$; $p < .05$. The follow-up tests indicated that 7 days of isolation followed by regrouping elicited higher levels of NE than remaining isolated without further disruption.

The levels of DA within the PFC were not affected by the experimental treatments, whereas DOPAC accumulation varied with the Social stressor condition, $F_{(5, 69)} = 2.62$; $p < .05$ (see Figure 10). Newman-Keuls multiple comparisons indicated that DOPAC levels were higher in animals isolated for 3, 7, and 14 days and then regrouped than among mice that remained isolated without the regrouping manipulation. Moreover, group-housed animals also had significantly higher DOPAC levels than isolated controls. In contrast to NE and DA alterations, no differences of 5-HIAA or 5-HT accumulation were observed among treatment groups.

Insert Figure 10 about here

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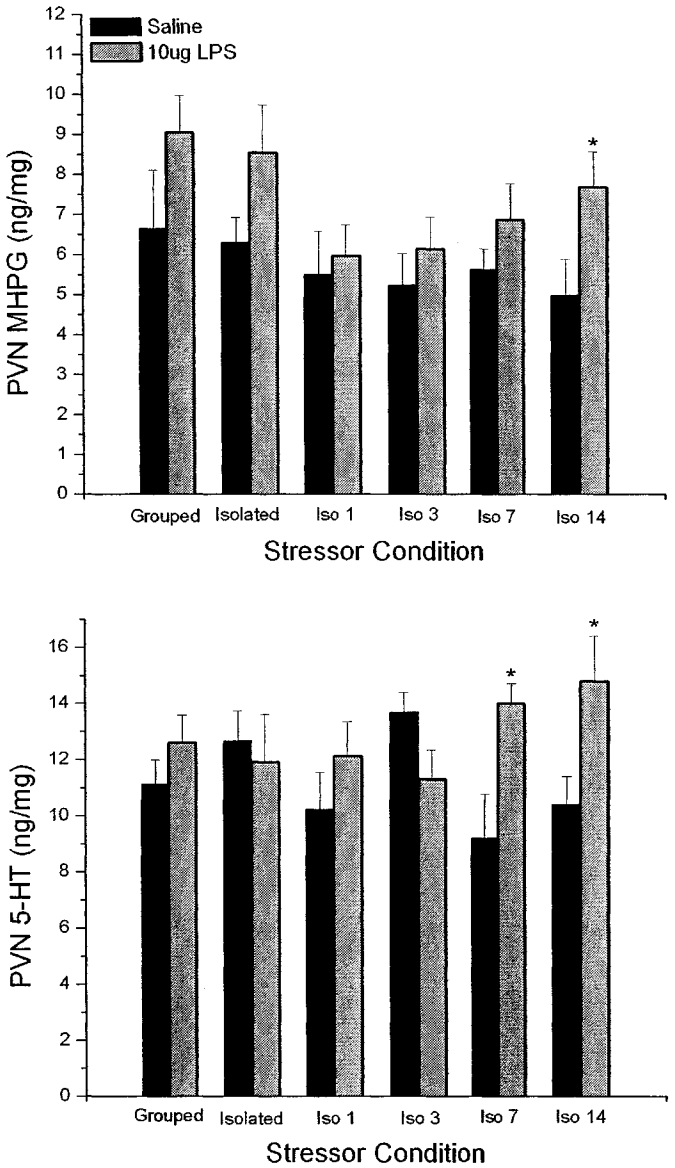

Figure 9. Mean (± SEM) MHPG (upper panel) and 5-HT (lower panel) within the PVN among mice that had been grouped housed, isolated for 14 days, or isolated for 1, 3, 7, or 14 days and regrouped for 1 hr. Mice were then treated with saline or 10ug of LPS. * $p < .05$ relative to similarly treated mice injected with saline.

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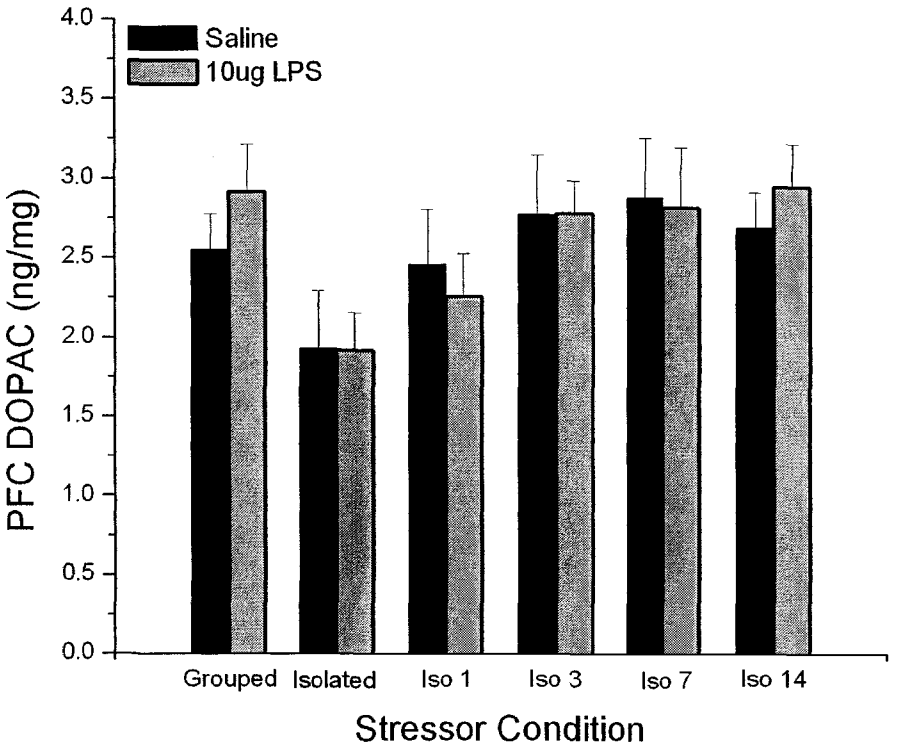

Figure 10. Mean (± SEM) DOPAC within the PFC among mice that had been grouped housed, isolated for 14 days, or isolated for 1, 3, 7, or 14 days and regrouped for 1 hr. Mice were then treated with saline or 10ug of LPS.

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3.3. Experiment 3: The behavioural, neuroendocrine and neurotransmitter effects of LPS coupled with social or non-social stressors.

Two studies were conducted to assess whether the interactive effects of social isolation and LPS would also be evident with non-social stressors. Experiment 3a compared the effects of LPS following 1-hr regrouping with those of either 5 min. restraint, isolated housing or grouped housing, whereas, Experiment 3b compared the effects of LPS following1-hr regrouping with 5 min. tail pinch, 6 min. startle, and isolated and group-housed controls.

Sickness behaviour: In Experiment 3a, sickness scores varied as a function of the interaction between Stressor condition and LPS treatment, $F_{(6,84)} = 2.37$; $p < .05$, as shown in Figure 11 (upper panel). Multiple comparisons revealed that the 5 and 10μg of LPS elicited higher sickness scores than did saline across all stressor conditions. The magnitude of the LPS effects were comparable in mice that had been restrained relative to those of isolated or group-housed mice. However, among mice that had been exposed to isolation and then regrouped (i.e., that had received the social disruption) the 10μg dose of LPS provoked greater sickness than in the isolated or group housed mice that had not experienced the social disruption prior to LPS administration. This finding suggests a possible synergism between social stressor exposure and endotoxin challenge on sickness behaviour, an effect not demonstrated following restraint.

In Experiment 3b, total sickness scores varied as a function of the Stressor condition, $F_{(4,105)} = 4.01$; $p < .005$ and LPS treatment, $F_{(2,105)} = 46.44$; $p < .0001$. In particular, as seen in Figure 11 (lower panel), the LPS treatment induced a dose-

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dependent increase of sickness scores, with 10μg of LPS inducing greater sickness than did a lower dose of drug or saline, and 5μg of LPS inducing greater sickness scores than saline. The 1-hr regrouping induced significantly higher sickness scores than did startle, tail pinch, isolation or group-housing.

Insert Figure 11 about here

Plasma Corticosterone: As seen in Figure 12 (upper panel), circulating corticosterone levels in Experiment 3a were significantly influenced by both the Stressor condition, $F_{(3,80)} = 5.06$; $p < .005$, and LPS treatment, $F_{(2,80)} = 23.62$; $p < .0001$. The follow-up comparisons revealed that 1-hr regrouping elicited significantly higher corticosterone levels than that evident in restraint and group-housed controls. The LPS treatment likewise increased plasma corticosterone levels, with a 10μg of LPS eliciting the greatest response.

Echoing the results of Experiment 3a, levels of plasma corticosterone in Experiment 3b were also significantly influenced by Stressor condition, $F_{(4,193)} = 13.16$; $p < .0001$, and LPS treatment, $F_{(2,103)} = 26.06$; $p < .0001$. As shown in Figure 12 (lower panel) and confirmed by Newman-Keuls multiple comparisons, the 1-hr regrouping elicited higher corticosterone levels than did the startle and tail pinch, or that apparent in isolated and group-housed controls. Furthermore, both 5μg and 10μg of LPS elicited higher corticosterone levels than saline. Once again, the nature of the stressor acted additively with the LPS in affecting circulating corticosterone levels.

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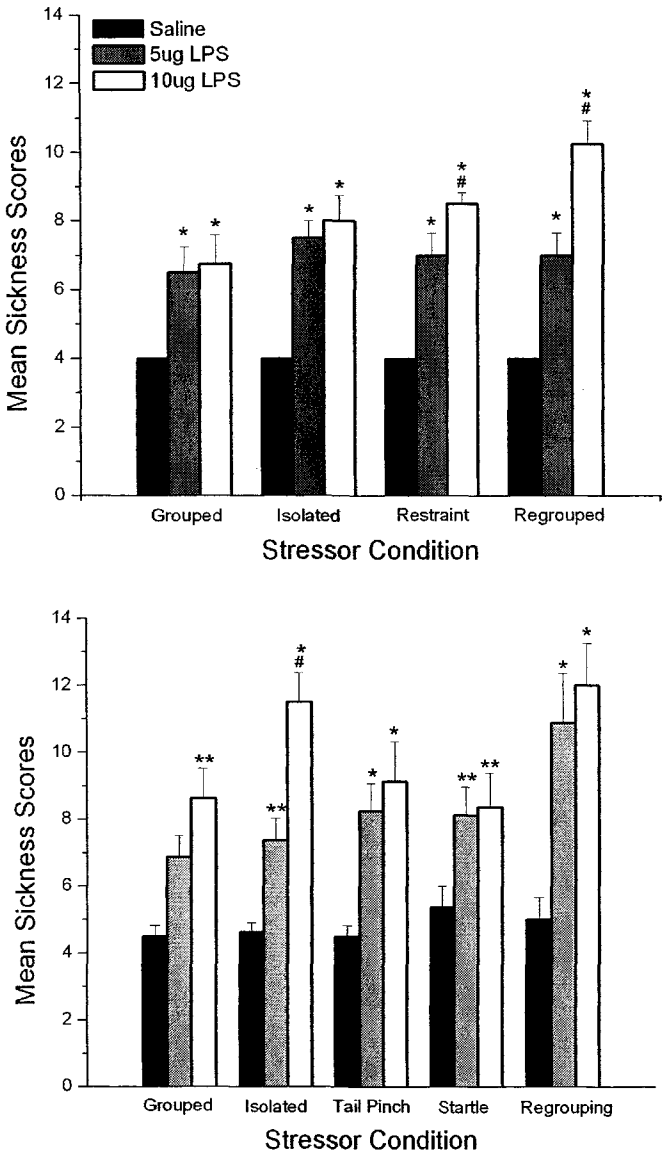

Figure 11. Mean sickness scores (± SEM) totaled over 4 ratings among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr (upper panel) and group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr (lower panel). Mice were then treated with saline or LPS (5 or 10ug). * $p < .01$ relative to similarly treated mice injected with saline ** $p < .05$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with 5ug of LPS.

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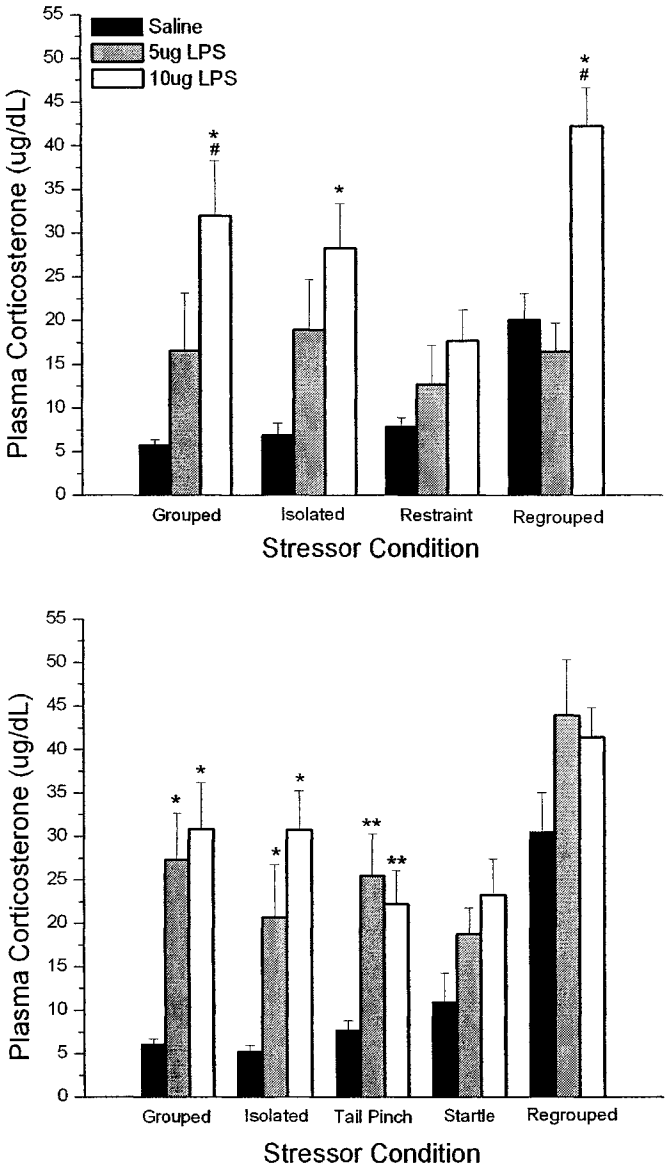

Figure 12. Mean plasma corticosterone (± SEM) among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr (upper panel) and group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr (lower panel). Mice were then treated with saline or LPS (5 or 10ug). * $p < .01$ relative to similarly treated mice injected with saline ** $p < .05$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with 5ug of LPS.

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Monoamine Variations: Within the PVN, LPS treatment significantly influenced MHPG accumulation in both Experiment 3a and 3b, F's $_{(2,79)}$= 3.88 and F $_{(2,101)}$= 3.54; $p < .05$, respectively. As shown in Figure 13, and confirmed by multiple comparisons, in Experiment 3a the 10μg dose of LPS elicited significantly higher MHPG levels than did saline, and in Experiment 3b both doses of LPS elicited significantly higher MHPG levels than did saline.

Insert Figure 13 about here

In addition to MHPG alterations, 5-HIAA levels in the PVN in Experiment 3a were significantly influenced by the Stressor condition, F $_{(3,77)}$= 3.19; $p < .05$, as shown in Figure 14 (top left panel). Subsequent analyses indicated that remaining group-housed elicited higher levels of 5-HIAA than did restraint. Likewise, at the 10μg dose, mice that were regrouped exhibited higher 5-HIAA levels than did mice that were restrained. In neither case, however, was 5-HIAA found to exceed that of mice that were continuously housed in isolation. Moreover, in Experiment 3b, 5-HIAA levels were unaffected by the stressor (see Figure 14, bottom left panel). Thus, it seems that the combination of the stressor and LPS treatment generally did not affect the utilization of 5-HT. However, it was observed in Experiment 3b that 5-HT varied as a function of the Stressor condition x LPS treatment interaction, F $_{(8, 103)}$= 2,42; $p < .05$ (see Figure 14, bottom left panel). In particular, in continuously isolated mice 5μg of LPS provoked an increase of 5-HT relative to mice treated with saline. Similar effects were not evident with respect to the other stressors. In fact, in these groups the LPS provoked a marginal decline ($p < .10$) of the 5-HT levels.

Insert Figure 14 about here

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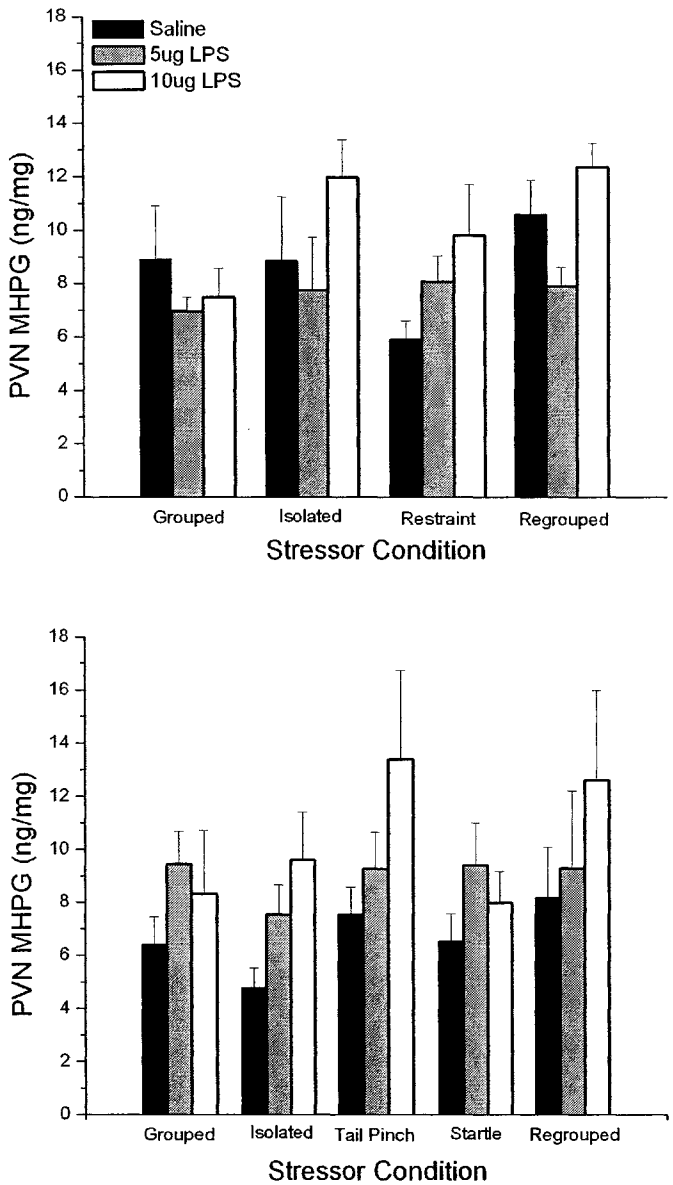

Figure 13. Mean (± SEM) MHPG within the PVN among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr (upper panel) and group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr (lower panel). Mice were then treated with saline or LPS (5 or 10ug).

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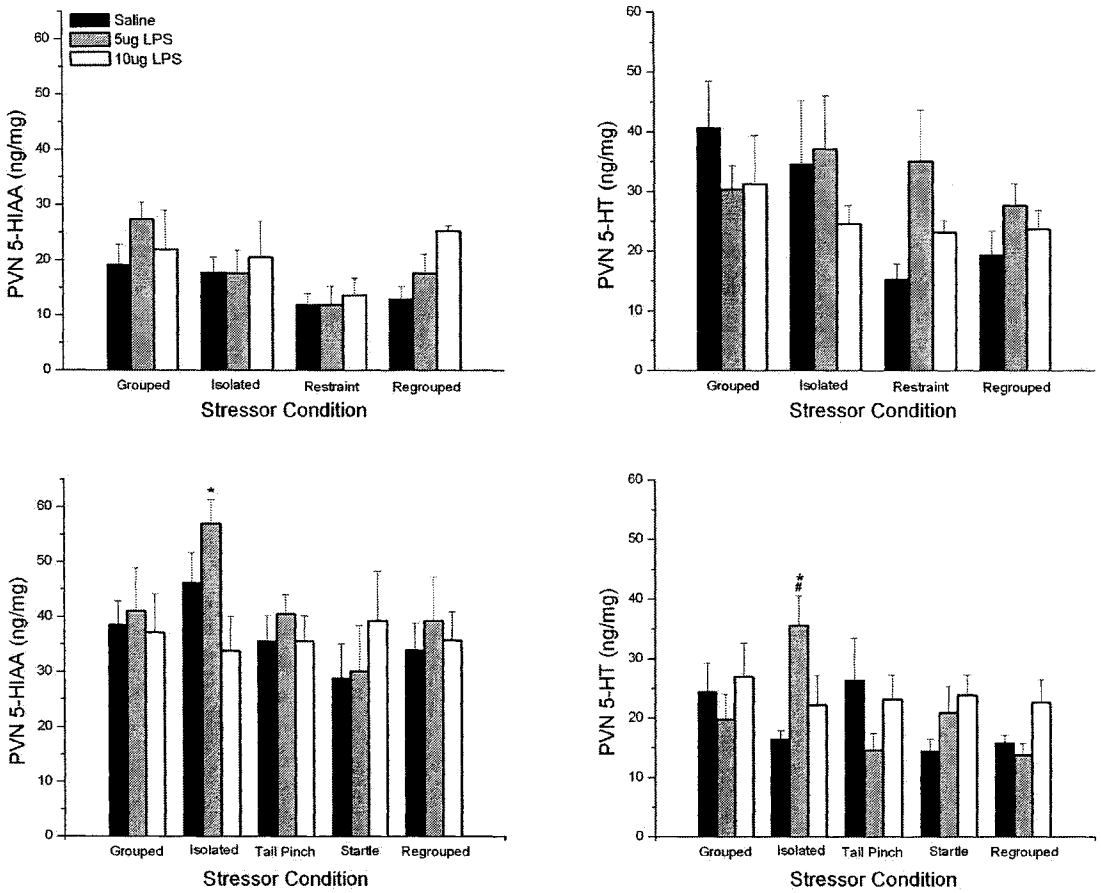

Figure 14. Mean (± SEM) 5-HIAA and 5-HT (left and right, respectively) within the PVN among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr (upper panels) and group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr (lower panels). Mice were then treated with saline or LPS (5 or 10ug). * $p < .05$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with 10ug of LPS

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Within the locus coeruleus of Experiment 3a, MHPG varied as a function of LPS treatment, $F_{(2,80)} = 4.03$; $p < .05$, although alterations of NE accumulation were not observed. The Newman-Keuls multiple comparisons indicated that 10μg of LPS elicited significantly higher MHPG levels than did saline. In Experiment 3b, both MHPG and NE levels were influenced by LPS treatment, $F_{(2,94)} = 3.81$ and $F_{(2,96)} = 5.10$; $p < .05$ and $p < .01$, respectively. In both instances, as shown in Figure 15, relative to saline treatment, 10μg of LPS elicited significantly higher levels of MHPG and NE.

Insert Figure 15 about here

Within the PFC of Experiment 3a, both MHPG and NE varied as a function of the interaction between Stressor Condition and LPS treatment, $F_{(6,83)} = 3.79$ and $F_{(6,84)} = 3.93$; $p < .01$, respectively (see Figure 16, upper panels). In saline treated animals, the 1-hr regrouping elicited markedly higher MHPG levels than did the 5 min restraint, or remaining isolated or group-housed. In mice that were continuously group-housed and in those that received the restraint, treatment with LPS significantly increased MHPG accumulation. Within the regrouped mice, where MHPG levels were already high, a further elevation was not induced by the endotoxin. With respect to NE, the follow-up analyses indicated that among mice exposed to the restraint stressor or those in continued isolation, a decline of NE was apparent following 10 ug of LPS. In contrast, no such effect was apparent among animals that were continuously group-housed or those that were regrouped.

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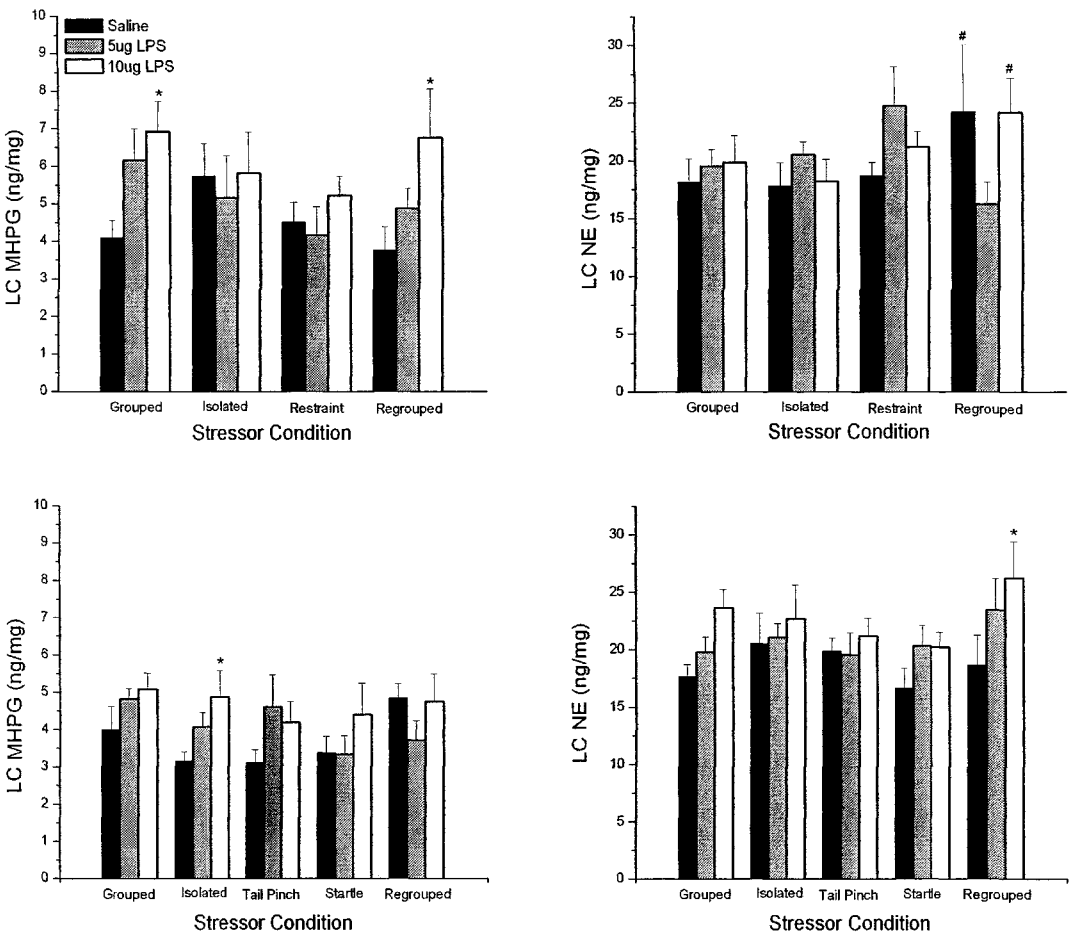

Figure 15. Mean (± SEM) MHPG and NE (left and right, respectively) within the locus coeruleus among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr (upper panels) and group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr (lower panels). Mice were then treated with saline or LPS (5 or 10ug). * $p < .05$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with 5ug of LPS

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The LPS effects on MHPG demonstrated in Experiment 3a were similar in Experiment 3b. Specifically, MHPG in the PFC varied as a function of LPS condition, $F_{(2,105)} = 5.69$; $p < .01$. The follow-up comparisons indicated that the 5μg and 10μg of LPS elicited higher MHPG levels within the PFC than did saline. Although the interaction involving stressor type was not significant, it is clear from Figure 16 (lower panels), and confirmed by Newman-Keuls comparisons of the simple effects for this interaction, that LPS, in fact, did not influence MHPG among mice that were continuously isolated or those that were grouped. Instead, the most pronounced effects of LPS were apparent in the regrouped mice or those exposed to the tail-pinch stressor. No significant differences of NE were observed in Experiment 3b.

Insert Figure 16 about here

In addition to NE changes in the PFC, DA and DOPAC levels varied as a function of the interaction between Stressor condition and LPS treatment, $F_{(6,79)} = 4.20$ and $F_{(6,81)} = 2.55$; $p < .001$ and $p < .05$, in Experiment 3a (see Figure 17, upper panels). Within the saline-treated animals, the 1-hr regrouping elicited markedly higher DA levels than restraint and remaining grouped. Although no significant differences were found within animals treated with 5μg of LPS, the 10μg dose of LPS increased DA levels in mice that had been restrained and in those that remained in an isolated condition. In contrast, in animals in the regrouping condition (which exhibited a marked DA rise relative to the non-stressed groups), LPS at the 10μg dose significantly reduced DA levels. The follow up tests regarding DOPAC accumulation roughly paralleled the DA results.

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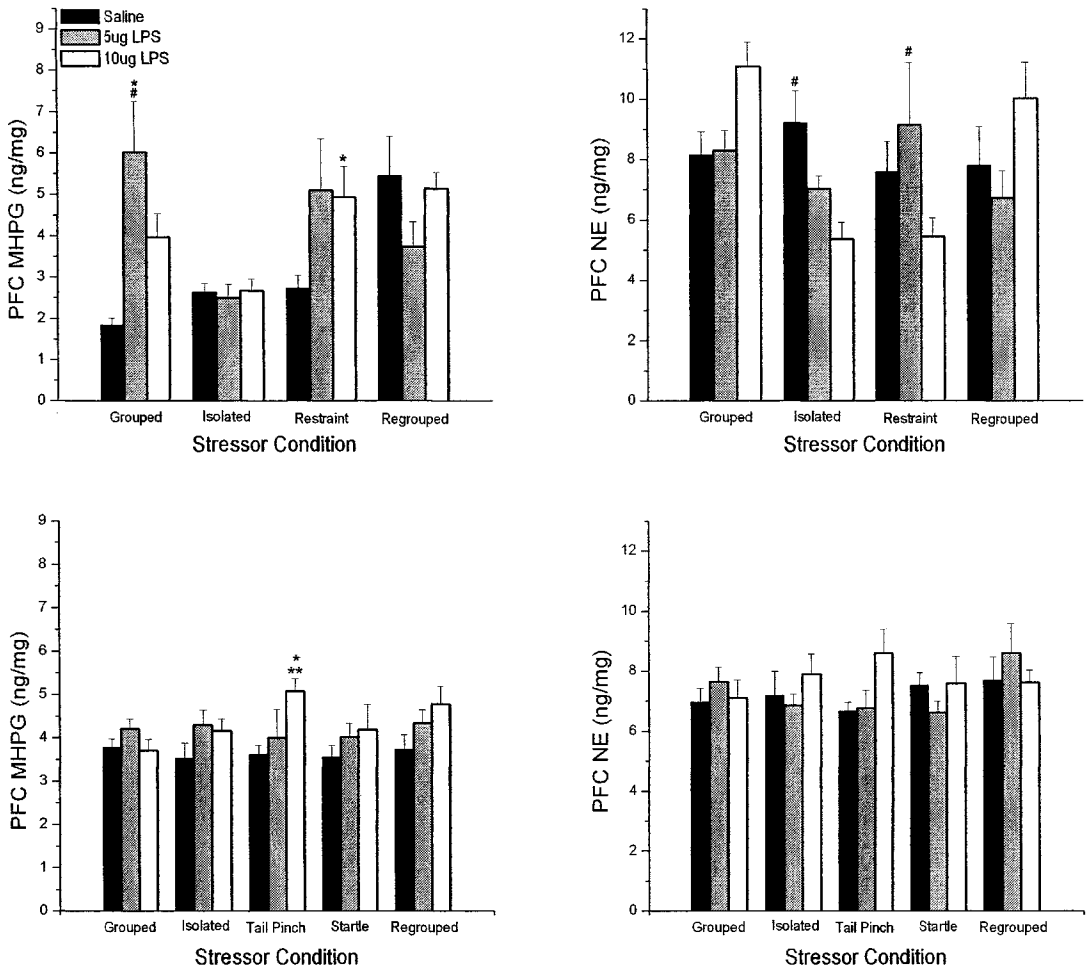

Figure 16. Mean (± SEM) MHPG and NE (left and right, respectively) within the PFC among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr (upper panels) and group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr (lower panels). Mice were then treated with saline or LPS (5 or 10ug).* $p < .05$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with 10ug of LPS ** $p < .05$ relative to similarly treated mice injected with 5ug of LPS

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Specifically, in saline treated animals, regrouping elicited higher DOPAC levels than restraint and remaining undisturbed (group-housed or isolated). Additionally, in response to 10μg of LPS, animals that remained isolated had higher DOPAC levels than those exposed to the 1-hr regrouping. In contrast, among regrouped mice, LPS had the effect of reducing DOPAC accumulation.

Although no significant interaction was found in Experiment 3b, DA and DOPAC were both influenced by LPS treatment, $F_{(2,99)} = 3.41$ and $F_{(2,101)} = 3.54$; $p < .05$, respectively (see Figure 17, lower panels). The multiple comparisons indicated that 10μg of LPS elicited significantly higher levels of DA than did saline. In contrast to the NE and DA alterations, 5-HIAA and 5-HT did not vary among treatment groups in Experiment 3a or 3b.

Insert Figure 17 about here

Within the central amygdala of Experiment 3a and 3b, the concentrations of NE were unaffected by any of the treatment conditions. In Experiment 3a, a main effect of LPS treatment was evident in regard to MHPG levels, $F_{(2,84)} = 6.06$; $p < .01$, as seen in Figure 18 (upper panel). Multiple comparisons indicated that 10μg of LPS elicited higher levels of MHPG accumulation than did saline. Although the interaction between Stressor condition x LPS did not reach an acceptable level of significance, $F_{(6, 84)} = 2.06$, $p < .07$, follow-up tests were conducted on the basis of *a priori* predictions. These comparisons indicated that in continuously isolated mice as well as those that were exposed to the restraint stressor, the administration of LPS (10μg) provoked a significant rise of MHPG.

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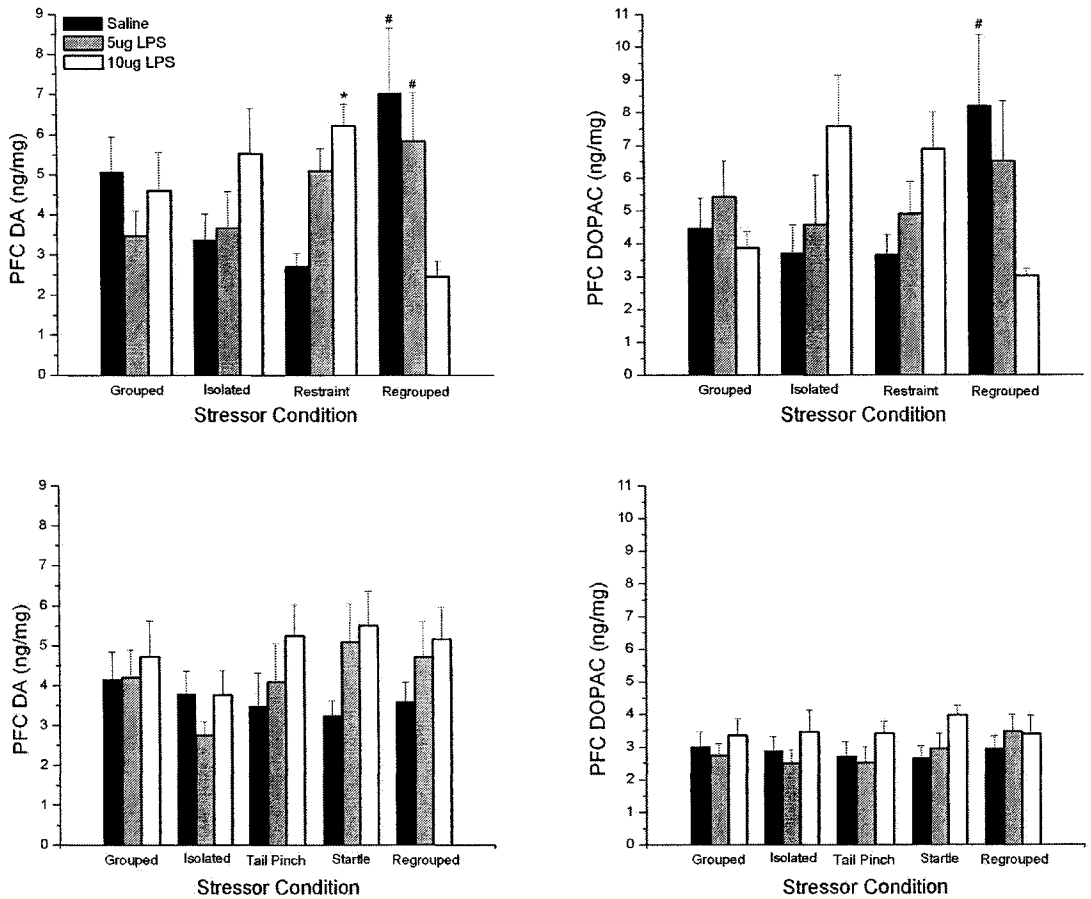

Figure 17. Mean (± SEM) DA and DOPAC (left and right, respectively) within the PFC among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr (upper panels) and group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr (lower panels). Mice were then treated with saline or LPS (5 or 10ug). * $p < .05$ relative to similarly treated mice injected with saline # $p < .01$ relative to similarly treated mice injected with 10ug of LPS

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In contrast, such an outcome was not apparent in grouped mice or those that were regrouped following a period of isolation.

In Experiment 3B, the LPS effect was just shy of significance, $F_{(2, 94)} = 2.57$, $p < .08$). Inspection of Figure 18 (lower panel) confirms that the effects of LPS were, at best, modest. However, among isolated mice an almost two-fold increase of MHPG was induced by LPS treatment.

Insert Figure 18 about here

In Experiment 3a, both DA and DOPAC within the central amygdala were significantly influenced by LPS treatment, $F_{(2,82)} = 3.84$ and $F_{(2,84)} = 9.95$; $p < .05$ and $p < .0001$, respectively (see Figure 19). The administration of LPS (5μg) increased DOPAC accumulation among those mice that had been restrained, housed in isolation, or housed in isolation and then regrouped, whereas such an effect was not apparent in the mice that lived in groups continuously. In contrast, elevations of DA were less notable, and reached significance only after the 5μg dose of LPS among mice that experienced regrouping. DOPAC accumulation also varied as a function of Stressor condition, $F_{(3,84)} = 3.49$; $p < .05$, where isolated animals had higher DOPAC levels within the central amygdala than regrouped or restrained animals.

Insert Figure 19 about here

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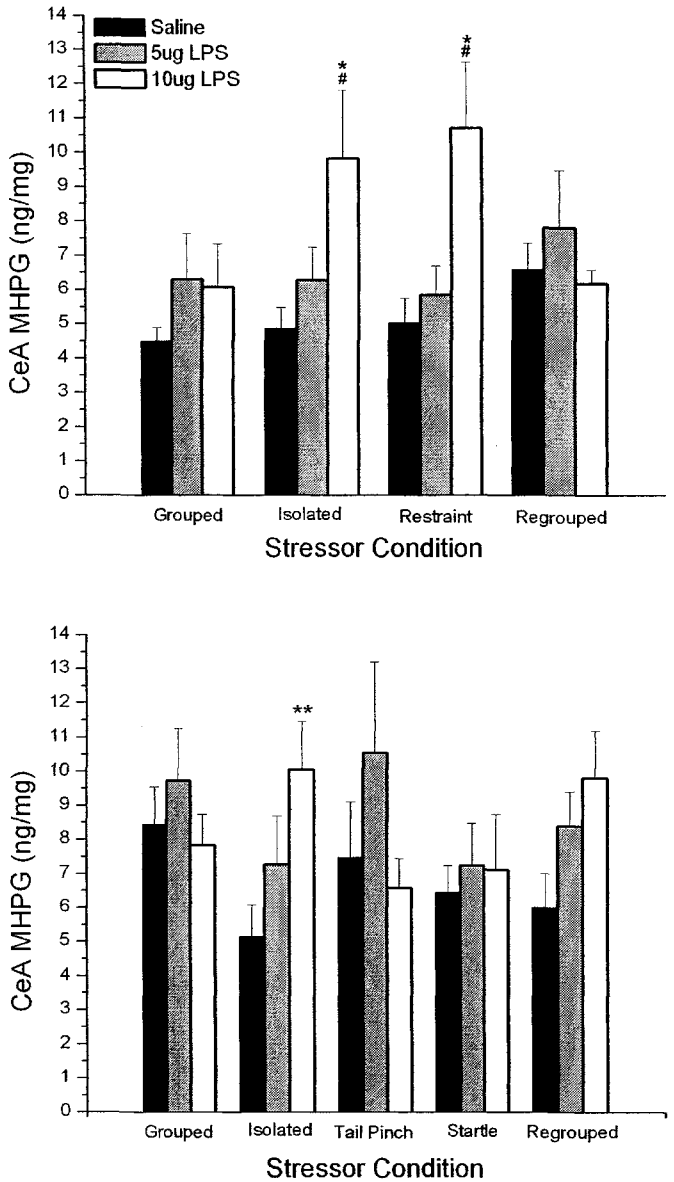

Figure 18. Mean (± SEM) MHPG within the central amygdala among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr (upper panel) and group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr (lower panel). Mice were then treated with saline or LPS (5 or 10ug). * $p < .01$ relative to similarly treated mice injected with saline ** $p < .05$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with 5ug of LPS.

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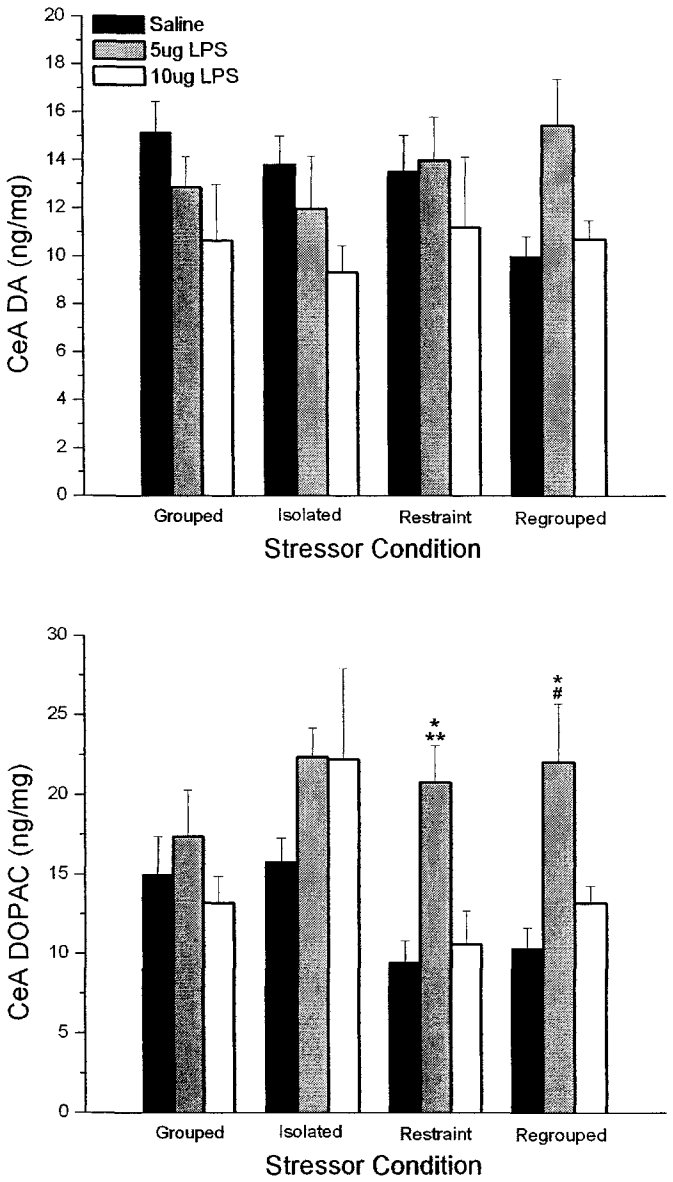

Figure 19. Mean (± SEM) DA (upper panel) and DOPAC (lower panel) among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr. Mice were then treated with saline or LPS (5 or 10ug). * $p < .01$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with 5ug of LPS ** $p < .05$ relative to similarly treated mice injected with 10ug of LPS

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The findings of Experiment 3b also revealed that LPS treatment did not affect DA levels, but influenced DOPAC accumulation, $F_{(2,92)} = 3.23$, $p < .05$. Once again, although the interaction with stressor type did not reach significance ($p = .12$), it is clear from Figure 20 that LPS markedly increased DOPAC in isolated mice, grouped housed mice, and mice exposed to tail pinch. In contrast, LPS did not affect DOPAC among mice exposed to a startle stimulus or those that experienced regrouping.

Insert Figure 20 about here

In addition to the NE and DA alterations in Experiment 3a, 5-HIAA varied as a function of Stressor condition, $F_{(3,82)} = 2.94$; $p < .05$, largely reflecting lower 5-HIAA levels in the restrained mice. It was also found that 5-HT varied as a function of the interaction between Stressor condition and LPS treatment, $F_{(6,84)} = 2.71$; $p < .05$. In particular, upon administration of 5μg of LPS, higher 5-HT levels were evident among mice that experienced restraint than among mice subjected to either regrouping, remaining isolated, or group-housing. No differences were found among animals that received saline or 10μg of LPS treatments. Furthermore, among animals that remained grouped or regrouped after a period of isolation, LPS (10 ug) had the effect of reducing 5-HT levels.

Within the hippocampus of Experiment 3a, MHPG levels were unaffected by the treatments, whereas NE levels varied as a function of LPS treatment, $F_{(2,83)} = 5.11$; $p < .01$ (see Figure 21, upper panels). Subsequent analyses revealed that both 5μg and 10μg elicited higher NE levels than that observed in saline-treated mice. In contrast to the drug

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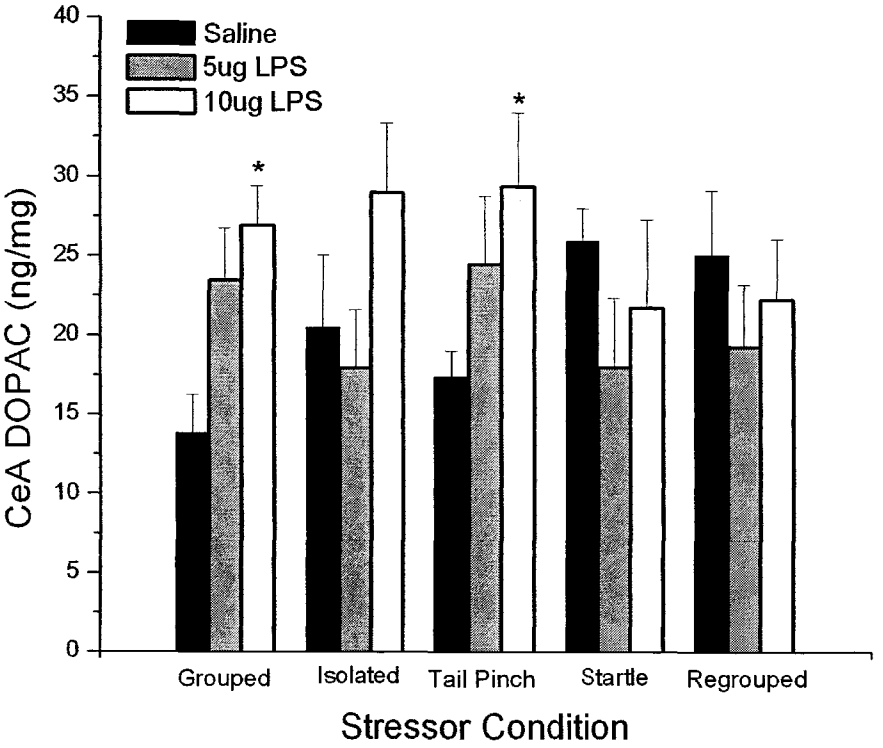

Figure 20. Mean (± SEM) DOPAC within the central amygdala among mice that had been group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr. Mice were then treated with saline or LPS (5 or 10ug). * $p < .05$ relative to similarly treated mice injected with saline

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effects in Experiment 3a, both MHPG and NE levels varied as a function of Stressor condition in Experiment 3b, $F_{(4,102)} = 2.89$ and $F_{(4, 100)} = 2.83$; $p < .05$, respectively (see Figure 21, lower panels). The multiple comparisons indicated that animals that received tail pinch had higher levels of MHPG and NE than group-housed controls. Furthermore, animals that received the 1-hr regrouping also had higher levels of NE than group-housed controls. Treatment with LPS, however, did not affect NE or MHPG levels.

Insert Figure 21 about here

In addition to the NE effects, 5-HIAA was significantly influenced by LPS treatment in both Experiment 3a and 3b, $F_{(2,83)} = 5.90$ and $F_{(2,95)} = 6.91$; $p < .01$, respectively. In Experiment 3a, 10μg of LPS elicited higher levels of 5-HIAA than saline, whereas the effects of 5μg were less pronounced, as shown in Figure 22 (upper panels). However, in Experiment 3b, 5μg of LPS elicited higher levels of 5-HIAA than 10μg of LPS or saline. Moreover, these effects were most pronounced among mice exposed to the stress of regrouping and those that were stressed by tail pinch. In addition to drug effects in Experiment 3b, 5-HIAA levels within the hippocampus also varied as a function of Stressor condition, $F_{(4,95)} = 4.22$; $p < .01$. As shown in Figure 22 (lower panels), and confirmed by Newman-Keuls, animals that received the 1-hr regrouping, tail pinch or remained grouped had higher 5-HIAA levels than animals that received startle. However, as already indicated, this main effect was primarily a reflection of the elevated metabolite levels associated with the LPS treatment in these stressor conditions. Finally, in Experiment 3a, 5-HT was significantly influenced by Stressor condition, $F_{(3,83)} = 4.06$; $p < .01$. Subsequent analyses indicated that group-housed animals had higher 5-HT

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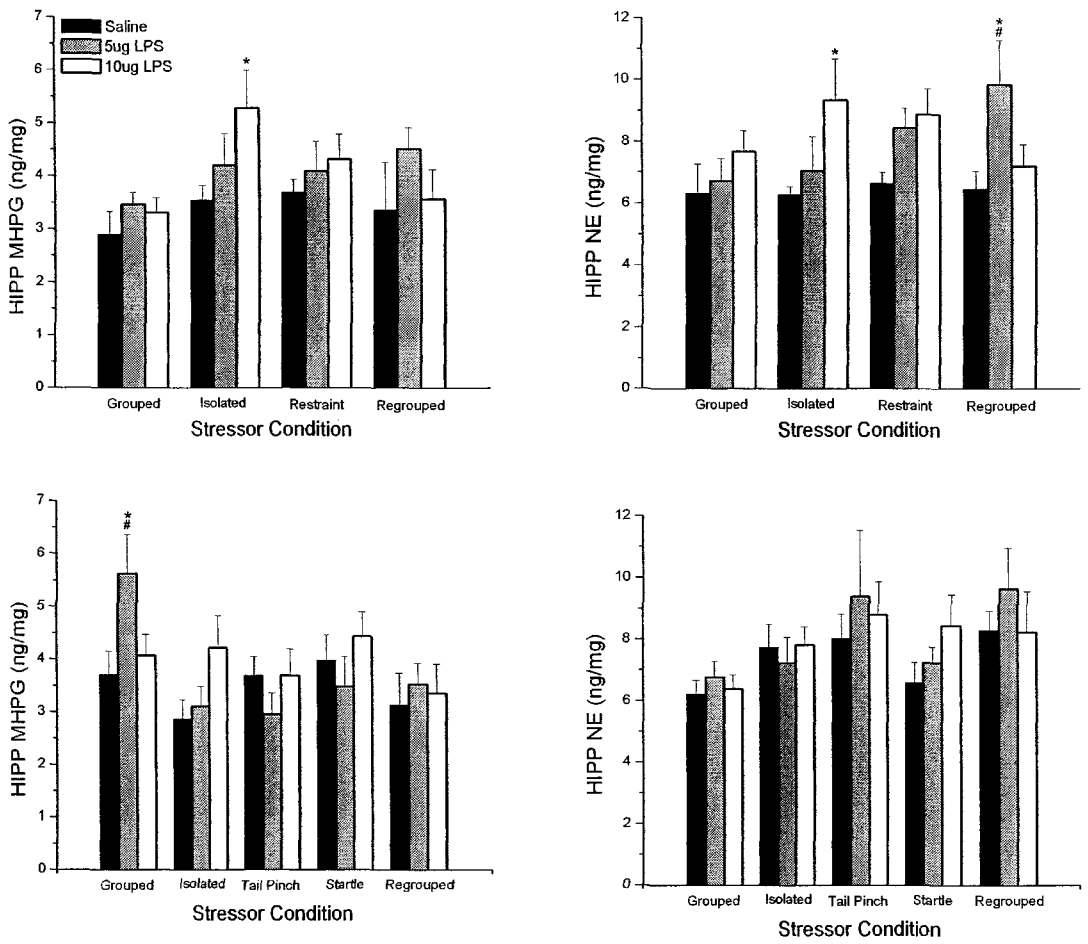

Figure 21. Mean (± SEM) MHPG and NE (left and right, respectively) within the hippocampus among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr (upper panels) and group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr (lower panels). Mice were then treated with saline or LPS (5 or 10ug). * $p < .05$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with 10ug of LPS

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accumulation than animals exposed to regrouping. No significant differences of 5-HT levels were evident in Experiment 3b.

Insert Figure 22 about here

3.4. Experiment 4: Physical or sensory contact combined with LPS challenge: behavioural and neurochemical effects

Sickness Behaviour: Total sickness scores varied as a function of Social stressor condition, $F_{(4,117)} = 3.77$; $p < .01$ and LPS treatment, $F_{(2,117)} = 69.80$; $p < .0001$. As seen in Figure 23, and confirmed by multiple comparisons, the social stress of the physical interaction induced greater sickness behaviour than remaining grouped or being exposed to the novel environment. In addition, both 10μg and 5μg of LPS provoked greater sickness scores than saline, although differences between the two doses were not observed. It is particularly interesting that although the interaction between the Stressor condition and the LPS treatment only approached significance, $F_{(8,117)} = 1.86$, $p = .07$, the LPS-elicited sickness was most profound in mice that experienced regrouping, and that the least pronounced was apparent among mice placed in the novel environment and those that were exposed to a conspecific, but physical contacted was prevented.

Insert Figure 23 about here

Plasma Corticosterone: As seen in Figure 24, circulating corticosterone levels were significantly influenced by the Social stressor condition, $F_{(4,115)} = 6.11$; $p < .001$, and LPS treatment, $F_{(2,115)} = 19.25$; $p < .0001$. Subsequent follow-up analyses revealed that

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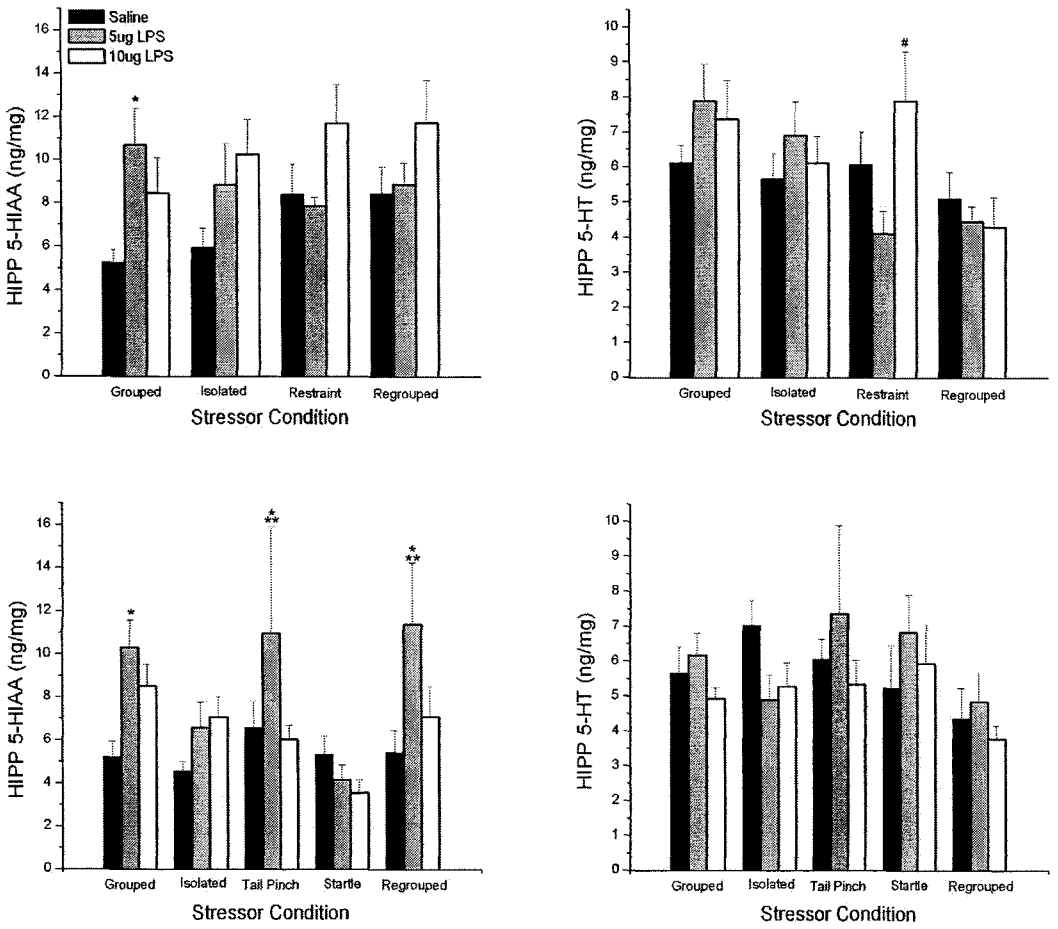

Figure 22. Mean (± SEM) 5-HIAA and 5-HT (left and right, respectively) within the hippocampus among mice that had been group housed, isolated for 14 days, restrained or isolated and regrouped for 1 hr (upper panels) and group housed, isolated for 14 days, exposed to tail pinch, startled or isolated and regrouped for 1 hr (lower panels). Mice were then treated with saline or LPS (5 or 10ug). * $p < .05$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with 5ug of LPS ** $p < .05$ relative to similarly treated mice injected with 10ug of LPS

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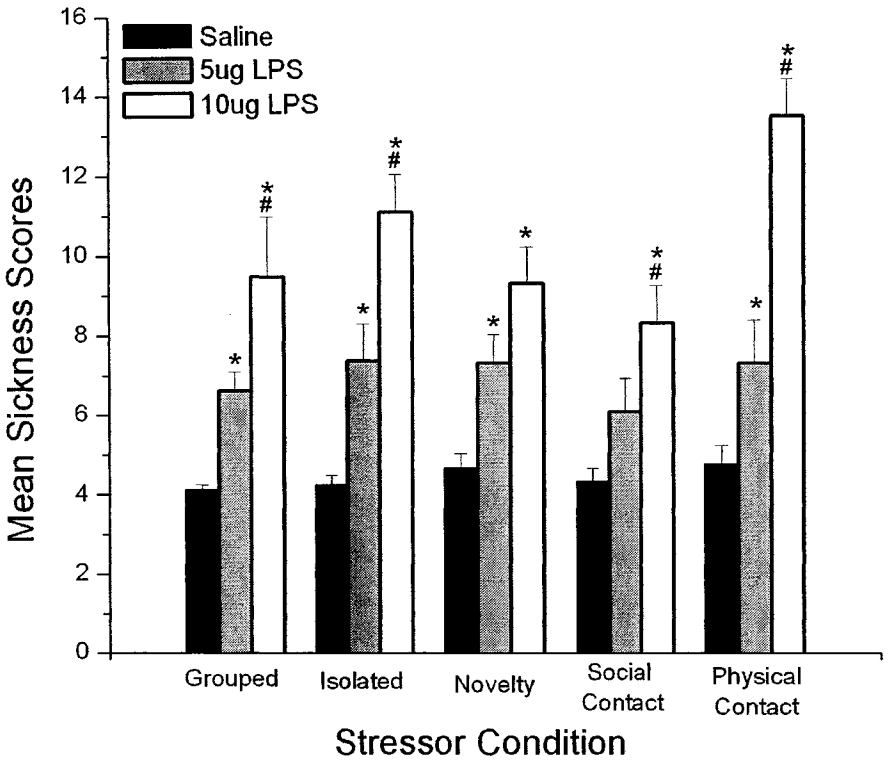

Figure 23. Mean sickness scores (±SEM) totaled over 4 ratings among mice that were group housed, isolated for 14 days, isolated and exposed to novelty, isolated and regrouped for 1 hr without physical contact, or isolated and regrouped for 1 hr permitting physical contact. Mice were then treated with saline or LPS (5 or 10ug). * $p < .01$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with saline

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1-hr regrouping permitting physical interaction induced greater corticosterone levels than either 1-hr regrouping without physical contact, exposure to the novel environment, remaining isolated or group-housed. In addition, the drug treatment was significant across all levels, with 10μg of LPS eliciting the highest corticosterone levels, followed by 5μg of LPS. As simply regrouping mice induced a marked increase of corticosterone, beyond that seen when contact was prevented, it is difficult to determine whether LPS differentially influenced corticosterone levels in these two groups. Certainly, corticosterone levels were higher among LPS animals that were regrouped than in those introduced to other mice without contact being possible, but this was largely a reflection of the stressor rather than the LPS treatment.

Insert Figure 24 about here

Monoamine Variations: Within the PVN, 5-HIAA was significantly influenced by LPS treatment, $F_{(2, 113)} = 3.39$; $p < .05$. Follow-up analyses revealed that 5μg of LPS elicited higher 5-HIAA levels than either saline or 10μg of LPS. MHPG, NE, DOPAC, 5-HIAA or 5-HT levels did not vary among treatment groups.

Within the locus coeruleus, MHPG accumulation was influenced by the Stressor condition, $F_{(4, 113)} = 4.43$; $p < .01$, and LPS treatment, $F_{(2, 113)} = 3.13$; $p < .05$. As shown in Figure 25 (upper panel), and confirmed by multiple comparisons, regrouped animals permitted physical contact demonstrated higher levels of MHPG than regrouped animals not permitted physical contact, and animals that remained isolated and undisturbed. Moreover, animals that remained grouped demonstrated higher MHPG levels than animals that were regrouped but not permitted physical interaction. Furthermore, both 5

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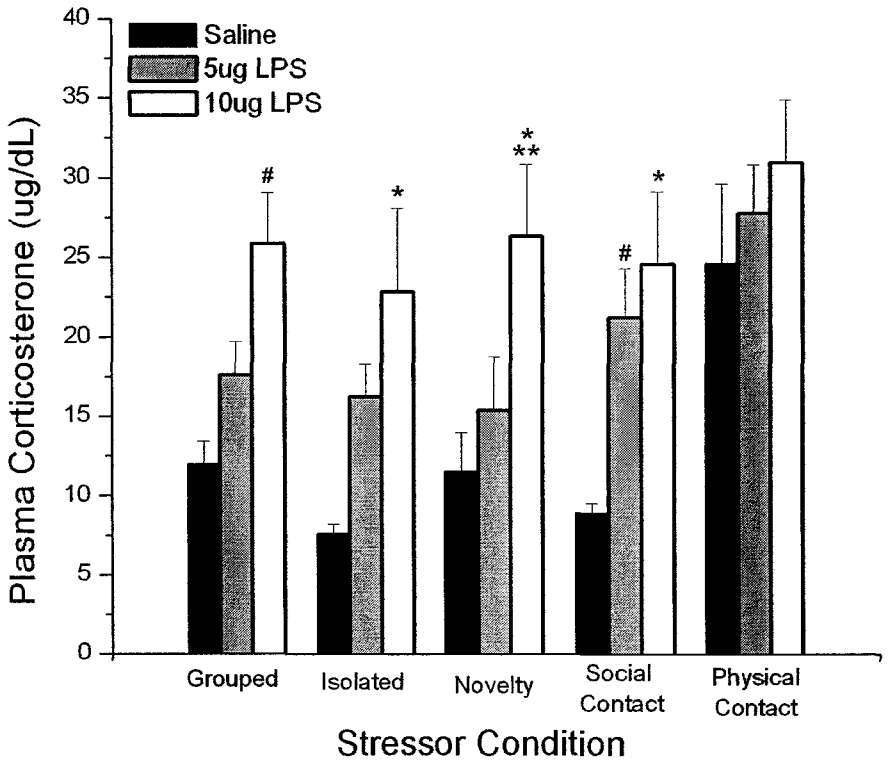

Figure 24. Mean plasma corticosterone (±SEM) among mice that were group housed, isolated for 14 days, isolated and exposed to novelty, isolated and regrouped for 1 hr without physical contact, or isolated and regrouped for 1 hr permitting physical contact. Mice were then treated with saline or LPS (5 or 10ug). * $p < .01$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with saline ** $p < .05$ relative to similarly treated mice injected with 5ug of LPS

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and 10μg of LPS elicited higher MHPG levels than saline. Levels of NE within the locus coeruleus also varied as a function of LPS treatment, $F_{(2, 113)} = 4.32$; $p < .05$. In particular, 10μg of LPS elicited significantly higher NE levels than did a lower dose of LPS or saline, as depicted in Figure 25 (lower panel).

Insert Figure 25 about here

Within the PFC, 5-HIAA varied as a function of Stressor condition, $F_{(4, 114)} = 4.99$; $p < .001$. Follow-up analyses revealed that animals permitted physical contact demonstrated significantly higher levels of 5-HIAA than animals restricted to social contact, and those exposed to the novelty of the partition. Furthermore, animals that remained isolated had higher levels of 5-HIAA than animals exposed to the novel environment. Aside from 5-HIAA alterations, levels of MHPG, NE, DA, DOPAC, and 5-HT in the PFC did not vary among treatment groups.

Within the central amygdala, NE levels were not altered by either the Stressor condition or LPS treatment. In contrast, MHPG levels varied as a function of LPS treatment, $F_{(2,115)} = 3.94$; $p < .05$. Follow-up analyses revealed that 10μg of LPS elicited higher levels of MHPG than saline, whereas the effects of the 5μg dose did not reach significance. It is interesting, as seen in Figure 26, that although the interaction between the stressor and the LPS treatment was not significant, LPS actually had no effect among animals that were simply exposed to the novel environment.

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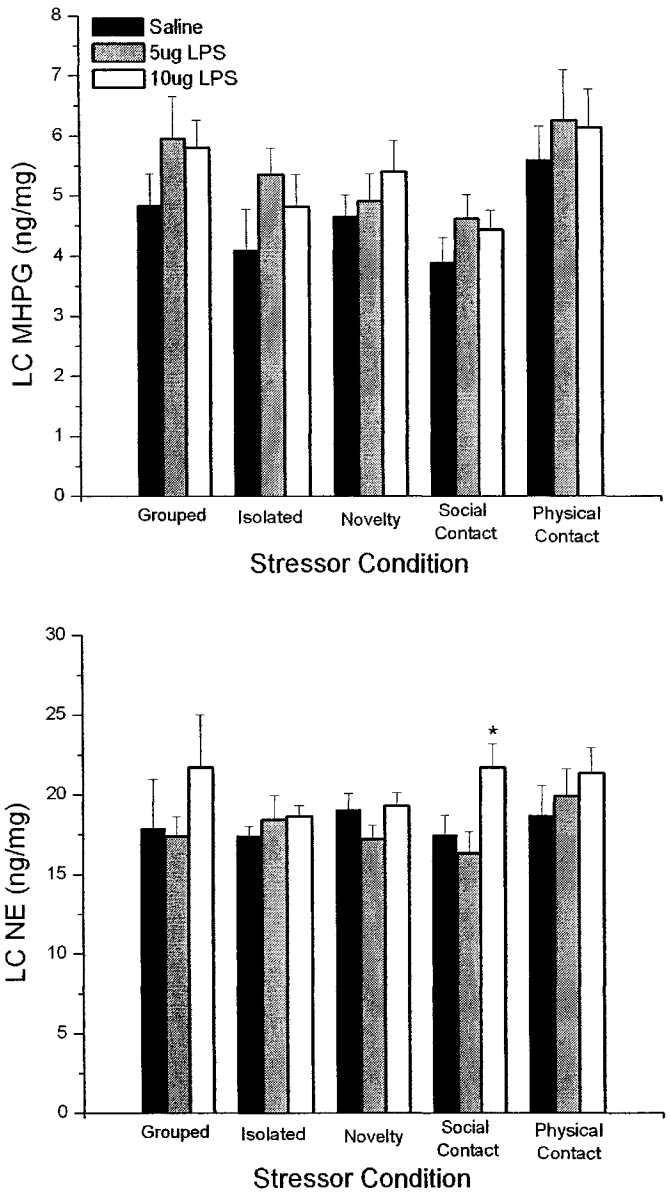

Figure 25. Mean (±SEM) MHPG (upper panel) and NE (lower panel) within the locus coeruleus among mice that were group housed, isolated for 14 days, isolated and exposed to novelty, isolated and regrouped for 1 hr without physical contact, or isolated and regrouped for 1 hr permitting physical contact. Mice were then treated with saline or LPS (5 or 10ug). * $p < .05$ relative to similarly treated mice injected with 5ug of LPS

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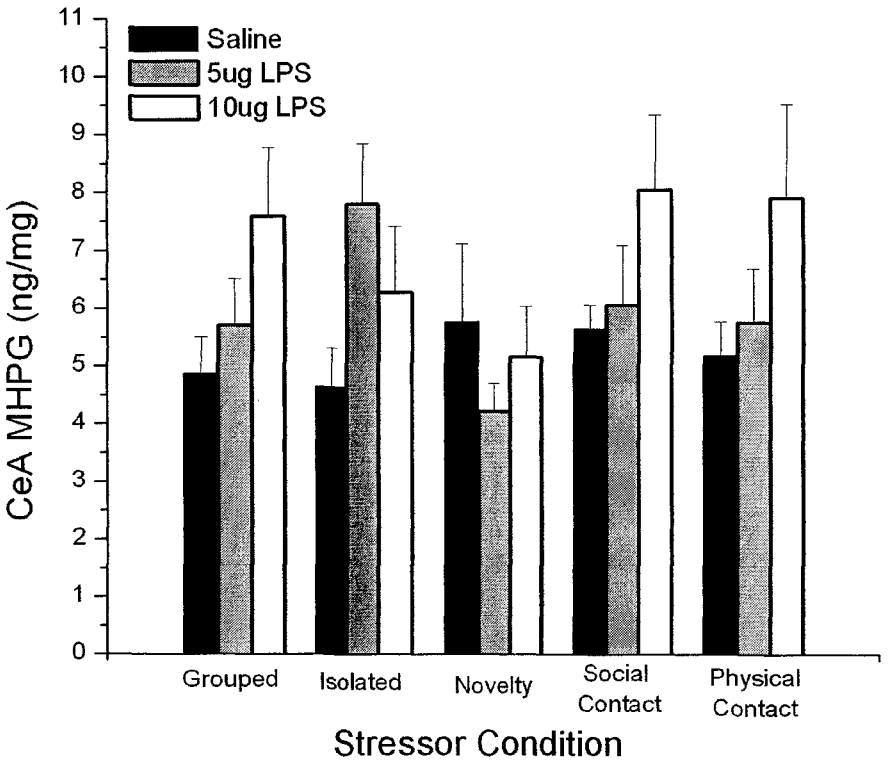

Figure 26. Mean (±SEM) MHPG within the central amygdala among mice that were group housed, isolated for 14 days, isolated and exposed to novelty, isolated and regrouped for 1 hr without physical contact, or isolated and regrouped for 1 hr permitting physical contact. Mice were then treated with saline or LPS (5 or 10ug).

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The levels of DA within the central amygdala were affected by the Stressor condition, $F_{(4, 115)} = 3.08$; $p < .05$ (see Figure 26, upper panel). The follow-up comparisons indicated that regrouped animals permitted physical contact had significantly higher DA levels than all other stressor conditions, namely regrouping without physical interaction, exposure to novelty, and remaining undisturbed in an isolated or group-housed setting. In contrast, levels of DOPAC varied as a function of LPS treatment, $F_{(2, 114)} = 3.10$; $p < .05$. Specifically, 5μg of LPS elicited higher DOPAC levels than 10μg of LPS (see Figure 26, lower panel). It ought to be noted that the effects of LPS on DOPAC were modest and were actually inconsistent across the stressor conditions. Insufficient power likely precluded detection of a significant interaction. Unlike NE and DA alterations, levels of 5-HT or its metabolite, 5-HIAA, were not influenced within the central amygdala.

Insert Figure 27 about here

Finally, within the hippocampus, MHPG levels were influenced by the LPS treatment, $F_{(2,117)} = 3.23$; $p < .05$. As shown in Figure 28, 10μg of LPS elicited higher MHPG levels than did saline. As in other brain regions, the grouping condition itself appeared to influence levels of MHPG, but the small N, and the resulting low power, did not permit detection of relevant interactions. No differences of NE, 5-HIAA, or 5-HT were observed within the hippocampus.

Insert Figure 28 about here

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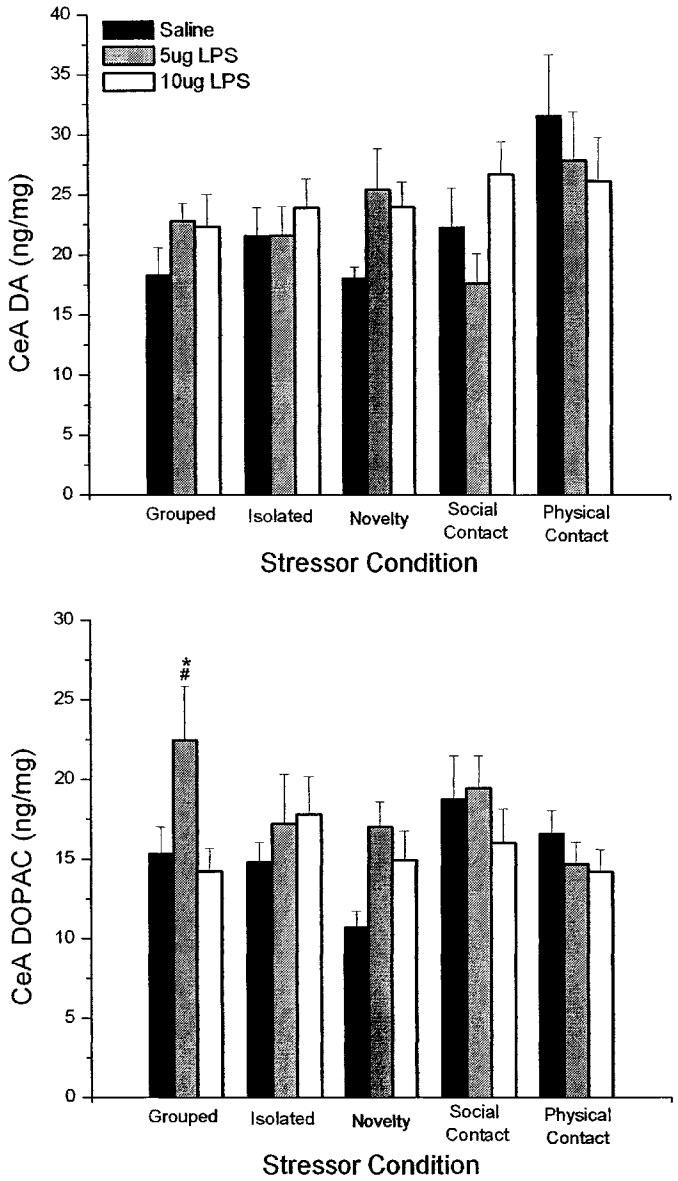

Figure 27. Mean (±SEM) DA (upper panel) and DOPAC (lower panel) within the central amygdala among mice that were group housed, isolated for 14 days, isolated and exposed to novelty, isolated and regrouped for 1 hr without physical contact, or isolated and regrouped for 1 hr permitting physical contact. Mice were then treated with saline or LPS (5 or 10ug). * $p < .05$ relative to similarly treated mice injected with saline # $p < .05$ relative to similarly treated mice injected with 10ug of LPS

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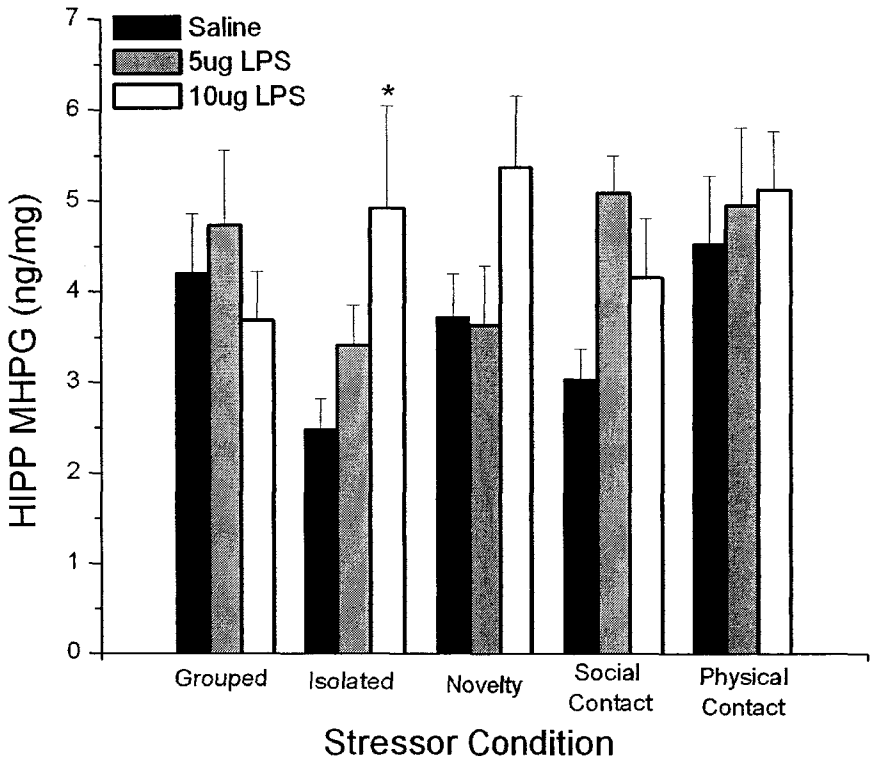

Figure 28. Mean (±SEM) MHPG within the hippocampus among mice that were group housed, isolated for 14 days, isolated and exposed to novelty, isolated and regrouped for 1 hr without physical contact, or isolated and regrouped for 1 hr permitting physical contact. Mice were then treated with saline or LPS (5 or 10ug). * $p < .05$ relative to similarly treated mice injected with saline

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4. Discussion

Activation of the inflammatory immune response, either through the administration of exogenous cytokines or LPS, produces several behavioural and neurochemical responses reminiscent of those elicited by traditional stressors. In this regard, it has been suggested that when applied concurrently, immune activation and social stressors may have additive or synergistic effects, which, if prolonged, can favour the evolution of affective disorders (Anisman et al., 2005). Supporting this contention, it was demonstrated in the present investigation that a social stressor (namely regrouping mice following a prolonged period of isolation) provoked enhanced behavioural, neurochemical and immune system responses when combined with LPS-induced immune activation. These combined effects were unique to social stressors, not being evident when the challenge involved other stressors, namely restraint, tail pinch or startle. It was also demonstrated that a relatively prolonged period of isolation (7 or 14 days) prior to the regrouping session was necessary to produce these effects, indicating that they were not simply the result of a change in housing condition. Finally, it appeared that physical contact between animals was necessary to produce the observed additive and synergistic effects, as simply regrouping animals without permitting physical contact and exposing mice to a novel environment did not produce effects beyond those of the individual and group-housed controls. In effect, the present findings suggest that endotoxin-induced immune activation and social stressors have interactive effects, which could have protracted consequences on subsequent psychological and physiological well-being.

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4.1 Behavioural Effects of Social Stress and LPS-induced Immune Activation

The constellation of physiological symptoms and behaviours that characterize an individual's response to inflammation (sickness behaviour), comprise non-specific symptoms including general malaise, decreased activity and social investigation, anorexia, and fever (Kent et al., 1992). Sickness behaviour was once described as a passive response reflecting the debilitating effects of an illness; however, it has more recently been characterized as an adaptive process geared towards recuperation (Aubert, 1999, Dantzer et al., 2001). Most changes that occur during immune activation function to either minimize heat loss (i.e. the curled body position), decrease energy expenditure (i.e. lethargy and reduced exploratory and social activity) or to produce the energy required to mount a febrile response (i.e. shivering; Maier, & Watkins, 1998). High core body temperatures stimulate the proliferation of immune cells necessary to combat the invasion, and concurrently slow the growth and proliferation of pathogens, making the febrile response essential to recuperation (Dantzer, 2001). These behaviours are therefore considered an organized strategy that is critical to the survival of the organism (Hart, 1988).

LPS has long been known to elicit sickness behaviour in otherwise healthy animals (Dantzer, 2001; Dantzer, et al., 1998; Deak, Bellamy, & Bordner, 2005; Hayley et al., 2001; Lacosta, et al., 1999; Linthorst, & Reul, 1998; Yirmiya, 1996). For instance, peripheral administration of LPS has been associated with decreased social interaction with a juvenile conspecific, reduced locomotor and exploratory activities, the induction of soporific effects, and increased anxiety-like behaviour (Dantzer, et al., 1998; Deak et al., 2005; Lacosta et al., 1999; Yirmiya, 1996). In line with this research, the present

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investigation demonstrated that LPS challenge engendered a sickness response. Moreover, it was the case that animals that received both a social stressor (1-hr of grouping) and various doses of endotoxic challenge (1μg, 5μg and 10μg of LPS) demonstrated increased sickness behaviour compared to animals that received only saline or a stressor of a different nature, such as restraint, startle or tail pinch. It has been reported that cytokines mediate the sickness behaviour observed following the administration of a bacterial endotoxin (Konsman, Parnet, & Dantzer, 2002; Larson, & Dunn, 2001). When LPS is systemically administered to an animal, the local immune cells expressing a particular type of receptor, namely Toll-like receptor-4 (TLR-4), are activated and release IL-1, which further potentiates release of this cytokine, along with a cascade of other cytokines such as IL-6 and TNF-α (Konsman, et al., 2002; Laflamme, & Rivest, 2001; Rivest, 2003). These peripheral cytokines are integral to the initiation of sickness behaviour through their influence in the CNS. For instance, LPS-induced behavioural alterations can be attenuated with the central administration of anti-inflammatory cytokines, such as IL-10 (Bluthe, Castanon, Pousset, Bristow, Ball, Lestage, Michaud, et al., 1999), as well as the soluble receptor antagonist, IL-1ra (Bluthe, Dantzer, & Kelley, 1992)

As previously mentioned, cytokines are relatively large molecules that do not readily penetrate the BBB, therefore the precise mechanisms by which peripheral cytokines mediate central processes have yet to be fully determined. Nevertheless, it has been shown that, at least two distinct communication pathways link the peripheral inflammatory response to CNS processing: a quick neural pathway and a slower humoral pathway (Konsman et al., 2002). The former comprises a rapid wave that begins in the

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peritoneum where local immune cells secrete proinflammatory cytokines, the most important being IL-1β, which activate vagal afferent fibers, originating in the abdominal cavity (Bluthe, Walter, Parnet, Laye, Lestage, Verrier, et al., 1994; Kelley, Bluthe, Dantzer, Zhou, Shen, Johnson, et al., 2003; Konsman, et al., 2002; Raison et al., 2006). Indeed, it has been demonstrated that systemically administered LPS induced sickness behaviors in sham-lesioned animals but not in vagotomized animals, emphasizing the role of the vagus nerve in the induction of sickness behaviour (Bluthe, et al., 1994; Wieczorek, Swiergiel, Pournajafi-Nazarloo, & Dunn, 2005). Once the cytokine signals reach the CNS, the nucleus of the solitary tract (located in the brainstem) serves as a hub, relaying signals to other brain regions important in inducing the behavioural effects that accompany the inflammatory immune activation. In response to LPS, IL-1 release in the brain has been most clearly demonstrated in the hypothalamus and hippocampus (Deak et al., 2005; Nguyen, Deak, Owens, Kohno, Fleshner, Watkins, & Maier, 1998). By studying early gene expression using c-fos, Wan et al. (1993) elucidated the vagal mediated pathway. Following the activation of the vagus nerve and nucleus solitary tract, the secondary projections of the vagus nerve are the parabrachial nucleus, PVN and supraoptic nuclei of the hypothalamus, the central amygdala, and the bed nucleus of the stria terminalis, as vagotomy blocked the c-fos expression in each of these brain regions (Wan, Janz, Vriend, Sorensen, Greenberg, & Nance, 1993; Wan, Wetmore, Sorensen, Greenberg, & Nance, 1994).

The second communication pathway, the humoral response, is a much slower process, whereby peripheral cytokines gain access to the CNS via the circumventricular organs (CVOs) and choroids plexus, and slowly diffuse by volume transmission into the

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brain parenchyma and subsequently to other brain targets such as the amygdaloid complex (Dantzer, 2001; Konsman et al., 2002). Furthermore, the humoral pathway involves a series of second messenger molecules, including the prostaglandins, which diffuse into the brain and act on EP_3 and EP_4 receptors to innervate the brain regions involved in HPA activation and body temperature control, such as the hypothalamic nuclei (Konsman et al., 2002). Irrespective of the mechanisms involved in their initiation, sickness behaviour has been proven an essential component in the inflammatory immune system response.

Although stressors and cytokines both induce behaviors that indicative of general discomfort that are usually considered distinctive, social stressors can provoke behavioural changes in animals reminiscent of those seen in response to endotoxic challenge. For instance, social stressors were found to elicit decreased grooming, decreased exploratory and social activities, anorexia, as well as increased anxiety-like behaviour (Bartolomucci, 2005; Kudryavtseva, et al., 1991; Martinez et al., 1998; Rygula, Abumaria, Flügge, Fuchs, Rüther, & Havemann-Reinecke, 2005). Similarly, in the present study, social stressors provoked mild signs of malaise that were increased appreciably when applied in combination with LPS administration. Inasmuch as these additive effects were not observed in animals exposed to other psychogenic and neurogenic stressors such as restraint, startle and tail pinch, it seems that social stressors may be influencing different neural substrates than other commonly used laboratory stressor paradigms.

Although the cause of the behavioural effects provoked by social stressors remain to be elucidated, it is possible that they may be induced by mechanisms similar to those

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involved in cytokine-induced sickness. For instance, stressors have been found to increase peripheral release of cytokines (O'Connor, Johnson, Hansen, Wieseler Frank, Maksimova, Watkins, & Maier, 2003). Moreover, paralleling the changes observed in the CNS following endotoxin administration, acute stressor exposure may increase the expression of IL-1β mRNA in the hypothalamus, hippocampus, and pituitary (O'Connor, et al., 2003). Thus, the combined neuronal actions of the stressors and LPS-induced variations of cytokine activity may result in the augmented sickness. However, the fact that social stressors uniquely provoked the augmented effect of LPS, and yet a variety of stressors may influence circulating cytokine levels (Nguyen, Deak, Will, Hansen, Hunsaker, Fleshner, et al., 2000; O'Connor, et al., 2000), suggests that factors beyond additive cytokine effects are responsible for the combined effects of the treatments.

In addition to inhibiting the behavioural and physiological effects of LPS, centrally administered IL-1ra also inhibits the behavioural effects associated with inescapable tail-shock, such as enhanced fear conditioning and learned helplessness (Maier, & Watkins, 1995). Although less research has focused exclusively on social stressors, Bartolomucci et al. (2003a) studied the effects of chronic social stress on central expression of cytokines. Contrary to the reports concerning acute physical stressors, chronic psychosocial stressor exposure induced a decrease of IL-1β in the hippocampus and a decrease of TNF-α in the hippocampus and striatum. However, they also reported a decrease of IL-1ra in the striatum and pituitary. Together, these findings suggest similar central mechanisms involved in provoking social stressor and LPS-induced sickness behaviour. Activating these mechanisms simultaneously could be responsible for the additive behavioural effects observed in the present study.

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It has been argued that the symptoms associated with sickness behaviour in animal are reminiscent of those seen in human depression (Bartolomucci, 2005). Although there certainly are profound differences between the behavioural alterations observed in animals administered exogenous cytokines, or a bacterial endotoxin, and the symptoms observed in a depressive episode provoked by immunotherapy (for a review, see Dunn et al., 2005b), it has been suggested that immune activation will have greater effects when superimposed on a social stressor backdrop, which can favour the evolution of depressive-like behaviour (Anisman & Merali, 2003). The findings of the present investigation showing the combined actions of stressors and LPS are certainly in line with this proposition.

4.2 Neuroendocrine Alterations Induced by the Combined Actions of LPS and Social Stress

LPS is a potent activator of the HPA axis, markedly increasing CRH release from the median eminence, and hence increasing circulation of ACTH and glucocorticoids (Carobrez, et al., 2002; Deak et al., 2005; Hayley et al., 2001; Lacosta et al., 1999; Quan et al., 2001; Reichenberg, et al., 2001;Yirmiya, 1996). In line with current research, the present investigation demonstrated a dose-dependent increase of plasma corticosterone following LPS challenge, an effect that was greatly enhanced with the combination of a social stressor. It will be recalled that peripherally administered LPS provokes the release of pro-inflammatory cytokines from local immune cells, which proliferate and infiltrate the CNS through a fast vagal-mediated pathway and a slower humoral pathway (Konsman et al., 2002). Once in the CNS, these cytokines (either directly or through second messenger systems) activate stressor sensitive brain regions. Contrary to

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processive stressors that require higher-order processing, systemic stressors such as LPS-induced immune activation bypass the limbic system and directly innervate the PVN, initiating the HPA activity (Herman, & Cullinan, 1997).

Psychosocial stressors are also known to prompt neuroendocrinological changes, and particularly hypersecretion of glucocorticoids. Studies using social stressor paradigms, such as social disruption, social reorganization or social defeat, have all consistently reported increased secretion of glucocorticoids (Bailey, Avitsur, Engler, Padgett, & Sheridan, 2004; Bartolomucci et al., 2001; Carobrez et al., 2002; Merlot, Moze, Dantzer, & Neveu, 2004; Pardon, Kendall, Perez-Diaz, Duxon, & Marsden, 2004; Sheridan et al., 2000). Interestingly, although these paradigms typically deal with interactions between different ranks of animals, no significant corticosterone differences were found between dominant and submissive animals, indicating that it is the nature of the situation that is influencing the neuroendocrine changes, rather than the subjective assessment of that situation (Bartolomucci et al., 2001, Bartolomucci et al., 2005). In line with these findings, in the present investigation, increased corticosterone secretion was elicited by exposure to a social stressor comprising 1 hr regrouping after a prolonged period of isolation. Furthermore, it appeared that a longer period of isolation (7 or 14 days) was optimal before the regrouping session in order to produce this effect. When comparing the effects of a social stressor to other psychogenic and neurogenic stressors, non-social stressors such as restraint, tail pinch and startle failed to produce corticosterone levels that were beyond those of the group-housed and isolated controls. Finally, exposure to a novel environment and 1-hr regrouping without physical contact failed to elicit elevated corticosterone levels in comparison to the physical interaction of

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1-hr the regrouping session. Taken together, these findings suggest that social stressors provoke marked neuroendocrine activity, which is greatly enhanced with the application of an endotoxin challenge.

The HPA alterations provoked by acute and chronic social stressors have been attributed, in part, to a dampened functioning of the negative HPA feedback loop. Once the HPA axis is activated and glucocorticoids are secreted, they bind to GC receptors on the hippocampus and PVN, which inhibit the further release of CRH, deactivating the HPA axis. The functioning of the negative feedback loop is typically investigated using the dexamethasone suppression test (DST), which assesses the ability of the CNS to inhibit the HPA axis. Using the DST, it was found that social stress impairs the ability of the CNS to terminate the HPA response (Bartolomucci, Pederzani, Sacerdote, Panerai, Parmigiani, & Palanza, 2004; Bartolomucci, Palanza, Sacerdote, Panerai, Sgoifo, Dantzer et al., 2005). Furthermore, it was found that social stressors downregulate GC receptors in the hippocampus, which could be responsible for the dysregulation of HPA functioning, and hypersecretion of glucocorticoids (Bartolomucci, Palanza, Parmigiani, Pederzani, Merlot, & Neveu, 2003a). Taken together, it appears that social stressors impair the proper functioning of the HPA axis, which might be responsible for the elevated corticosterone levels observed in mice following the 1-hr regrouping.

Interestingly, isolated control animals demonstrated somewhat lower corticosterone levels compared to group-housed controls. Mice are considered extremely social animals, and it is believed that social isolation is relatively stressful (Bartolomucci, 2001; Kim & Kirkpatrick, 1996; Weiss, et al., 2004). Furthermore, it has been reported (Chida et al., 2005) that eliminating social interaction between mice can induce various

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pathophysiological changes, such as altering the response of the HPA axis and accelerating autoimmune disease progression as well as increasing the growth of transplanted tumors (Liu, & Wang, 2005; Wu, Yamaura, Murakami, Murata, Matsumoto, Watanabe, et al., 2000). It appears that although prolonged isolation does not affect basal cort levels and immune functioning, it does provoke higher levels of emotional reactivity and a hyperactive HPA response to subsequent psychosocial stressors (Bartolomucci et al., 2003b; Weiss et al., 2004). It is possible that the prolonged isolation in the present study primed neural circuits so that the subsequent regrouping elicited larger corticosterone effects than would have been observed otherwise.

Reports concerning the impact of social stressors have made the distinction between physical attack and the threat of attack (for a review see Martinez et al., 1998). Also called sensory contact, animals under the threat of attack, in the absence of physical encounters, demonstrate the emotional effects of the attack by having olfactory, visual and auditory contact with conspecifics. Studies using physical and sensory contact typically show elevated corticosterone levels in response to physical contact in comparison to just having sensory contact (Bailey, et al., 2004; Merlot et al., 2004; Pardon, et al., 2005; Stefanski, 2000). For instance, animals permitted to fight had higher corticosterone levels and higher circulating interleukin-6 levels compared to animals separated by a mesh (Merlot et al. 2004). This raises the question of whether the observed neuroendocrine alterations in the current investigation were a direct result of the psychosocial effects of regrouping, physical contact, or the wounds inflicted by fighting.

To ascertain the influence of injuries on stress responses, Stefanski (2000) compared the behavioural and neuroendocrinological differences between bitten and non-

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bitten male rats after a period of physical aggression. Bitten males demonstrated an increase of plasma corticosterone levels, passive behaviours, and a decrease in testosterone and body weight, all indicative of higher stress levels. These findings raise the possibility that the higher corticosterone levels seen in the physical contact group were due to the injuries sustained during the regrouping period, as opposed to the stress of physical contact. Subsequent studies, however, found conflicting results. For instance, it was reported that wounded and non-wounded animals did not differ with respect to their corticosterone levels following social defeat (Merlot et al., 2004; Sheridan et al., 2000). However, the role of injuries in the development of GC resistance is uncertain, as contradictory results have been reported. At this point, the possibility ought to be considered that the psychological stress of the physical interaction is responsible for the elevated corticosterone levels, as opposed to the injuries themselves; however, firm conclusions are unwarranted at this time.

It will be recalled that social stressors, but not physical stressors, can decrease the sensitivity of immune cells to the anti-inflammatory effects of corticosterone (known as functional GC resistance), leading to enhanced production of proinflammatory cytokines, and subsequently to immunopathology (Engler et al., 2005; Merlot et al., 2004; Sheridan et al., 2000; Sheridan et al., 2000; Stark et al., 2001). As in the case of the corticosterone differences between wounded and non-wounded animals, there is debate over the role of injury in the provocation of GC resistance (Merlot et al., 2004; Sheridan et al., 2000; Stark et al., 2001). For instance, Merlot et al. (2003, 2004) reported that a social stressor induced GC resistance, regardless of the presence of injury, concluding that it was the psychological distress associated with the physical interaction that provoked the decrease

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in sensitivity. In contrast, Sheridan et al., (2000), using a similar SDR paradigm, reported that wounding was a necessary component to the development of GC resistance. It was proposed that social, but not physical stressors, elicit the release of nerve growth factor (NGF), which could be responsible for reducing the sensitivity of immune cells to corticosterone. During physical attacks, corticosterone levels are dramatically increased, which would normally be immuno-suppressant. However, if wounding occurs, a compromised immune system could be hazardous, making GC resistance vital for survival and recovery. As wounding induces high levels of NGF, Sheridan et al (2000) speculated that wounding was necessary for the development of functional GC resistance through processes related to NGF.

The findings that social stressors reduce the sensitivity of immune cells to the anti-inflammatory effects of corticosterone, which could explain the additive effects of social stress and LPS-induced immune activation demonstrated in the present study. In particular, social stressors activate the HPA axis, leading to increased secretion of corticosterone, which ultimately might result in the impairment of the negative feedback loop. The resulting hypersecretion of corticosterone, among other factors, can then provoke a decrease in sensitivity of immune cells to corticosterone, leading to elevated levels of pro-inflammatory cytokines. When LPS is administered, it provokes the release of additional cytokines, instigating HPA activation and further corticosterone elevations. This hypothesis is supported by findings that social stressors prime neurological circuits so that subsequent LPS administration induces enhanced effects and increased mortality rates (Carobrez et al., 2002; Quan et al., 2001). As secretion of glucocorticoids normally provide defense against endotoxic shock by inhibiting the production and action of

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cytokines, these findings suggest that social stressors can cause functional GC resistance, resulting in the failure to inhibit the production of proinflammatory cytokines, rendering animals susceptible to endotoxic shock upon LPS administration (Quan et al., 2001). Inasmuch as social stressors and LPS administration elicit their own independent neuroendocrine alterations, their simultaneous application may provoke cross-sensitization effects leading to an additive effect on corticosterone secretion.

The view has been offered that depression can result from the dysregulation of HPA functioning provoked by stressors and immune challenges, leading to glucocorticoid hypersecretion, which then leads to disruptions of monoaminergic neurotransmission and immune system reactivity (Anisman, et al., 2005). In line with this view, the present investigation demonstrated that social stressors and LPS-induced immune activation have additive neuroendocrine effects. It is conceivable that such effects, if maintained, could provoke behavioural pathologies such as depression.

4.3 Catecholamine Variations Provoked by the Combined Actions of Social Stressors and Endotoxin Exposure

Processive and systemic stressors are known to provoke a cascade of neurotransmitter alterations, including monoamine release at several hypothalamic and extrahypothalamic sites, including the PVN, median eminence, locus coeruleus, PFC, central amygdala, hippocampus, and nucleus accumbens, (Anisman & Merali, 1999). Although systemic stressors, such as immune activation, are thought to have their effects independent of the limbic system, directly innervating the PVN (Herman, & Cullinan, 1997), it is evident that they effectively induce widespread neurochemical alterations.

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For instance, both *in vivo* and post-mortem analyses indicated that LPS increased the release of NE, DA and 5-HT at several hypothalamic and extra-hypothalamic sites (Borowski, et al., 1998; Dunn, 2002, Lacosta, et al.,1999; Lavicky, & Dunn, 1995; Linthorst, Flachskamm, Müller-Preuss, Holsboer, & Reul, 1995; Linthorst, & Reul, 1998; Wieczorek et al., 2005). The present results are consistent with these findings, in that LPS elicited a dose-dependant increase of NE turnover (reflected by elevated levels of MHPG accumulation) within the PVN, locus coeruleus, PFC, central amygdala, and hippocampus. Furthermore, LPS dose-dependently increased DA turnover (elevated DOPAC accumulation) within the PFC and central amygdala. In contrast in the central amygdala, LPS reduced DA turnover. Finally, LPS treatment increased 5-HIAA accumulation within the PVN and hippocampus, and reduced 5-HT accumulation within the central amygdala and hippocampus. Taken together, these findings indicate that LPS increased monoamine neuronal activity in stressor-sensitive brain regions. It will be recognized that many of these changes (notably disturbances of NE and 5-HT neurotransmission in brain regions such as the hypothalamus, hippocampus, amygdala and PFC) have also been implicated in major depression (Schiepers, et al., 2005). However, whereas depression is typically thought to evolve relatively slowly, or in response to a chronic stressor or major trauma, the LPS treatment in the present investigation was acutely administered, and it is essential to assess the impact of chronic immune activation on these neurochemical processes.

The mechanisms by which LPS produces alterations of catecholamine neurotransmission have not been determined (Hayley et al., 2001; MohanKumar, MohanKumar, & Quadri, 2000; Wieczorek, et al., 2005). Although IL-1β stimulation of

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the vagus nerve mediates many of the LPS-induced behavioural and neuroendocrine alterations, its role in mediating the increased monoamine release is uncertain. For instance, in comparison to sham-lesioned animals, sub-diaphragmatic vagotomy attenuated the anhedonic and sickness behaviours as well as the ACTH and corticosterone secretion following peripheral LPS administration. The vagotomy, however, had little effect on monoamine release, suggesting that other mechanisms are responsible for the observed amine alterations (MohanKumar, et al., 2000; Wieczorek, et al., 2005). It is thought that central IL-1β mediates some of the neurochemical effects of peripheral immune activation, as intracerebroventricular (i.c.v.) injection of IL-1ra abolished the LPS-induced increases of monoamine neurotransmission in various brain regions (Linthorst, et al., 1995; Linthorst, & Reul, 1998).

In addition to a role for IL-1β, it has been reported that endotoxin-induced neurochemical changes may be due to second messenger systems (Lavicky, & Dunn, 1995; Linthorst, & Reul, 1998). It will be recalled that, in addition to the vagal-mediated pathway, LPS activates a humoral pathway, which involves a series of second messenger molecules, including the prostaglandins, which act on specialized receptors to innervate various brain regions (Konsman et al., 2002). The intermediary role of prostaglandins in LPS-induced neurochemical disturbances was supported by the finding that the cyclo-oxygenase inhibitor, indomethacin, abolished several of the monoamine alterations produced by the endotoxin, (Lavicky, & Dunn, 1995; Linthorst, & Reul, 1998) although monamine changes are induced by subpyrogenic doses of cytokines (Anisman & Merali, 1999). Regardless of the mechanisms involved, it is clear that LPS induces widespread

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neurochemical variations, which could have protracted consequences and may result in behavioural disturbances.

Although similar in some respects, the monoamine variations induced by social stressors are readily distinguishable from those provoked by LPS. In particular, social stressors increased NE utilization in the locus coeruleus, increased DA utilization in the PFC, and 5-HT utilization in the hypothalamus, amygdala and hippocampus (Blanchard, et al., 2001; Blanchard et al., 1993; Tidey, & Miczek, 1996). As multiple social stressor paradigms have been used across studies, it is difficult to compare them with the current results. Regardless, social stressor exposure in the present investigation elicited monoamine alterations similar to those reported in other investigations. For instance, regrouping following a period of isolation increased NE turnover in the locus coeruleus, PFC and hippocampus. The social stressor also increased DA utilization in the PFC and central amygdala. As well, the social stressors increased 5-HT utilization within the PFC.

In addition to altering monoamine turnover, it has been reported that both acute and chronic social stressors can provoke the downregulation or desensitization of NE and 5-HT receptor subtypes (Berton, Durand, Aguerre, Mormede, & Chaouloff, 1999; Blanchard, et al., 2001; Flügge, 1995; Korte, Buwalda, Meijer, De Kloet, & Bohus, 1995, McKittrick, Blanchard, Blanchard, McEwan, & Sakai, 1995). For instance, acute social defeat resulted in downregulated 5-HT_{1A} receptor binding as measured using the administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (Korte, et al., 1995). Furthermore a single social defeat was sufficient to decrease binding to the 5-HT transporter in the hippocampus (Berton et al., 1999). Alterations of NE receptor subtypes, namely β_1- and β_2-adrenoceptors, have also been reported; however, these

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occur mainly in response to chronic social stress (Flügge, Ahrens, & Fuchs, 1997). Unlike 5-HT and NE variations, stressor-induced DA alterations have only been recently investigated. A hyposensitivity of the D_2receptor sub-type was found along with decreased binding capacity in subordinate female Cynomolgous monkeys (Shively, Laber-Laird, & Anton, 1997). In effect, it is possible that the 1-hr regrouping following a prolonged period of isolation lead to a decrease in 5-HT, NE and/or DA receptor sensitivity, possibly secondary to the marked increase of amine utilization.

In addition to their influence on monoaminergic receptor sensitivity, both social and non-social stressors may provoke alterations of central cytokine expression in several brain regions, including the hypothalamus, hippocampus and pituitary (Bartolomucci et al., 2003a; Bartolomucci, et al., 2005; O'Connor et al., 2003). Although it has been reported that chronic social stressors down-regulate central cytokine expression (Bartolomucci et al., 2003a), it has been consistently found that acute stressor exposure (i.e. footshock and restraint) increases the central expression of IL-1β, IL-6 and TNF-α (Nguyen, Deak, Will, Hansen, Hunsaker, Fleshner et al., 2000; O'Connor et al., 2003). As IL-1β is thought to mediate some of the monoaminergic alterations, at least in response to immune activation (Linthorst, et al., 1995; Linthorst, & Reul, 1998), it is possible that the stressor-induced increases of neurotransmission could be the result of alterations of central cytokine activity, especially when combined with the LPS-induced immune activation. Indeed, it has been reported that IL-1ra may preclude monoamine changes associated with footshock administration (Shintani, Nakaki, Kanba, Kato, & Asai, 1995; Shintani, Nakaki, Kanba, Sato, Yagi, Shiozawa, et al., 1995; Suzuki,

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Shintani, Kanba, Asai, & Nakaki, 1997). Whether a similar outcome would be evident in response to a psychosocial stressor has not yet been reported.

In some brain regions, both social and non-social (restraint, tail pinch, noise) stressors elicited comparable monoamine variations. In particular, these stressors increased NE turnover within the PFC and hippocampus, and decreased DA accumulation within the central amygdala compared to the group-housed and isolated controls. However, in addition to their analogous effects, social and non-social stressors induced their own independent effects. For instance, compared to restraint, tail pinch and startle, the 1-hr regrouping elicited greater NE turnover within the locus coeruleus, increased turnover of DA and 5-HT in the PFC and decreased 5-HT levels relative to controls within the hippocampus. In contrast, restraint increased 5-HT accumulation within the central amygdala, and tail pinch induced lower NE levels within the hippocampus relative to controls. In effect, it appears that the social stressor (regrouping) provoked more pronounced and widespread neurochemical alterations than did the non-social stressors. These findings raise the possibility, as previously argued that different challenges engage different neural circuits (e.g., Herman, & Cullinan, 1997; Merali, Khan, Michaud, Shippy, & Anisman, 2004). Moreover, it is possible that psychosocial stressors involve pre-wired circuits that are distinct from those associated with learned stressors, although the present results do not speak to this directly. Of course, these stressors may also differ from one another in terms of the organism's appraisal of them, (e.g., perceived severity, threat, control, etc). Thus, these stressors cannot actually be compared to one another. Nonetheless, the findings of the present investigation are

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consistent with reports showing that naturalistic stressors may involve neural circuits that differ from those activated by other stressors (Merali et al., 2004).

Beyond their independent effects, it appeared that the social stressor and LPS treatment synergistically influenced 5-HT accumulation within the central amygdala. It is significant that earlier studies found that a social stressor followed by LPS treatment increased circulating IL-1β, IL-6 and TNF-α, and also resulted in reduced sensitivity to the inhibitory effects of glucocorticoids on spleen cell viability (Avitsur et al., 2005; Quan, et al., 2001). From this perspective, it is possible that the augmented response to the bacterial endotoxin following the exposure to a social stressor may reflect augmented cytokine release coupled with suppression of glucocorticoid actions that might otherwise inhibit the monoaminergic alterations normally mediated by the cytokines. In addition, as mentioned previously, social stressors have been found to alter central cytokine expression in several brain regions (Bartolomucci, et al., 2003a; Bartolomucci, et al., 2005; O'Connor, et al., 2003). As LPS has also been known to induce central cytokine secretion, this may account for the synergy between the endotoxin and stressor treatments. Furthermore, as this synergy occurred with 5-HT, it is tempting to postulate that the social stressor decreased the sensitivity of 5-HT_{1a} receptors within the central amygdala, so that upon LPS administration, the 5-HT response was increased.

It was of interest to determine in the present investigation, whether the physical interaction, as opposed to the psychogenic component related to social change, during the regrouping was responsible for the behavioural and neurochemical disturbances. It will be recalled that although the physical interaction followed by LPS treatment elicited marked sickness behaviour and HPA activity, these effects were less pronounced if

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animals were regrouped but separated by a metal partition. In fact, the widespread neurochemical alterations provoked by the physical interaction were not evident in response to the non-physical social contact. Interestingly, simply exposing mice to a novel situation (i.e., exposure to the metal partition) elicited considerable monoamine alterations, which were at least as great as those provoked by the physical interaction and social contact. For instance, novelty elicited increased DA accumulation in the PVN, as well as increased NE accumulation in the central amygdala. It has been suggested that disturbances of DA neurotransmission are the result of the anxiety or emotional arousal induced by the stressor, rather than the physical impact of the aversive stimulus (Anisman, et al., 1993). It is possible that exposure to the novel environment triggered the high emotional arousal, leading to the increased DA turnover. Taken together, these findings suggest that physical contact, as opposed to the threat of physical aggression, is a necessary component in the development of depressive-like symptoms and neurochemical alterations. However, the psychological stress provoked by the threat of physical contact should not be underestimated. It has been reported that animals that live under the constant threat of physical contact, interrupted by brief periods of social defeat are far more likely to develop immunological and behavioural pathologies than mice that return to their home cage following defeat (Merlot et al., 2004).

Behavioural pathologies such as depression are characterized by a reduction of monoamine transmission, particularly in the limbic system (Schiepers et al., 2005). Although acute stressor exposure typically provokes increased monoamine activity, it has been suggested that if maintained, it can lead to excessive and prolonged utilization, resulting in decreased amine stores, and subsequently to pathology (Anisman, & Merali,

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1997). In the present investigation, both LPS treatment and social stressor exposure elicited their own independent effects on monoamine secretion within several stressor-sensitive brain regions. In addition, however, they also provoked synergistic effects on 5-HT functioning, a fundamental neurotransmitter implicated in depression. Although the processes governing this interaction are unclear, it is evident that LPS-induced immune activation in combination with social stressors may produce enhanced neurochemical effects, favoring the evolution of behavioral pathologies.

4.4 Cytokine Alterations Provoked by Social Stressor and LPS Exposure

Systemically administered LPS has been shown to elicit increased circulating levels of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α (Dantzer, et al., 2001; Goujon et al., 1995; Johnson et al., 2002). It will be recalled that when administered, LPS binds to the TLR-4 receptor of local macrophages, initiating a cascade of cytokine signaling including IL-1β, which further potentiates release of this cytokine, along with other cytokines such as IL-6 and TNF-α (Konsman, et al., 2002; Laflamme, & Rivest, 2001; Rivest, 2003). Interestingly, in the present investigation, no differences of circulating IL-1β and IL-6 levels were found between LPS and saline-treated animals. Although contrary to several studies (Dantzer, et al., 2001; Goujon, et al., 1995; Johnson et al., 2002), this finding was supported by other studies that failed to find significant alterations of IL-1β following the systemic administration of LPS (Deak et al., 2005; Turrin, Gayle, Ilyin, Flynn, Langhans, Schwartz, & Plata-Salaman, 2001). For instance, while plotting an IL-1β time-course, Deak et al. (2005) found that although central IL-1β was significantly increased in the hypothalamus, hippocampus, pituitary, spleen, and

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adrenal gland for at least 24 hours following an i.p. LPS challenge, plasma IL-1β levels were unaffected.

In contrast to IL-1β and IL-6, LPS administration significantly altered plasma TNF-α levels. This finding is consistent with other reports demonstrating that LPS increased peripheral TNF-α levels (Turrin et al., 2001). Interestingly, although the stressor did not significantly alter TNFα levels, animals that received the 1-hr regrouping had consistently lower plasma TNF-α levels, especially at the highest dose of LPS, compared to isolated and group-housed controls. As glucocorticoids are typically immunosuppressant, it is possible that the additive effects of the social stressor and endotoxin challenge on corticosterone levels were responsible for the reduced concentration of circulating TNF-α. Indeed, it was reported that animals exposed to both a stressor (15 min restraint) and bacterial challenge had reduced TNF-α mRNA expression in several peripheral and central tissues compared to non-stressed animals (Goujon et al., 1995). Furthermore, although circulating TNF-α levels were not measured, plasma IL-1β levels were reduced in stressed-mice relative to non-stressed mice after LPS administration (Connor, et al., 2005; Goujon et al., 1995). Despite the fact that the overall interaction in the present investigation was not significant, it appeared that LPS induced substantial increases of circulating TNF-α in mice that remained undisturbed in their home cages, yet produced only modest increases in mice that were exposed to a social stressor.

The reduced concentration of TNF-α following the social stressor exposure and LPS challenge can potentially be explained by the observed elevations of IL-10. Anti-inflammatory cytokines such as IL-10 have been found to abolish LPS-induced

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production of IL-1β and TNF-α (Bogdan, Paik, Vodovotz, & Nathan, 1992; Fiorentino, Zlotnik, Mosmann, Howard, & O'Garra, 1991; Gerard, Bruyns, Marchant, Abramowicz, Vandenabeele, Delvaux, et al., 1993). Thus, it is possible that the lack of IL-1β alterations, and suppressed TNF-α levels at a high dose of LPS can be attributed to the increase of IL-10.

In addition to LPS challenge, exposure to acute stressors has also been reported to provoke increased circulating proinflammatory cytokines (Johnson, Campisi, Sharkey, Kennedy, Nickerson, Greenwood et al., 2005; Nguyen, et al., 2000; O'Connor et al., 2003; Shintani et al, 1995). For instance, intense stressors such as inescapable shock (Johnson et al., 2005; Nguyen et al., 2000; O'Connor et al., 2003) and immobilization (Shintani et al, 1995; Zhou, Kusnecov, Shurin, DePaoli, & Rabin, 1993) enhanced plasma levels of IL-1β and IL-6. Furthermore, although few studies have assessed the effects of social stressors on peripheral cytokine release, psychosocial stressors such as exposure to an open field, social isolation and social defeat were found to increase plasma IL-1β and IL-6 (LeMay, Vander, & Kluger, 1990; Merlot et al., 2003; Puch, Nguyen, Gonyea, Fleshner, Watkins, Maier, et al., 1999). In contrast to these findings, the social stress of the 1-hr regrouping did not affect circulating levels of IL-1β, IL-6 or TNF-α. However, it is important to note that in the present study cytokines were measured at only a single time following treatment, and it is possible that dynamic variations of circulating cytokines may have been missed.

In addition to the variations of TNF-α, IL-10 was synergistically affected by social stressor exposure and LPS treatment. IL-10 has anti-inflammatory properties, by inhibiting the synthesis of proinflammatory cytokines, and plays a major role in

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inhibiting the development of septic shock (Kronfol & Remick, 2000). LPS has been found to provoke elevated levels of IL-10 (Connor, et al., 2005), which may act as a defense against endotoxic shock. Although limited research has focused on stressor or LPS-induced alterations of IL-10, an increase of IL-10 was found in response to forced swim stress followed by LPS challenge (Connor et al., 2005). Furthermore, the increase of IL-10 was accompanied by a suppression of IL-1β and TNF-α, which, again, could explain the lack of significant alterations of IL-1β, and only modest increases in TNF-α following the regrouping session. In sum, the 1-hr regrouping following a prolonged period of social isolation, particularly when combined with LPS-induced immune activation had synergistic effects on circulating IL-10 levels, which could have had suppressive effects on proinflammatory cytokines.

It has been suggested that the observed alterations of circulating cytokines may be mediated by the increased secretion of catecholamines (namely epinephrine and NE) in response to the stressor (Connor et al., 2005; Johnson et al, 2005). For instance, it was found that blocking α-adrenergic receptors attenuated the increases of IL-1β and IL-6 following inescapable shock, while stimulation of the β-adrenergic receptors increased these cytokines (Johnson et al, 2005). Furthermore, it was reported that the enhanced IL-10 following forced swim stress and LPS challenge were mediated by β-adrenergic receptors (Connor et al., 2005). Although catecholamines may be involved in mediating peripheral cytokine secretion, other mechanisms have also been implicated, such as Substance P and CRH (Johnson et al., 2005). Thus, it is premature to ascribe the cytokine variations solely to catecholamine release.

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Major depressive disorder has been associated with a variety of immunological disturbances, such as elevated levels of circulating blood lymphocytes and monocytes, increased production of cytokines (such as IL-1, IL-2, IL-6 and INF-γ) in mitogen-stimulated cells, and elevated plasma concentrations of IL-1β, sIL-2R, IL-1Ra, IL-6, sIL-6R and INF-γ (Maes et al., 1995). Although stressors were once thought to be immuno-suppressant, repeated stress can lead to chronic elevations of several proinflammatory cytokines despite the hypersecretion of glucocorticoids, which can eventually lead to the development of depression (Avitsur et al., 2005; Engler et al., 2005). More specifically, as mentioned, social stressors can lead to the resistance of immune cells to the anti-inflammatory properties of glucocorticoids, resulting in enhanced release of proinflammatory cytokines, which can engender profound effects if maintained. It remains to be determined whether the stressor regimen used in the present investigation would functionally be expressed as depressive-like behaviors.

4.5 Conclusion

Until recently, the brain has been considered an immuno-privileged organ, distinct from the involvement of the immune system and the inflammatory immune response. It is now be appreciated that the CNS is part of a multi-directional system of communication involving the immune system, endocrine system, and autonomic nervous system (Schiepers et al., 2005; Anisman et al., 2005). In this respect, both stressor exposure and immune activation can influence behavioural, neurochemical, and immune system activity. Indeed, the present investigation demonstrated that when applied concurrently, a social stressor (namely 1-hr regrouping following a prolonged period of

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isolation) and LPS-induced immune activation, have additive or synergistic effects on behavioural, neuroendocrine, neurotransmitter and immune system functioning. If prolonged, it is possible that these effects may provoke or exacerbate stress-related behavioural pathologies such as depression.

Given the combined action of these treatments, it may be important to consider such effects in the provocation of affective disorders among individuals undergoing immunotherapy. The administration of the cytokine treatment in combination with the stress of having the disorder may be responsible for the impact of immunotherapy on depression. In this regard, it may be advisable to begin antidepressant treatment prior to the immunotherapy, as a prophylactic measure against depression.

5. References

Anisman, H., Hayley, S., Turrin, N., & Merali, Z. (2002). Cytokines as a stressor: Implications for depressive illness. *International Journal of Neuropsychopharmacology*, 5, 357-373.

Anisman, H., & Matheson, K. (2005). Stress, depression and anhedonia: Caveats concerning animal models. *Neuroscience and Biobehavioral Reviews*, 29, 525-546

Anisman, H., & Merali, Z. (2003). Cytokines, stress and depressive illness: Brain-immune interactions. *Annals of Medicine, 35*(1), 2-11.

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

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Anisman, H., & Merali, Z. (1997). Chronic stressors and depression: distinguishing characteristics and individual profiles. *Psychopharmacology*, 134(4), 371-377.

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*(8), 963-972.

Anisman, H., Zalcman, S., & Zacharko, R.M. (1993). The impact of stressors on immune and central transmitter activity: Bidirectional communication. *Reviews in the Neurosciences*, 4, 147-180.

Aubert, A. (1999). Sickness and behaviour in animals: A motivational perspective. *Neuroscience and Biobehavioral Reviews,* 23(7), 1029-1036.

Avitsur, R., Kavelaars, A., Heijnen, C., & Sheridan, J. F. (2005). Social stress and the regulation of tumor necrosis factor-alpha secretion. *Brain, Behavior, and Immunity, 19*(4), 311-317.

Avitsur, R., Stark, J. L., & Sheridan, J. F. (2001). Social stress induces glucocorticoid resistance in subordinate animals. *Hormones and Behavior, 39*(4), 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(5), 416-424.

Ban, E., Haour, F., & Lenstra, R. (1992). Brain interleukin 1 gene expression induced by peripheral lipopolysaccharide administration. *Cytokine*, 4(1), 48-54.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Banks, W.A., Farr, S.A., Morley, J.E. (2003). Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. *Neuroimmunomodulation,* 10(6), 319-327.

Bartolomucci, A. (2005). Resource loss and stress-related disease: Is there a link? *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 11*(5), RA147-154.

Bartolomucci, A., Palanza, P., Gaspani, L., Limiroli, E., Panerai, A. E., Ceresini, G., et al. (2001). Social status in mice: Behavioral, endocrine and immune changes are context dependent. *Physiology & Behavior, 73*(3), 401-410.

Bartolomucci, A., Palanza, P., Parmigiani, S., Pederzani, T., Merlot, E. & Neveu, P. (2003a). Chronic psychosocial stress downregulates central cytokines mRNA. *Brain Research Bulletin,* 62(3), 173-178.

Bartolomucci, A., Palanza, P., Sacerdote, P., Ceresini, G., Chirieleison, A., Panerai, A. E., et al. (2003b). Individual housing induces altered immuno-endocrine responses to psychological stress in male mice. *Psychoneuroendocrinology, 28*(4), 540-558.

Bartolomucci, A., Palanza, P., Sacerdote, P., Panerai, A.E., Sgoifo, A., Dantzer, R., et al. (2005). Social factors and individual vulnerability to chonic stress exposure. *Neuroscience and Biobehavioral Reviews,* 29(1), 67-81.

Bartolomucci, A., Pederzani, T., Sacerdote, P., Panerai, A.E., Parmigiani, S., & Palanza, P. (2004). Behavioral and physiological characterization of male mice under chronic psychosocial stress. *Psychoneuroendocrinology,* 29(7), 899-910.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Berton, O., Durand, M., Aguerre, S., Mormede, P., & Chaouloff, F. (1999). Behavioural, neuroendocrine and serotonergic consequences of single social defeat and repeated fluoxetine pretreatment in the Lewis rat strain. *Neuroscience,* 92, 327-341.

Blanchard, R.J., McKittrick, C.R., & Blanchard, D.C. (2001). Animal models of social stress: effects of behavior and brain neurochemical systems. *Physiology and Behavior*, 73(3), 261-271.

Blanchard, D. C., Sakai, R. R., McEwen, B., Weiss, S. M., & Blanchard, R. J. (1993). Subordination stress: Behavioral, brain, and neuroendocrine correlates. *Behavioural Brain Research, 58*(1-2), 113-121.

Bluthe, R.M., Castanon, N., Pousset, F., Bristow, A., Ball, C., Lestage, J., Michaud, B., et al. (1999). Central injection of IL-10 antagonizes the behavioural effects of lipopolysaccharide in rats. Psychoneuroendocrinology, 24, 301-311.

Bluthe, R.M., Dantzer, R., & Kelley, K.W., (1992). Effects of interleukin-1 receptor antagonist on the behavioural effects of lipopolysaccharide in rat. Brain Research, 573, 318-320

Bluthe, R.M., Dantzer, R., & Kelley, K.W. (1997). Central mediation of the effects of interleukin-1 on social exploration and body weight in mice. *Psychoneuroendocrinology*, 22(1), 1-11.

Bluthe, R.M., Walter, V., Parnet, P., Laye, S., Lestage, J., Verrier, D., et al., (1994). Lipopolysaccharide induces sickness behaviour in rats by a vagal mediated mechanism. C.R. Academy of science III, 317, 499-503.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Bogdan, C., Paik, J., Vodovotz, Y., & Nathan, C. (1992). Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor-β and interleukin-10. *Journal of Biological Chemistry,* 267, 23301-23308.

Borowski, T., Kokkinidis, L., Merali, Z. & Anisman, H. (1998). Lipopolysaccharide, central in vivo biogenic amine variations, and anhedonia. *NeuroReport*, 9, 3797-3802.

Brebner, K., Hayley, S., Zacharko, R., Merali, Z., & Anisman, H. (2000). Synergistic effects of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha: central monoamine, corticosterone, and behavioral variations. *Neuropsychopharmacology*, 22(6), 566-580.

Capuron, L., Ravaud, A., Neveu, P. J., Miller, A. H., Maes, M., & Dantzer, R. (2002). Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Molecular Psychiatry, 7*(5), 468-473.

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

Chandler, S., Miller, K.M., Clements, K.M., Lury, J., Corkill, D., Anthony, D.C.C., et al. (1997). Matrix metalloproteinase, tumor necrosis factor and multiple sclerosis: an overview. *Journal of Neuroimmunology*, 72, 155-161.

Chida, Y., Sudo, N., & Kubo, C. (2005). Social isolation stress exacerbates autoimmune disease in MRL/lpr mice. *Journal of Neuroimmunology, 158*(1-2), 138-144.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Connor, T.J., Brewer, C., Kelly, J.P., & Harkin, A. (2005). Acute stress suppresses pro-inflammatory cytokines TNF-alpha and IL-1 beta independent of a catecholamine-driven increase in IL-10 production. *Journal of Neuroimmunology,* 159(1-2), 119-128.

Dantzer, R. (2001). Cytokine-induced sickness behavior: Mechanisms and implications. *Annals of the New York Academy of Sciences*, 933, 222-234.

Dantzer, R., Bluthe, R. M., Laye, S., Bret-Dibat, J. L., Parnet, P., & Kelley, K. W. (1998). Cytokines and sickness behavior. *Annals of the New York Academy of Sciences, 840*, 586-590.

D'Arbe, M., Einstein, R., & Lavidis, N.A. (2002). Stressful animal housing conditions and their potential effect on sympathetic neurotransmission in mice. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 282, R1422-1428.

Deak, T., Bellamy, C., Bordner, K.A. (2005). Protracted increases in core temperature and interleukin-1 following acute administration of lipopolysaccharide: Implications for the stress response. *Physiology & Behavior*, 85(3), 296-307.

Dunn, A.J. (1992). Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: Comparison with interleukin-1. *The Journal of Pharmacology and Experimental Therapeutics,* 261(3), 964-969.

Dunn, A.J. (2005a). Cytokine activation of the hypothalamo-pituitary-adrenal axis. In: Steckler, T., Kalin, N, editors. *Handbook on Stress, Immunology and Behaviour.* New York, Elsevier. Amsterdam. 157-174.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

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

Engler, H., Engler, A., Bailey, M. T., & Sheridan, J. F. (2005). Tissue-specific alterations in the glucocorticoid sensitivity of immune cells following repeated social defeat in mice. *Journal of Neuroimmunology, 163*(1-2), 110-119.

Fiorentino, D.F., Zlotnik, A., Mosmann, T.R., Howard, M., & O'Garra, A. (1991). IL-10 inhibits cytokine production by activated macrophages. *Journal of Immunology,* 147, 3815-3822.

Flügge, G. (1995). Dynamics of central nervous 5-HT1A-receptors under psychosocial stress. *Journal of Neuroscience,* 15, 7132-40.

Flügge, G., Ahrens, O., & Fuchs, E. (1997). Beta-adrenoceptors in the tree shrew brain: II. Time-dependent effects of chronic psychosocial stress on iodocyanopindolol binding sites. *Cellular and Molecular Neurobiology,* 17, 417-432.

Franklin, K.B.J., Paxinos, G. (1997). *A Stereotaxic Atlas of the Mouse Brain.* Academic Press, San Diego, CA.

Gerard, C., Bruyns, C., Marchant, A., Abramowicz, D., Vandenabeele, P., Delvaux, A., et al. (1993). Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *Journal of Experimental Medicine*, 177, 547-550.

Goujon, E., Parnet, P., Laye, S., Combe,C., Kelley, K.W., & Dantzer, R. (1995). Stress downregulates lipopolysaccharide-induced expression of proinflammatory

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

cytokines in the spleen, pituitary, and brain of mice. *Brain, Behavior, and Immunity,* 9, 292-303.

Hart, B.L., (1988). Biological basis of the behavior of sick animals. *Neuroscience and Biobehavioural Reviews,* 12, 123-137.

Hayley, S., & Anisman, H. (2005). Multiple mechanisms of cytokine action in neurodegenerative and psychiatric states: neurochemical and molecular substrates. *Current Pharmaceutical Design*, 11, 947-962.

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. *The Journal of Neuroscience*, 19(13), 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. (2002). The acute and sensitization effects of tumor necrosis factor-α: implications for immunotherapy as well as psychiatric and neurological conditions. *Acta Neuropsychiatrica*, 14(6), 322-335.

Hayley, S., Merali, Z., & Anisman, H. (2003). Stress and cytokine-elicited neuroendocrine and neurotransmitter sensitization: implications for depressive illness. *Stress*, 6(1), 19-32.

Hayley, S., Poulter, M.O., Merali, Z., & Anisman, H. (2005). The pathogenesis of clinical depression: Stressor- and cytokine-induced alterations of neuroplasticity. *Neuroscience*, 135, 659-678.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

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

Johnson, J.D., Campisi, J., Sharkey, C.M., Kennedy, S.L., Nickerson, M., Greenwood, B.N. et al. (2005). Catecholamines mediate stress-induced increases in peripheral and central inflammatory cytokines. *Neuroscience*, 135, 1295-1307.

Johnson, J.D., O'Connor, K.A., Deak, T., Stark, M., Watkins, L.R., & Maier, S.F. (2002). Prior stressor exposure sensitizes LPS-induced cytokine production. *Brain, Behavior, and Immunity*, 16, 461-476.

Johnson, J.D., O'Connor, K.A., Watkins, L.R., & Maier, S.F. (2004). The role of IL-1β in stress induced sensitization of proinflammatory cytokine and corticosterone responses. *Neuroscience*, 127, 569-577.

Kamata, M., Higuchi, H., Yoshimoto, M., Yoshida, K., & Shimizu, T. (2000). Effect of single intracerebroventricular injection of alpha-interferon on monoamine concentrations in the rat brain. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology, 10*(2), 129-132.

Kelley, K.W., Bluthe, R.M., Dantzer, R., Zhou, J.H., Shen, W.H., Johnson, R.W., et al. (2003). Cytokine-induced sickness behavior. *Brain, Behavior, and Immunity, 17 Suppl 1, S112-8.*

Kent, S., Bluthe, R., Kelley, K.W., & Dantzer, R. (1992). Sickness behavior as a new target for drug development. *Trends in Pharmacological Science*, 13, 24-28.

Kim, J.W., & Kirkpatrick, B. (1996). Social isolation in animal models of relevance to neuropsychiatric disorders. *Biological Psychiatry,* 40(9), 918-922.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Konsman, J.P., Parnet, P., & Dantzer, R. (2002). Cytokine-induced sickness behaviour: Mechanisms and implications. *Trends in Neurosciences*, 25(3), 154-159.

Korte, S.M., Buwalda, B., Meijer, O., De Kloet, E.R., & Bohus, B. (1995). Socially defeated male rats display a blunted adrenocortical response to a low dose of 8-OH-DPAT. *European Journal of Pharmacology,* 272, 45-50.

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

Kudryavtseva, N. N., Bakshtanovskaya, I. V., & Koryakina, L. A. (1991). Social model of depression in mice of C57BL/6J strain. *Pharmacology, Biochemistry, and Behavior, 38*(2), 315-320.

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*(4), 351-361.

Lacosta, S., Merali, Z., & Anisman, H. (1999). Behavioral and neurochemical consequences of lipopolysaccharide in mice: anxiogenic-like effects. *Brain Research*, 818(2), 291-303.

Laflamme, N., & Rivest, S. (2001). Toll-like receptor 4: The missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology,* 15(1), 155-163.

Larson, S.J., & Dunn, A.J. (2001). Behavioral effects of cytokines. *Brain, Behavior, and Immunity*, 15(4), 371-387.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Lavicky, J., & Dunn, A.J. (1995). Endotoxin administration stimulates cerebral catecholamine release in freely moving rats as assessed by microdialysis. *Journal of Neuroscience Research*, 40, 407-413.

LeMay, L.G., Vander, A.J., & Kluger, M.J. (1990). The effects of psychological stress on plasma interleukin-6 activity in rats. *Physiology and Behavior,* 47, 957-961.

Leonard, B.E. (2001). The immune system, depression and the action of antidepressants. *Progress in Neuropsychopharmacological and Biological Psychiatry,* 25(4), 767-780.

Linthorst, A.C.E., Flachskamm, C., Müller-Preuss, P., Holsboer, F., & Reul, J.M.H.M. (1995). Effect of bacterial endotoxin and interleukin-1β on hippocampal serotonergic neurotransmission, behavioral activity, and free corticosterone levels: An *in vivo* microdialysis study. *Journal of Neuroscience,* 15(4), 2920-2934.

Linthorst, A.C.E., & Reul, J.M.H.M. (1998). Brain neurotransmission during peripheral inflammation. *Annals of the New York Academy of Sciences,* 840, 139-152.

Liu, H., & Wang, Z. (2005). Effects of social isolation stress on immune response and survival time of mouse with liver cancer. *World Journal of Gastroenterology,* 11(37), 5902-5904.

Lu, Z.W., 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(1), 55-70.

Maes, M, Sharpe, S., Bosmans, E., Vandewoude, M., Suy, E., Uyttenbroeck, W., et al. (1992). Disturbances in acute phase plasma proteins during melancholia:

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additional evidence for the presence of an inflammatory process during that illness. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 16(4), 501-515.

Maes, M., Smith, R., & Scharpe, S. (1995). The monocyte T-lymphocyte hypothesis of major depression, *Psychoneuroendocrinology*, 20, 111-116.

Maier, S.F., & Watkins, L.R. (1995). Intracerebroventricular interleukin-1 receptor antagonist blocks the enhancement of fear conditioning and interference with escape produced by inescapable shock. Brain Research, 695, 279-282

Maier, S.F. & Watkins, L.R. (1998). Cytokines for psychologists: implications of bi-directional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychological Review*, 105(1), 83-107.

Martinez, M., Calvo-Torrent, A., & Pico-Alfonso, M. A. (1998). Social defeat and subordination as models of social stress in laboratory rodents: A review. *Aggressive Behavior, 24*, 241.

McEwen, B.S. (2000). The neurobiology of stress: from serendipity to clinical relevance. *Brain Research*, 886(1-2), 172-189.

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

McKittrick, C.R., Blanchard, 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(6), 383-393.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Menkes, D. B., & MacDonald, J. A. (2000). Interferons, serotonin and neurotoxicity. *Psychological Medicine, 30*(2), 259-268.

Merali, Z., Khan, S., Michaud, D.S. Shippy S. & Anisman H. (2004). Does amygdaloid corticotropin-releasing hormone (CRH) mediate anxiety-like behaviors? Dissociation of anxiogenic effects and CRH release. *European Journal of Neuroscience*20, 229-239.

Merlot, E., Moze, E., Dantzer, R., & Neveu, P.J. (2004). Cytokine production by spleen cells after social defeat in mice: Activation of T cells and reduced inhibition by glucocorticoids. *Stress (Amsterdam, Netherlands),* 7(1), 55-61.

Merlot, E., Moze, E., Dantzer, R., & Neveu, P. J. (2003). Importance of fighting in the immune effects of social defeat. *Physiology & Behavior, 80*(2-3), 351-357.

Merrill, J.E., & Murphy, S.P. (1997). Inflammatory events at the blood brain barrier: regulation of adhesion molecules, cytokines, and chemokines by reactive nitrogen and oxygen species. *Brain, Behaviour and Immunity*, 11, 245-263.

Mohankumar, S.M.J., Mohankumar, P.S., & Quadri, S.K. (2000). Effects of bacterial lipopolysaccharide on central monoamines and fever in the rat: Involvement of the vagus. *Neuroscience Letters*, 284, 159-162.

Musselman, D. L., Lawson, D. H., Gumnick, J. F., Manatunga, A. K., Penna, S., Goodkin, R. S. et al. (2001). Paroxetine for the prevention of depression induced by high-dose interferon alpha. *The New England Journal of Medicine, 344*(13), 961-966.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Nguyen, K.T., Deak, T., Owens, S.M., Kohno, T., Fleshner, M., Watkins, L.R., & Maier, S.F. (1998). Exposure to acute stress induces brain interleukin-1beta protein in the rat. *Journal of Neuroscience*, 18(6), 2239-2246.

Nguyen, K.T., Deak, T., Will, M.J., Hansen, M.K., Hunsaker, B.N., Fleshner, M. (2000). Timecourse and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1beta protein response to acute stress. *Brain Research*, 859(2), 193-201.

O'Connor, K.A., Johnson, J.D., Hansen, M.K., Wieseler Frank, J.L., Maksimova, E., Watkins, L.R., & Maier, S.F. (2003). Peripheral and central proinflammatory cytokine response to a severe acute stressor. *Brain Research, 991, 123-132.*

O'Connor, T.M., O'Halloran D.J., & Shanahan, F. (2000). The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. *Quarterly Journal of Medicine*, 93, 323-333.

Pardon, M.C., Kendall D.A., Perez-Diaz, F., Duxon, M.S., & Marsden, C.A. (2004). Repeated sensory contact with aggressive mice rapidly leads to an anticipatory increase in core body temperature and physical activity that precedes the onset of aversive responding. *The European Journal of Neuroscience,* 20(4), 1033-1050.

Pizarro, J. M., Lumley, L. A., Medina, W., Robison, C. L., Chang, W. E., Alagappan, A. et al. (2004). Acute social defeat reduces neurotrophin expression in brain cortical and subcortical areas in mice. *Brain Research, 1025*(1-2), 10-20.

Puch, C.R., Nguyen, K.T., Gonyea, J.L., Fleshner, M., Watkins, L.R., Maier, S.F. et al., (1999). Role of interleukin-1 beta in impairment of contextual fear conditioning caused by social isolation. *Behavioral Brain Research*, 106, 109-118.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Quan, N., Avitsur, R., Stark, J. L., He, L., Shah, M., Caligiuri, M. et al. (2001). Social stress increases the susceptibility to endotoxic shock. *Journal of Neuroimmunology, 115*(1-2), 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(1), 24-31.

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

Rivest, S. (2003). Molecular insights on the cerebral innate immune system. *Brain Behavior, and Immunity,* 17(1), 13-19.

Rygula, R., Abumaria, N., Flügge, G., Fuchs, E., Rüther, E., & Havemann-Reinecke, U. (2005). Anhedonia and motivational deficits in rats: Impact of chronic social stress. *Behavioural Brain Research*, 162, 127-134.

Sapolsky, R.M., Romero, M., & Munck, A.U. (2000). How do glucocorticoids influence the stress response? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, 21(1), 55-89.

Schiepers, O.J.G., Wichers, M.C., & Maes, M. (2005). Cytokines and major depression. *Progress in Neuropsychopharmacology & Biological Psychiatry*, 29, 201-217.

Schmidt, E.D., Binnekade, R., Janszen, A.W., & Tilders, F.J. (1996). Short stressor induced long-lasting increases of vasopressin stores in hypothalamic corticotrophin-releasing hormone (CRH) neurons in adult rats. *Journal of Neuroendocrinology*, 8(9), 703-712.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Schmidt, E.D., Janszen, A.W., Wouterlood, F.G., & Tilders, F.J. (1995). Interleukin-1-induced long-lasting changes in hypothalamic corticotropin-releasing hormone (CRH)--neurons and hyperresponsiveness of the hypothalamus-pituitary-adrenal axis. *Journal of Neuroscience*, 15(11), 7417-7426.

Sheridan, J. F., Stark, J. L., Avitsur, R., & Padgett, D. A. (2000). Social disruption, immunity, and susceptibility to viral infection. Role of glucocorticoid insensitivity and NGF. *Annals of the New York Academy of Sciences, 917*, 894-905.

Shintani, F., Nakaki, T., Kanba, S., Kato, R., & Asai, M. (1995) Role of interleukin-1 in stress responses. A putative neurotransmitter. *Molecular Neurobiology*, 10(1), 47-71.

Shintani, F., Nakaki, T., Kanba, S., Sato, K., Yagi, G., Shiozawa, M. et al. (1995) Involvement of interleukin-1 in immobilization stress-induced increase in plasma adrenocorticotropic hormone and in release of hypothalamic monoamines in the rat. *Journal of Neuroscience, 15(3), 1961-70.*

Shively, C.A., Laber-Laird, K., & Anton, R.F. (1997). Behavior and physiology of social stress and depression in female cynomolgus monkeys. *Biological Psychiatry,* 41(8), 871-882.

Shuto, H., Kataoka, Y., Horikawa, T., Fujihara, N., & Oishi, R. (1997). Repeated interferon-alpha administration inhibits dopaminergic neural activity in the mouse brain. *Brain Research, 747*(2), 348-351.

Simmons, D.A., & Broderick, P.A. (2005). Cytokines, stressors, and clinical depression: Augmented adaptation responses underlie depression pathogenesis. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 29, 793-807.

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Stark, J.L., Avitsur, R., Padgett, D.A., Campbell, K.A., Beck, F.M., & Sheridan, J.F. (2001). Social stress induces glucocorticoid resistance in macrophages. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology,* 280(6), R1799-805.

Stefanski, V. (2000). Social stress in laboratory rats: Hormonal responses and immune cell distribution. *Psychoneuroendocrinology*, 25(4), 389-406.

Stefanski, V., & Engler, H. (1999). Social stress, dominance and blood cellular immunity. *Journal of Neuroimmunology, 94*(1-2), 144-152.

Suzuki, E., Shintani, F., Kanba, S., Asai, M., & Nakaki, T. (1997). Immobilization stress increases mRNA levels of interleukin-1 receptor antagonist in various rat brain regions.*Cellular Molecular Neurobiology,* 17(5), 557-62.

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

Tilders, F.J., Schmidt, E.D., de Goeij, D.C. (1993). Phenotypic plasticity of CRF neurons during stress. *Annals of the New York Academy of Sciences*, 697, 39-52.

Tidey, J.W., & Miczek, K.A. (1996). Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. *Brain Research*, 721(1-2), 140-149.

Tornatzky, W., & Miczek, K. A. (1993). Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiology & Behavior, 53*(5), 983-993.

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Turner, E. H., & Blackwell, A. D. (2005). 5-hydroxytryptophan plus SSRIs for interferon-induced depression: Synergistic mechanisms for normalizing synaptic serotonin. *Medical Hypotheses, 65*(1), 138-144.

Turrin, N.P., & Plata-Salaman, C.R. (2000). Cytokine-cytokine interactions and the brain. *Brain Research Bulletin*, 51(1), 3-9.

Turrin, N.P., Gayle, D., Ilyin, S.E., Flynn, M.C., Langhans, W., Schwartz, G.J., & Plata-Salaman, C.R. (2001). Pro-inflammatory and anti-inflammatory cytokine mRNA induction in the periphery and brain following intraperitoneal administration of bacterial lipopolysaccharide. *Brain Research Bulletin,* 54(4), 443-453.

Wan, W., Janz, L., Vriend, C.Y., Sorensen, C.M., Greenberg, A.H., & Nance, D.M. (1993). Differential induction of c-fos immunoreactivity in hypothalamus and brain stem nuclei following central and peripheral administration of endotoxin. *Brain Research Bulletin,* 32, 581-587.

Wan, W., Wetmore, L., Sorensen, C.M., Greenberg, A.H., & Nance, D.M. (1994). Neural and biochemical mediators of endotoxin and stress-induced c-fos expression in the rat brain. *Brain Research Bulletin*, 34, 7-14.

Watanobe, H., & Takebe, K. (1993). Intrahypothalamic perfusion with interleukin-1-beta stimulates the local release of corticotropin-releasing hormone and arginine vasopressin and the plasma adrenocorticotropin in freely moving rats: a comparative perfusion of the paraventricular nucleus and the median eminence. *Neuroendocrinology*, 57(4), 593-599.

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Watkins, L.R., Maier, S.F., & Goehler, L.E. (1995). Cytokine-to-brain communication: a review and analysis of alternative mechanisms. *Life Sciences*, 57(11), 1011-1026.

Weiss, I.C., Pryce, C.R., Jongen-Relo, A.L., Nanz-Bahr, N.I., & Feldon, J. (2004). Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. *Behavioural Brain Research,* 152(2), 279-295.

Wieczorek, M., Swiergiel, A.H., Pournajafi-Nazarloo, H., & Dunn, A.J. (2005). Physiological and behavioral responses to interleukin-1beta and LPS in vagotomized mice. *Physiology and Behavior,* 85(4), 500-511.

Wu, W., Yamaura, T., Murakami, K., Murata, J., Matsumoto, K., Watanabe, H., et al., (2000). Social isolation stress enhanced liver metastasis of murine colon 26-L5 carcinoma cells by suppressing immune responses in mice. *Life Sciences,* 66(19), 1827-38.

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

Yokoyama, A., Kimura, Y., & Shigemura, J. (1996). Psychiatric side effects of interferon. *The Journal of Toxicological Sciences, 21*(1), 93-96.

Zalcman, S., Green-Johnson, J., Murray, L., Nance, D.M., Dyck, D., Anisman, H., et al. (1994). Cytokine-specific central monoamine alterations induced by interleukin (IL)-1, -2 and –6. *Brain Research*, 643, 40-49.

Zhou, D., Kusnecov, A.W., Shurin, M.R., DePaoli, M., & Rabin, B.S. (1993). Exposure to physical and psychological stressors elevates plasma interleukin 6:relationship to the activation of hypothalamic-pituitary-adrenal axis. *Endocrinology*, 133, 2523-2530.

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