**CENTRAL ADMINISTRATION OF INTERFERON-ALPHA
INDUCES NEUROCHEMICAL, NEUROENDOCRINE,
AND BEHAVIOURAL ALTERATIONS**

by

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Abstract

Exogenous cytokines can lead to depressive behaviour in humans as well as in animal models of depression. Interferon-alpha (IFN- α) used in the treatment of several diseases is frequently accompanied by depressive symptoms although the underlying mechanism is unknown. Likewise, it is uncertain whether IFN- α administered directly into the brain would have obvious behavioural effects or alter the functioning of neurotransmitters implicated in depression. The present investigation was conducted to determine whether central infusion of IFN- α could induce variations of central cytokine and serotonin receptor mRNA expression, neuroendocrine response, and behavioural alteration involved in depressive disorders. Results demonstrated the ability of IFN- α to modify serotonin receptor mRNA expression in the PFC and hippocampus. Also, IFN- α treatment induced significant cytokine expression in the brain as well as increased plasma corticosterone. It is suggested that increased cytokine activity concomitant with elevated corticosterone and altered serotonergic system is involved in the onset of depressive-like behaviour.

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GLOSSARY OF TERMS

Cytokine: A small protein released by cells that has a specific effect on the interactions between cells, on communications between cells or on the behaviour of cells. The cytokines includes the interleukins, lymphokines and cell signal molecules, such as tumour necrosis factor and the interferons, which trigger inflammation and respond to infections.

Endogenous: Produced or synthesized within the organism or system.

Endotoxin: A toxin that forms an integral part of the cell wall of certain bacteria and is only released upon destruction of the bacterial cell.

Exogenous: Originating from outside; derived externally.

Immunotherapy: Treatment of disease by inducing, enhancing, or suppressing an immune response.

Innate immune response: The cells and mechanisms that defend the host from infection by other organisms, in a non-specific manner. This means that the cells of the innate system recognize, and respond to, pathogens in a generic way, but unlike the adaptive immune system, it does not confer long-lasting or protective immunity to the host. Innate immune systems provide immediate defence against infection,

Interferon: Natural proteins produced by the cells of the immune system of most vertebrates in response to challenges by viruses, parasites and tumour cells. Interferons are produced by a wide variety of cells in response to the presence of double-stranded RNA, a key indicator of viral infection. Interferons assist the immune response by inhibiting viral replication within host cells, activating natural killer cells and macrophages, increasing antigen presentation to lymphocytes, and inducing the resistance of host cells to viral infection.

Interleukin: Cytokines that were first seen to be expressed by white blood cells (leukocytes, hence the *-leukin*) as a means of communication (*inter-*). It has since been found that interleukins are produced by a wide variety of bodily cells.

Neurotrophin: A family of proteins that induce the survival of neurons. They belong to a class of growth factors, secreted proteins, which are capable of signalling particular cells to survive, differentiate, or grow. Growth factors such as neurotrophins that promote the survival of neurons are known as neurotrophic factors.

1. Introduction

The view that activation of the inflammatory immune system may promote depressive illness (Dantzer, 2006) has been supported by several lines of research. Specifically, depressive-like symptoms can be provoked in laboratory animals by activation of the immune system, through the direct administration of proinflammatory cytokines, such as interleukin-1b (IL-1 β) (Anisman et al., 2008) and by tumour necrosis factor alpha (TNF- α) (Reynold et al., 2004), or indirectly by the potent inducer of cytokines, lipopolysaccharide (LPS) (Frenois et al., 2007). Likewise, the behaviour induced by a bacterial endotoxin treatment in humans closely resembles idiopathic depression (Reichenberg et al., 2001). Further support for the involvement of inflammation in the development of depression is seen in the high comorbidity of depression that often accompanies medical illness with an inflammatory component, such as heart disease, Type II diabetes, or rheumatoid arthritis (Evans et al., 2005).

The strongest support for a link between depression and cytokine activation has come from reports that depressive symptoms frequently develop in patients undergoing immunotherapy with cytokines, such as interferon alpha (IFN- α) and interleukin-2 (IL-2), for the treatment of some types of cancer or chronic viral diseases, such as hepatitis C (Capuron & Miller, 2004). To a considerable extent, the symptoms associated with depression induced by immunotherapy are characterized by vegetative symptoms (fatigue, feelings of sickness, soporific-like effects) as well as poor mood. Moreover, just as these features are apparent in depressed patients (Capuron & Miller, 2004, Capuron et al., 2002), these symptoms diminish with cessation of immunotherapy (Loftis & Hauser,

2004) and can be limited by pretreatment with antidepressants (Raison et al., 2005; Kraus et al., 2005).

Animal research concerning cytokine-induced behavioural alterations has primarily dealt with IL-1 β , IL-6 and TNF- α , and limited attention has been devoted to the role of IFN- α in promoting depressive-like symptoms. There are two main reasons why this might be the case. First, as considerable data regarding IFN- α have been obtained studying the influence of immunotherapy in human patients, it might be thought that studies with animals have little to add. Second, most studies with animals that have assessed the influence of IFN- α in animal models of depression have actually indicated that the impact of IFN- α was limited (Anisman et al., 2007). In fact, it has even been suggested that using a mouse model to assess the depressogenic action of IFN- α is not productive (Loftis et al., 2006). Although it has been shown that IFN- α activates hypothalamic-pituitary-adrenal (HPA) functioning, this effect is modest relative to other cytokines, such as IL-1 β or TNF- α (Menzies et al, 1996). However, contrary to these perspectives concerning the use of IFN- α in mouse models of depression, it was reported that when applied on a backdrop of an ongoing stressor, the effects of IFN- α are far more pronounced (Anisman et al., 2007). Thus, it is possible that the effects of IFN- α seen in humans might actually reflect the conjoint effects of the cytokine coupled with the distress being experienced by cancer or hepatitis C patients.

Although the behavioural effects of IFN- α in mice are modest, it is unclear whether, and to what extent, IFN- α reaches the brain following systemic administration, and whether this would be altered under conditions where the blood brain barrier might be compromised (e.g., following stressful events and/or immunologic challenges).

Likewise, it is uncertain whether IFN- α administered directly into the brain would have obvious behavioural effects (supporting central involvement of IFN- α in eliciting these outcomes) or alter the functioning of neurotransmitters that have been implicated in depression. Accordingly, the present investigation was conducted to determine whether central infusion of IFN- α could induce depressive-like behaviour as well as biological changes that might accompany major depressive disorder.

1.1. Inflammatory immune response and depression

According to the macrophage theory of depression (Smith, 1991), excessive secretion of inflammatory cytokines such as IL-1 β , TNF- α and IFN- α are a cause of some cases of major depression. Correlative data relating inflammatory markers with depressive symptoms is substantial. In particular, inflammatory markers have frequently been reported to be elevated in the plasma and cerebrospinal fluid (CSF) of a melancholic depressive patients (Maes, 1995). Furthermore, associations have been identified between inflammatory markers and individual depressive symptoms such as fatigue, cognitive dysfunction, and impaired sleep (Bower et al., 2009, Bermejo et al., 2008, Motivala et al., 2005). More recent pharmacological studies revealed that anti-inflammatory drugs are effective as an adjunctive treatment for depression. For example, celecoxib, a drug that inhibits cyclooxygenase-2 (COX-2), an enzyme responsible for inflammation, was observed to have antidepressant actions when given alone. However, the beneficial effects of the drug are still more pronounced when given in conjunction with standard pharmaceutical treatments, such as selective serotonin reuptake inhibitors

(SSRI) (Akhondzadeh et al., 2009), further supporting the inflammatory depressive model.

Considerable evidence, based on both clinical and experimental studies, have supported the role of cytokines in depressive disorders. In this regard, experimental animal models have been used to explore the relationship between immune activation and depression. Acute activation of the peripheral innate immune system in laboratory animals through LPS administration (a potent inducer of cytokines) provokes depressive-like behaviour. This has been demonstrated through various measures, such as immobility in the forced-swim test and tail suspension test, decreased consumption of a sweetened solution (Frenois et al., 2007), and suppression of sexual behaviour (Avitsur & Yirmiya, 1999), all of which can be attenuated by antidepressant administration (Yirmiya, 2001).

The depressive-like effects of immune activation can also be achieved through the direct treatment with proinflammatory cytokines. Administration of IL-1 β or TNF- α to rodents leads to behavioural changes like those elicited by LPS administration. These cytokine-induced behaviours include anorexia, fever, anxiety, reduced motor activity, and memory impairment, and have collectively been referred to as “sickness behaviours” (Dantzer et al., 2008). The behaviours observed after immune activation are, in some respects, reminiscent of neurovegetative symptoms of depressive illness as seen in human patients. In addition, these treatments have also been shown to promote anhedonia in rodents, a key feature of depression, as well as signs of anxiety, a characteristic often comorbid with depression (Anisman et al., 2008).

Animal and human studies suggest that inflammatory cytokines play a central role in mediating sickness and other behaviours by communicating peripheral inflammatory signals to the brain. Cytokines are induced at the site of inflammation and then signal to the brain by one of four mechanisms: 1) directly across the blood–brain barrier; 2) through sites where the blood–brain barrier is compromised or relatively porous; 3) via the vagus nerve following systemic inflammation (Konsman et al., 2000); or 4) direct activation of brain endothelium by bacterial endotoxins (Ek et al., 1998).

It is of particular significance that the central inhibition of IL-1 β or TNF- α blocked sickness behaviour following a peripheral injection of LPS, indicating that sickness behaviour is likely mediated through central nervous system processes (Abraham & Johnson, 2009). These cytokine-induced sickness behaviours might account for the high incidence of depression in medically ill patients, particularly those with medical illness involving an inflammatory component such as coronary heart disease, Type II diabetes, or rheumatoid arthritis (Evans et al., 2005).

Another line of study linking immune activation and depression is the use of genetically engineered animal models. For instance, TNF- α receptor knockout mice exhibit reduced anxiety in an open field (Silverman et al., 2007), implicating it in mediating cytokine-induced behavioural changes. Likewise, IL-1 receptor null mice exhibit decreased anxiety-related behaviours, that are often associated with depression (Koo & Duman, 2009). Another inflammatory cytokine, interferon-gamma (IFN- γ), also appears to be involved in behavioural alterations. When exposed to a chronic stressor, only wild-type mice demonstrated increased anxiety and depressive symptoms, whereas

deficiency of IFN- γ failed to provoke these effects, supporting the potential involvement of inflammatory components in depressive-like behaviours (Littlejohn et al., 2009).

Selective serotonin reuptake inhibitors ordinarily used as antidepressant treatments can be used to counteract cytokine-induced depressive symptoms in animal models. A decrease in the intake of sweetened solution in LPS-treated rats is reversed with repeated fluoxetine intervention (Yirmiya et al., 2001). Likewise, antidepressants effectively countered the decreased performance of IL-1 β -treated rats in a task in which they had to progressively increase their rate of responding in order to obtain a sucrose solution reward (progressive ratio performance) (Merali et al., 2003). Thus, it appears that the behavioural changes exerted by cytokines are not simply due to sickness, and that antidepressants can act on the anhedonic effects elicited by cytokines. Additionally, pretreatment with the endogenous IL-1 receptor antagonist prevented development of the behavioural deficits in a learned helplessness model of depression (Maier & Watkins 2003). These findings in animal models confirm the observation in humans indicating that activation of the immune system can provoke depression, and point to the fact that the behavioural disturbances in animals elicited by inflammatory factors can be ameliorated by the same treatments that act as antidepressants in humans.

The strongest support for a link between depression and cytokine activation comes from reports that depressive symptoms frequently develop in patients undergoing immunotherapy with cytokines, such as IFN- α or IL-2. IFN- α , an innate immune cytokine that has both antiviral and antiproliferative properties, is used in the treatment of chronic viral infections and some cancers. However, treatment with IFN- α induces psychiatric disturbances including cognitive dysfunction, memory disturbances, as well

as severe depression in 30 – 50% of patients receiving immunotherapy (Capuron et al., 2002). These symptoms can become severe and even lead to interruption of treatment (Kirkwood et al., 1996), but diminish with the cessation of treatment (Loftis & Hauser, 2004) or pretreatment with antidepressants (Kraus et al., 2005). The depressive symptoms resemble idiopathic depression and likewise respond to treatment with antidepressants (Musselman et al., 2001). As with idiopathic depression, IFN- α -induced depressive symptoms are associated with increases in blood levels of IL-6 and TNF- α (Wichers et al., 2006). Similarly, proinflammatory cytokines are induced following IFN- α administration (Raber et al., 1997) suggesting that the depressive effect of IFN- α is indeed mediated by inflammatory cytokines.

1.2. IFN- α and depression

Most vertebrate cells respond to viral infection by producing and sensing type I interferon (like IFN- α), which establishes an antiviral state characterized by inhibition of viral replication, apoptosis of infected cells, and stimulation of innate immune mechanisms that augment subsequent adaptive immune responses. The molecular cascade for IFN- α postreceptor signaling involves Janus kinase (JAK) and signal transducer and activator of the transcription (STAT) signalling pathway (Darnell et al., 1994). Signalling begins with the binding of IFN- α to the type I IFN receptor triggering receptor subunit dimerization, and JAK-dependent tyrosine phosphorylation of receptor proteins as well as transcription factors STAT1 and STAT2.

Phosphorylated STAT1 and STAT2 then form heterodimers, and migrate into the nucleus and associate with another molecule, IFN regulatory factor-9 (also called p48 or

ISGF-3g), forming IFN-stimulated gene factor 3 (ISGF-3). This biologically active ISGF-3 then interacts with a specific DNA sequence called the IFN-stimulated response element present in the promoter region of type I IFN-stimulated genes (ISGs) to activate ISG transcription, resulting in IFN- α -induced gene expression. Among the most highly expressed genes are IFN-induced 15 kDa protein (ISG15), ubiquitin-specific proteinase 18 (USP18), IFN-induced 10 kDa protein (IP-10 or CXCL10), STAT1, and IFN-induced guanylate-binding protein 3 (GBP3), all of which are associated with the biological effects of IFN- α (Stark et al., 1998).

As indicated earlier, although IFN- α has been used as an effective treatment for hepatitis C and some cancers, IFN- α immunotherapy induces a wide-range of untoward behavioural changes, such as fatigue, anorexia, and depression (Reichenberg et al., 2001). In lab animals, administration of IFN- α induces increased immobility time in the forced swimming test (Orsal et al., 2008, Makino et al., 2000), a standard screening test for antidepressant effects. Consistent with the view that inflammatory factors contribute to depressive symptoms, intracerebroventricular administration of IFN- α increases serotonin turnover in prefrontal cortex and increases dopamine turnover in the hippocampus, while pre-treatment with diclofenac, a non-steroidal anti-inflammatory drug, completely prevented these neurochemical changes (De La Garza et al., 2003). These changes were recorded in brain regions (e.g., prefrontal cortex and hippocampus) known to be involved in depression and antidepressant action.

Acute IFN- α administration in healthy volunteers and in hepatitis patients significantly increases adrenocorticotrophic hormone (ACTH), corticotropin-releasing hormone (CRH), as well as circulating IL-6 (Cassidy et al., 2002). In rodents, IFN- α

alters monoaminergic neurotransmission (Kamata et al, 2000) as well as the activity of the HPA axis (Gisslinger et al., 1993) both of which are strongly associated with depression. The link between HPA activation and the onset of depression is supported by the ability to predict which patients receiving immunotherapy are most likely to experience depressive symptoms. Patients who initially showed the greatest neuroendocrine response to IFN- α , as measured by ACTH and cortisol elevation, were most likely to develop major depression (Capuron et al., 2003). Furthermore, patients exhibiting subsyndromal levels of depression, low self-directedness, and history of mood disorders prior to immunotherapy were most likely to develop depressive illness in response to the treatment (Castellvi et al., 2009). However, the mechanism by which IFN- α induces these effects is not fully understood.

1.3. Mechanism of IFN- α -induced depression

The mechanisms responsible for cytokine-induced (IFN- α -induced) depression are becoming better understood, and several possible mechanisms have been proposed in this regard. Pro-inflammatory cytokines influence glucocorticoids, serotonin and dopamine, and even growth factors, all of which have been implicated in the onset of depression. Either individually or synergistically, these systems can be involved in the psychiatric disorders that follow immunotherapy. Alternatively, IFN- α may be inducing depressive symptoms through actions on indoleamine 2,3-dioxygenase (IDO), the rate limiting enzyme in the kynurenine pathway. Ordinarily, IDO converts tryptophan into kynurenine and quinolinic acid and high levels of activity of IDO can lead to decreased synthesis of serotonin (O'Connor et al., 2009). It was thus suggested that the

depressogenic effects of immunotherapy are due to IDO activation and the consequent tryptophan depletion (Capuron et al., 2002; Capuron & Miller, 2004; Musselman et al., 2001). In addition, a direct role of kynurenine in depressive-like behaviour has also been suggested, possibly stemming from the neurotoxic actions of quinolinic acid (O'Connor et al., 2009).

1.3.1. Impact of IFN- α on HPA-axis

Cytokines stimulate the HPA-axis, provoking increased expression and release of CRH as well as ACTH, both of which are elevated in depressed patients (Pariante & Miller 2001). Like other cytokines, acute IFN- α administration to healthy volunteers and to hepatitis patients significantly increases ACTH, CRH, as well as circulating IL-6 (Cassidy et al., 2002). Furthermore, IL-6 has an additive effect with CRH, where IL-6 appears to induce the secretion of CRH and, thereby, ACTH (Matta et al., 1992), which could lead to a spiral into depressive symptomatology. As mentioned earlier, patients who initially showed the greatest neuroendocrine response to IFN- α , as reflected by ACTH and cortisol elevation, were most likely to develop major depression (Capuron et al., 2003), suggesting a close relationship between HPA-axis activation and depression. Indeed, the expression of glucocorticoid receptor (GR) in cells treated with IFN- α was greatly reduced, while, co-incubation with desipramine or fluoxetine attenuated the effect of IFN- α on GR (Cai et al., 2005).

In non-human primates, IFN- α treatment increases peripheral ACTH and IL-6 in association with the development of anxiety and depressive symptoms (Felger et al., 2007). These alterations, which included hyperactivity of the HPA axis and increased

activity of CRH-containing circuits, resemble those present in depressed adult individuals (Sanchez, 2006). The HPA alteration observed in depressed patients could be the result of biological anomalies that lead to a predisposition to depression. Indeed, IFN- α treatment of a mouse hippocampal cell line disrupts glucocorticoid receptor functioning, a process that may be a factor in the development of depression in patients receiving IFN- α treatment (Hu et al., 2009). In this regard, it was reported that atypical antidepressants as well as SSRIs (e.g., fluoxetine) attenuated glucocorticoid action by lowering hippocampal receptor levels in rats (Szymanska et al., 2009).

1.3.2. IFN- α and monoamine alterations

Abnormal serotonergic neural transmission has been thought to be a susceptibility factor for major depressive disorder. In line with this view, chronic administration of IFN- α reduces serotonin (5-HT) levels in the prefrontal cortex of rodents and increased 5-HT turnover within the amygdala (De La Garza et al., 2005), as well as lowers dopamine (DA) utilization (Shuto et al., 1997), possibly affecting reward (hedonic) processes. The same researchers also reported that chronic administration of IFN- α induced small reductions of whole brain DA, while acute treatment showed no change. Likewise, repeated subcutaneous treatment with IFN- α for 7 days increased the DA and norepinephrine (NE) contents of the cortex, hypothalamus and medulla, but not of the hippocampus or thalamus (Kumai et al., 2000). Reports on the effects of IFN- α on brain catecholamines have been varied and the mechanism by which IFN- α alters dopamine metabolism is unclear.

The serotonin 1A receptor (5-HT_{1A}) is of special interest because of its key role in autoregulation of the brain 5-HT system. Specific activation of the 5-HT_{1A} receptor reduces anxiety-like behaviour in high-anxiety-bred rats (Brunelli 2009). Interestingly, a genetic polymorphism of the 5-HT_{1A} has been associated with the development of IFN- α -induced depression (Kraus et al., 2007). Furthermore, cells treated with IFN- α showed a significant decrease in the expression of 5-HT_{1A}. However, co-incubation with antidepressants attenuated the effect (Cai et al., 2005). In addition to effects related to 5-HT_{1A} receptors, IFN- α also promotes 5-HT_{2C} receptor mRNA editing, resulting in receptor down regulation (Yang et al., 2004). This outcome may be particularly relevant, as 5-HT_{2C} receptor function has been associated with anxiety and depressive symptoms (Merali et al., 2006).

The major involvement of 5-HT in depression is further indicated by the finding that depressed patients show lower hippocampal 5-HT_{2A} receptor binding (Sheline et al., 2004) and animals with disruption of the 5-HT_{2A} receptor display modulation of conflict anxiety (Weisstaub et al., 2006). Alterations of 5-HT activity influence the functioning of other neuronal systems. For instance, activation of 5-HT_{2C} receptors attenuates prefrontal cortex DA release in rodent models (Li et al., 2005) showing that the dysregulation of one system can lead to multiple downstream sites being altered as well.

As indicated earlier, there is considerable evidence suggesting that IFN- α may come to affect depression through its actions on 5-HT functioning. Specifically, IFN- α -induced depression could be due to decreased tryptophan levels available for the production of 5-HT through the activation of the enzyme indoleamine 2, 3-dioxygenase (IDO). Although IFN- γ is a stronger inducer of IDO, peripheral administration of IFN- α

can also successfully activate IDO (Raison et al., 2009). Furthermore, when IDO is activated in conditions of chronic inflammation, its degree of activation is correlated to the intensity of depressive symptoms, as observed in cancer patients chronically treated with IFN- α (Capuron et al., 2002).

1.3.3. IFN- α and neurotrophins: alterations in neuroplasticity involved in depression

Growth factors, like brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), are essential for the neuronal growth and development involved in the neuroplasticity of the adult nervous system. In fact, an important component of the mechanism of action of antidepressants is the increase in neurotrophins like VEGF and BDNF (Warner-Schmidt & Duman, 2007). Cytokines such as IL-1, IL-6, and TNF- α appear to cause abnormalities in the pathways relevant in depression, including diminished neurotrophic support, and decreased neurogenesis (Goshen et al., 2008). Activation of the peripheral innate immune system, due to either an immune challenge or stress, leads to increased proinflammatory cytokine production. What follows is decreased neurotrophic support and neurogenesis in brain areas important to behaviour and cognition (Ben Menachem-Zidon et al., 2008). For example, peripheral LPS administration produces cognitive impairment and increased hippocampal concentrations of TNF- α and IL-1, which are associated with decreased hippocampal expression of BDNF, all of which are correlated with reduced hippocampal neurogenesis (Wu et al., 2007). A causal role of inflammatory mediators comes from studies showing that administration of the IL-1 receptor antagonist (IL-1Ra) blocks the effects of stressors

on behaviour, cognition, neurotrophic factors, and neurogenesis (Koo & Duman, 2008; Goshen & Yirmiya, 2009).

As described earlier, IFN- α can elicit the production of proinflammatory cytokines and indirectly alter neurogenesis. However, there is evidence that IFN- α is directly associated with neurodegeneration and hence might be a factor in IFN- α -induced depression (Wang et al., 2002). It has been shown that chronic IFN- α administration results in diminished hippocampal neurogenesis, and this outcome is attenuated by an IL-1 antagonist (Kaneko et al., 2006) indicating a direct link between IFN- α and decreased neurogenesis.

1.4. Present investigation

Even though behavioural effects of IFN- α in mice are modest, clear physiological alterations are present. It has been shown that IFN- α administered peripherally leads to increased IFN- α in the cerebrospinal fluid in human patients (Raison et al., 2009). However, it is not known whether IFN- α crosses the blood-brain barrier, or whether it induces further production of endogenous IFN- α . It is also uncertain whether IFN- α administered directly into the brain would promote behavioural disturbances or alter the functioning of neurotransmitters that have been implicated in depression. Accordingly, the present investigation was conducted to determine whether central infusion of IFN- α could induce depressive-like behaviour as well as biological changes that might accompany major depressive disorder. In effect, the present study sought to elaborate on the parallels between human and mouse behavioural effects of IFN- α as well as biological mechanisms driving these effects. To this end, in the present investigation we

assessed to what extent central infusion of IFN- α would induce an immune response, and would immune activation lead to depressive behaviour as determined by sucrose consumption.

2. Materials and methods

2.1. Subjects

Naïve, male CD-1 strain mice obtained from Charles River Canada (St. Constant, PQ) at 6–7 weeks of age and were accustomed to the vivarium for 2 weeks prior to being used as experimental subjects. Mice were housed in groups of four in standard (27×21×14 cm) polypropylene cages and maintained on a 12-h light–dark cycle (light phase: 0800–2000 h), with temperature (22°C) and humidity (63%) kept constant, and were permitted free access to food (Ralston Purina, St. Louis, MO, USA) and water. To limit variability associated with diurnal rhythms, all experimental procedures were conducted between 0800 and 1200 h. The studies met the guidelines set out by the Canadian Council on Animal Care and were approved by the Carleton University Animal Care Committee.

2.2. Surgery

Animals were anaesthetised with 2.5% isoflurane and stereotactically implanted with a 26-gauge stainless steel guide cannula (Plastic One, Roanoke, VA, USA) in the lateral ventricle (ML +1.0, AP –0.22, DV –2.5 mm) beneath the surface of the skull, according to coordinates from Paxinos and Franklin (Paxinos & Franklin, 2004). The guide cannula was anchored to the skull with three stainless steel screws and dental

cement. A cannula dummy was inserted into the guide cannula to prevent blockage of the guide cannula prior to intracerebroventricular (ICV) injection. Following surgery, animals were administered analgesia and were individually housed and allowed a 7-day recovery period prior to behavioural testing.

2.3. Drugs and injections

Recombinant mouse IFN- α (R&D Systems, Minneapolis, MN, USA) was dissolved in 0.5% bovine serum albumin (BSA) in saline solution as a stabilizing agent and carrier protein while LPS was dissolved in saline solution. The control animals received equal amounts of 0.5% BSA saline alone (vehicle). A single dose of IFN- α (100 or 1000 IU), lipopolysaccharide (100 ng) or vehicle was microinjected into the lateral ventricle in a 2- μ l volume, infused over 5 minutes, via an injection cannula connected to an infusion pump with polyethylene tubing (Harvard Apparatus, Holliston, MA, USA). Following drug infusion, the injector was left in place for an additional 60 seconds to ensure drug diffusion.

2.4. Experiment 1: acute infusion of IFN- α or LPS

Mice (N=10/group) received infusion of IFN- α or vehicle into the lateral ventricle. As a positive control of immune activation, a separate group of mice were infused with LPS. Immediately following drug administration, mice were returned to their home cages. The cages were part of a Micromax motor activity monitoring system allowing home-cage motor activity to be recorded for 90 minutes. Measuring activity in the home cage permitted assessment of behaviour relatively uncontaminated by

experimental procedures (e.g., handling, novel environment) that could influence the response to treatments.

Ninety minutes after infusion, mice were rapidly decapitated, and free-flowing plasma trunk blood was collected in tubes containing 10 μ g of EDTA for subsequent determinations of corticosterone. Blood samples were centrifuged for 15 min at 3,600 rpm, and the supernatant was stored in separate aliquots at -80°C for subsequent analyses of corticosterone levels.

Brains were rapidly removed and placed on a stainless steel brain matrix ($1 \times 1.5 \times 0.75$ in.) situated on top of a block of ice. The brain blocker had a series of slots (spaced 500 μm apart) that served as guides for razor blades to provide coronal brain sections. Tissue from hippocampus and PFC were collected by multiple micro-punches using a hollow 20-gauge microdissection needle following the mouse atlas of Franklin and Paxinos (1997). In the case of the PFC, eight punches were used to form an inverted triangle, whereas hippocampal punches comprised four punches extending approximately 2 mm on either side of the midline. Tissue was stored at -80°C for subsequent neurochemical determinations.

2.5. Experiment 2: repeated infusion of IFN- α

Mice ($N=10/\text{group}$) received intracranial infusion of IFN- α or vehicle into the lateral ventricle (as described in Exp. 1) on each of 6 consecutive days. After each infusion, mice were returned to their individual cages. Following the fifth infusion, sickness behaviours were recorded for 1 h at 20-min intervals following administration of IFN- α or vehicle. The overall appearance of each animal was rated to assess degree of

sickness exhibited. Sickness measurements were scored on a four-point scale (0 = no symptom, 1 = one symptom present, 2 = two symptoms presents, 3 = three or more symptoms) with respect to absent exploration and locomotion, curled body posture, ptosis, ragged fur, lethargy, pilo erection, drooping eyelids, and overall nonresponsiveness. We previously observed that this procedure yielded better than 90% agreement between two raters blind to the treatment mice received. Moreover, the results obtained using this procedure were highly correlated with the more common procedure in which each symptom was independently scored for severity on a four-point scale (Anisman et al. 2007).

On the sixth day of treatment, two hours after the final infusion, mice were rapidly decapitated; blood and brain collection procedures were the same as described in experiment 1.

2.6. Experiment 3: Sucrose preference and consumption following repeated central IFN- α administration

To examine if administration of IFN- α provoked depressive-like behaviours, a sucrose preference test that has been used as a measure of anhedonia (Willner et al., 1992) was conducted over a six-day period. In this test, mice (N=9 or 10/group) were provided several days of pre-training to establish a stable baseline of sucrose consumption. To this end, mice had access to two 200 ml bottles, one containing 2% sucrose, and the other tap water, with the position altered on a predetermined random schedule. Bottles were weighed and changed daily. Intake volume of each was determined on the basis of the bottle weights prior to vs. after the 24 h test period.

Following the training phase, mice received intracranial infusion of IFN- α or vehicle into the lateral ventricle (as described in Exp. 1) on each of 6 consecutive days. Additionally, mice were weighed every day prior to infusion.

2.7. Plasma corticosterone determination

Plasma corticosterone levels were measured in Experiment 1 and 2. Levels were determined in duplicate using a commercially available radioimmunoassay kit (ICN Biomedicals, CA, USA). Assays were conducted in a single run precluding inter-assay variability, and the intra-assay variability was less than 8%.

2.8. Reverse transcription-quantitative polymerase chain reaction analysis in brain

In Experiment 1 and 2, RNA from within the hippocampus and PFC was isolated and purified by standard methodologies employing Trizol according to the manufacturers' protocol (Invitrogen; Burlington, ON, Canada). The RNA was then reverse transcribed using Superscript II reverse transcriptase (Invitrogen; Burlington, ON, Canada), and aliquots of this reaction were used in simultaneous quantitative polymerase chain reactions (QPCR). For QPCR, SYBR green detection was used according to the manufacturer's protocol (Stratagene Brilliant QPCR kit). A Stratagene MX-4000 real time thermocycler was used to collect the data. All PCR primer pairs used generated amplicons between 129 and 200 base pairs. Amplicon identity was checked by restriction analysis. Primer efficiency was measured from the slope relation between absolute copy number or RNA quantity and the cycle threshold determined using the MX-4000 software. All primer pairs had a minimum of 90% efficiency. Primers that

amplify synaptophysin mRNA were used as a control to normalize the data. This mRNA species, even under extreme perturbations (static epilepsy), is a stably expressed “house-keeping gene” (Chen et al., 2001), and we have found this gene stable in human suicide brain (Merali et al. 2004) and in mouse brain of stressed mice (Anisman et al. 2007). Moreover, we have observed that the cycle thresholds for synaptophysin, cyclophilin, and β -actin were highly correlated (r = ranging from 0.85 to 0.92), attesting to the validity of using synaptophysin alone. Importantly, in the present investigation, the Ct values for synaptophysin did not differ as a function of any of the treatments (F 's<1). To compensate for inter-individual variability that ordinarily exists within the assay, the expression of each species within the hippocampus and PFC was normalized by subtracting its Ct from the synaptophysin Ct, thus providing the normalized Ct values (Ctn) for each mRNA species. A difference between Ctn's for an mRNA species represents the fold change (i.e., as power of two) in abundance. To simplify data presentation, the nCt values were converted to fold changes relative to mice in the naïve group, following the procedure described by Livak and Schmittgen (2001). Primer sequences were as follows: synaptophysin, forward: GGACGTGGTGAATCAGCTGG, reverse: GGCGAAGATGGCAAAGACC; Mus IL-1 β , forward: TGTCTGAAGCAGCTATGGCAAC, reverse: CTGCCTGAAGCTCTTGTGATG; Mus IL-1R1, forward: ATGAGTTACC CGAGGTCCAGTG, reverse: TACTCGTGTGACCGGA TATTGC; Mus IL-6, forward: TCTTGGGACTGATGCTGGTG, reverse: CAGAATTGCCATTGCACAACCTC; Mus IL-6R, forward: CTCTCCAACCACGAA GGCTG, reverse: TGCAACGCACAGTGACACTATG; Mus TNF- α , forward:

CTCAGCCTCTTCTCATTCCTGC reverse: CCATAGAACTG ATGAGAGGG; Mus IL-10, forward: AATTCCCTGGGTGAGAAGCTG, reverse: TCATGGCCTTGTAGACACCTTG; Mus 5-HT1A (Htr1a), forward: TCACCTTG AGTTTGCAGCCTC, reverse: GCAGGAGTTGGAAGCACTTAGG; Mus 5-HT1B (Htr1b), forward: GTCAAAGTGCGAGTCTCAGACG; reverse: ACAGATAGGCATCACCAGGGAG; Mus 5-HT2A (Htr2a), forward: TGCCACCAACTATTTTCCTG ATG, reverse: ACATCCAGGTAAATCCAGACGG; Mus 5-HT2C (Htr2c), forward: GTTCAATTTCGC GGACTAAGGC, reverse: GTCAACGGGATGAAGAATGCC.

2.9. Statistical analyses

Data for corticosterone, as well as mRNA changes for each of the cytokines, and 5-HT receptor subtypes were analyzed using one-way between-group analyses of variance (ANOVA). Motor activity, sickness scoring, and sucrose preference/consumption over days (and periods within days) was assessed through repeated measures ANOVA, with time as a within-group factor. In all instances, follow-up t tests were conducted using Bonferroni corrections to control for family-wise error.

3. RESULTS

3.1. Experiment 1. Effects of acute administration of IFN- α or LPS

Plasma corticosterone: Plasma corticosterone levels, shown in Figure 1, were dependent on treatment administered, $F(3,36) = 5.44$, $p = .0035$. A single treatment of central IFN- α moderately, but significantly increased levels of plasma corticosterone at both 100 and

1000 IU doses, compared to vehicle. Infusion of LPS also increased plasma corticosterone relative to vehicle treated mice, as well as mice treated with 1000 IU IFN- α . In effect, a single infusion of IFN- α (100 IU or 1000 IU) elicited corticosterone changes just as LPS was effective in this regard.

Insert Figure 1 about here

Cytokine mRNA expression: Cytokine mRNA expression was markedly influenced by treatment in both the PFC and hippocampus (Figure 2). Specifically, a Treatment effect was observed in the PFC with respect to mRNA expression of IL-1 β and IL-6, $F(3,31) = 2.99, 7.65$, $p < .05$ and $.001$, respectively, whereas there was no main effect with regard to TNF- α or IL-10 expression. Follow up t-tests showed that relative to the vehicle treatment, the administration of 100 IU of IFN- α did not elicit significantly more mRNA expression of IL-1 β , IL-6 and TNF- α . However, 1000 IU induced a significant increase of mRNA expression of IL-1 β , IL-6 and TNF- α compared to vehicle treatment. Neither dose impacted IL-10 expression.

Within the hippocampus, cytokine mRNA response varied with Treatment, IL-1 β , $F(3,32) = 23.57$, $p < .0001$, IL-6, 5.47 , $p < .005$, TNF- α , $F(3,31) = 15.23$, $p < .0001$. The follow-up tests indicated that 100 IU of IFN- α was once again unable to elicit significantly more mRNA expression of IL-1 β and TNF- α , although this dose induced an increase in IL-6 expression compared to vehicle. The 1000 IU IFN- α produced a significant inflammatory response with IL-1 β , IL-6 and TNF- α all greatly increased compared to vehicle. Neither dose impacted IL-10 expression.

Figure 1. Concentrations of plasma corticosterone among mice that had received i.c.v. infusion of IFN- α (100 or 1000 IU), LPS (100 ng) or vehicle ($M \pm SEM$). * $p < 0.05$, relative vehicle. ** $p < 0.025$, relative to vehicle-treated mice.

Infusion with LPS was included in the experiment as a positive control of immune activation. Indeed, LPS clearly induced significant proinflammatory cytokine expression (Figure 2b). Follow up t-tests showed that LPS infusion significantly elevated expression of all proinflammatory cytokines when compared to vehicle-treated mice. However, in the hippocampus, LPS elicited robust expression of IL-1 β and TNF- α which was significantly greater than vehicle-treated mice, as well as both IFN- α -treated groups.

Insert Figure 2a about here

Insert Figure 2b about here

Serotonin receptor mRNA expression: Given that depressive illness has been associated with variations of 5-HT receptors, it was of interest to assess the impact of IFN- α infusion on these receptors. Analyses of the 5-HT receptor variations confirmed that there was an overall treatment effect on 5HT_{1A} mRNA expression in the hippocampus, $F(3,32) = 3.45$, $p = .02$ (Figure 3). The follow-up t tests revealed decreased 5-HT_{1A} expression at both doses, 100 IU and 1000 IU, compared to vehicle. There was no change of 5HT_{1A} expression in the PFC as a function of the treatment mice received.

A treatment effect also occurred with 5HT_{1B} mRNA expression in the hippocampus, $F(3,32) = 3.78$, $p = .02$, and approached significance in the PFC, $F(3,32) = 2.55$, $p = .07$. The follow-up t tests revealed decreased 5-HT_{1B} expression in LPS-treated mice compared to vehicle in the PFC, and hippocampus, as well as decreased expression in 1000 IU-treated mice compared to their respective controls.

Figure 2a. Changes in cytokine mRNA expression in the PFC and hippocampus following acute i.c.v. administration of IFN- α (100 or 1000 IU) or vehicle ($M \pm SEM$). * $p < 0.05$ compared to vehicle.

Figure 2b. Changes in cytokine mRNA expression in the PFC and hippocampus following acute i.c.v. administration of IFN- α (100 or 1000 IU), LPS (100 μ g) or vehicle ($M \pm SEM$). * $p < 0.01$ compared to vehicle. ** $p < 0.001$ compared to IFN- α (100 and 1000 IU) and vehicle.

A main effect of treatment on 5HT_{2A} mRNA expression was apparent in the PFC, $F(3,32) = 4.32$, $p = .01$, and nearly in the hippocampus, $F(3,32) = 2.53$, $p = .07$. The follow-up t tests revealed that in the PFC, IFN- α 100 IU and LPS significantly lowered expression, compared to vehicle, while in the hippocampus, 5HT_{2A} receptor expression in IFN- α 100 IU-treated mice approached significance and was higher than vehicle-treated subjects.

Insert Figure 3 about here

mRNA expression. CRH, CRH₁, BDNF, Trk2. Irrespective of the brain region (PFC or hippocampus), acute IFN- α treatment did not alter expression of CRH receptors, CRH₁, BDNF mRNA or expression of its receptor gene NTRK2 compared to vehicle (Data not shown).

Locomotor activity following acute IFN- α administration: There was no main effect of Treatment following acute administration of IFN- α , and it did not significantly alter motor behaviour as seen in the first 90 minutes following infusion (Figure 4). However, follow-up t tests conducted on the basis of a priori hypotheses, revealed reduced motor behaviour following LPS infusion only in the third interval (minutes 31 – 45), $t = 2.34$, $p = .03$.

Insert Figure 4 about here

Figure 3. Normalized mRNA expression of 5HT receptors in the PFC and hippocampus following i.c.v. administration of IFN- α (100 or 1000 IU), LPS (100 ng), or vehicle. ($M \pm SEM$) * $p < .05$, relative vehicle. ** $p < .0025$, relative to vehicle-treated mice.

Figure 4. Locomotor activity following acute i.c.v. administration of IFN- α , LPS, or vehicle. ($M \pm SEM$) * $p < .05$, relative vehicle.

3.2. Experiment 2. Effects of repeated administration of IFN- α

Plasma corticosterone: Repeated administration of IFN- α significantly increased corticosterone levels (See Figure 5). After 6 repeated infusions of IFN- α , plasma corticosterone levels were nearly three times that of vehicle-treated mice, $F(1,17) = 16.06$, $p < .001$.

Insert Figure 5 about here

Cytokine mRNA expression: Repeated infusion of IFN- α increased proinflammatory cytokine mRNA expression in the PFC. As seen in Figure 6, IL-1 β expression was greater in IFN- α -treated than in that of vehicle-treated mice, $F(1,16) = 5.95$, $p = 0.03$, as was the expression of TNF- α , $F(1,16) = 4.53$, $p = 0.05$. Furthermore, PFC IL-6 expression was also increased by IFN- α $F(1,16) = 4.61$, $p = 0.05$, but this outcome was marginal and less marked than the effect in hippocampus. Interestingly, anti-inflammatory IL-10 expression was increased in the PFC, $F(1,16) = 9.14$, $p = .008$.

Similar to the PFC, repeated infusion of IFN- α increased proinflammatory cytokine mRNA expression in the hippocampus. IFN- α increased IL-1 β , IL-6, and TNF- α mRNA expression compared to vehicle, $F(1,15) = 5.74$, $p = 0.03$; $F(1,15) = 6.64$, $p = .02$, $F(1,15) = 4.31$, $p = .05$. Unlike the effect in the PFC, IL-10 mRNA expression remained unchanged in the hippocampus.

Insert Figure 6 about here

Figure 5. Concentrations of plasma corticosterone ($M \pm SEM$) among mice that had received i.c.v. infusion of IFN- α (1000 IU), LPS or vehicle. * $p < .001$, relative to vehicle.

Figure 6. Expression of cytokine mRNA ($M \pm SEM$) in the PFC and hippocampus following repeated i.c.v. administration of 1000 IU of IFN- α or vehicle. * $p < 0.05$, ** $p < 0.01$ compared to vehicle-treated mice.

Serotonin receptor mRNA expression. There was some evidence of 5-HT receptor alterations in the hippocampus of IFN- α -treated mice, as seen in Figure 7. Although all modifications did not reach significance, the trends are an indication of IFN- α involvement in modifying 5-HT system functioning. There was a nearly significant increase in 5HT_{2A} receptor mRNA expression in the IFN- α -treated mice, $F(1,10) = 4.13$, $p = .07$. Also present was a nearly significant decrease in 5HT_{2C} receptor mRNA expression, $F(1,14) = 4.16$, $p = .06$, and to a lesser extent a decrease in 5HT_{1B} expression, $F(1,14) = 2.87$, $p = .11$, compared to vehicle-treated mice.

Insert Figure 7 about here

mRNA expression. CR1, CRH, BDNF, Trk2. As in the case of acute administration, repeated IFN- α treatment did not alter expression of CRH₁ receptors, nor that of BDNF expression or that of its receptor gene NTRK2. This was the case in both the PFC and hippocampus (Data not shown).

Sickness behaviour. Sickness scores were recorded for 60 minutes following the fifth day of treatment. As seen in Figure 8, ANOVA revealed a main effect of Treatment, $F(1,34) = 16.32$, $p < 0.001$, indicating that IFN- α affected sickness at all times (20, 40, 60 minutes) with IFN- α -treated mice showing greater signs of sickness compared to vehicle-treated mice. The overall sickness scoring (data not shown) further demonstrated the difference in sickness behaviour between the two groups with IFN- α -treated mice displaying more sickness behaviour than vehicle treated mice, $F(1,55) = 31.79$, $p < .0001$.

Figure 7. Normalized mRNA expression of 5HT receptors in the PFC and hippocampus following repeated i.c.v. administration of IFN- α (1000 IU) or vehicle ($M \pm SEM$).

Insert Figure 8 about here

3.3. *Experiment 3. Anhedonia following repeated administration of IFN- α*

At the end of the habituation period and before treatment, sucrose and water intake were comparable in both the vehicle and IFN- α -treated groups.

Sucrose consumption was equally lowered in both groups following the first infusion of either vehicle or IFN- α (Figure 9a). Both treatment groups continued at this lowered level. After this initial decrease, a main effect was present for Treatment on sucrose consumption, $F(1,48) = 10.63$, $p < 0.01$. Furthermore, a significant change in sucrose consumption was apparent following the fifth infusion. The IFN- α -treated mice significantly lowered their consumption compared to vehicle-treated mice, and also when compared to consumption following the first infusion.

There was no main effect or interaction effect on sucrose preference. However, follow-up t test revealed a significant decrease in sucrose preference was apparent after the fifth infusion (Figure 9b). The IFN- α -treated mice significantly lowered their consumption, compared to baseline levels. The sucrose preference of vehicle-treated mice remained at baseline levels.

Insert Figure 9 about here

Figure 8. Sickness symptoms following 5 days of i.c.v. administration of IFN- α (1000 IU) or vehicle. ($M \pm SEM$)

Figure 9. Sucrose consumption and preference following repeated i.c.v. administration of IFN- α (1000 IU) or vehicle. ($M \pm SEM$) * $p < 0.05$

4. DISCUSSION

Activation of the inflammatory immune system has been implicated in the development of depressive disorders (Anisman, 2009; Dantzer et al., 2008; Maes, 1995). Moreover, there has been increasing evidence indicating that cytokines, through their actions on central neurochemical processes, may play a pivotal role in this regard (Anisman et al., 2008). Specifically, it has been shown that, the introduction of exogenous cytokines can lead to depressive behaviour similar to depression in humans as well as in animal models of depression. The use of IFN- α in the treatment of several diseases has become more prevalent owing to its ability to assist in viral clearance as well as boosting the immune system. However, a large sub-group of patients receiving IFN- α treatment manifest depressive symptoms (Capuron & Miller, 2004). The frequent occurrence of depression during IFN- α immunotherapy lends support to the possibility that interference with biological mechanisms related to immunity are involved in the onset of depression. Furthermore, it is this interference that through direct or indirect actions of this cytokine on CNS processes leads to depression.

Thus, the present investigation was undertaken to assess some of the effects of central administration of IFN- α on variations of central cytokine mRNA expression, neuroendocrine responses, and behavioural alteration involved in depressive disorders.

4.1. Behavioural effects induced by IFN- α

Rodents have been used to study the neurophysiologic modifications as well as the behavioural changes induced by IFN- α . However, the results that have been reported have been inconsistent. Whereas some investigators reported sickness behaviours and

reduced locomotor activity following treatment, others reported no such effects (Fahey et al., 2007; De Le Garza et al., 2005). This inconsistency was paralleled by reports regarding the effects of IFN- α on plasma corticosterone concentrations (De Le Garza et al., 2005; Anisman et al., 2007). The disparity reported can partly be due to the use of both human and rodent forms of IFN- α in animal experiments. Human IFN- α has inhibitory activity on mouse tumour cell growth (Tabata et al., 1991), but has been unable to produce consistent behavioural results; some reports showed dramatic depressive-like behavioural activity (Dunn & Crnic, 1993) whereas other indicated none at all (De La Garza et al., 2005). It would be expected that due to species differences, human IFN- α would not be able to have any actions in the mouse. This might be accurate since expression of IFN-regulated genes in brain parenchymal cells in mice was possible with the administration of mouse IFN- α , while human IFN- α had no such activity (Wang et al., 2008), demonstrating species-specific actions of cytokines. However, even the use of species-specific IFN- α has not yielded consistent behavioural outcomes (Orsal et al., 2008; Wang et al., 2009). Importantly, most studies regarding the behavioural (or biological) impact of IFN- α have actually not simulated the human condition that accompanies immunotherapy. Specifically, none of the studies used the extended series of treatments ordinarily given to humans. Moreover, the depressive outcome of patients undergoing treatment might reflect the synergistic actions of IFN- α and the emotional and/or physical stress that accompanies chronic illness. Indeed, when mice are exposed to a psychosocial stressor, the effects of IFN- α -induced behaviour, neuroendocrine, and neurochemical effects are greatly enhanced (Anisman et al., 2007).

The present investigation demonstrated that sickness behaviour was induced by repeated central infusions of IFN- α , whereas vehicle-treated mice showed practically no signs of sickness. Sickness behaviour, as mentioned earlier, closely resembles the vegetative behaviours associated with major depression. However, in this experiment, the precipitator of sickness behaviour cannot be known since IFN- α administration elevated glucocorticoid levels as well as activated an inflammatory response characterised by multiple cytokine variations. Furthermore, the administration of either IL-1 β or TNF- α (both of which were elevated in the experiment) is sufficient to induce sickness, however, when administered concurrently they act in a synergistic fashion (Brebner et al., 2000).

Of interest is the fact that sickness behaviour has not been reported following a single administration of IFN- α systemically or centrally. Reports have indicated that even though an active immune response is present, as indicated by elevated cytokines (specifically IL-1 β , which is central to sickness behaviour) and corticosterone levels, these factors have been unable to elicit sickness. As mentioned earlier, the combination of a social stressor in conjunction with a single IFN- α treatment is able to produce signs of sickness. However, as seen in the present study, following acute administration, only LPS-treated mice demonstrated a significant reduction in motor activity, an indication of sickness behaviour, whereas with repeated administration of IFN- α sickness behaviour emerged. The underlying reason for this is unknown but it is possible that unlike LPS, IFN- α is unable to elicit a large enough immune and neuroendocrine response necessary to alter behaviour. It is only with repeated exposure to elevated levels of proinflammatory cytokines and corticosterone that sickness behaviour becomes apparent.

Decreased consumption of sucrose has been used as a measure of anhedonia in rodents, a key component of depressive illness. In the present investigation, IFN- α induced a significant decrease in sucrose consumption after 5 days of infusions. This supports previous reports that systemic IFN- α in a rat was able to induce anhedonia, and was subsequently reversed with antidepressants (Sammut, et al., 2002). However, the magnitude of the effect observed in the present study was admittedly small. The concentration of sucrose used in the present investigation was one that leads to an exceptionally high preference for sucrose, which might have obscured the impact of IFN- α . This is clearly speculative and additional studies are obviously necessary to assess the influence of the cytokine under these conditions. Alternatively, as signs of anhedonia were evident on the last day of IFN- α treatment, it might be the case that more sustained treatment is needed to induce significant anhedonia. Indeed, in humans, the neurovegetative effects of IFN- α appear relatively soon after treatment begins (i.e., within a few weeks) and the mood changes, including anhedonia, appear later (Capuron et al., 2002). Thus, with a more chronic regimen the anhedonic effects of IFN- α might have become more notable.

4.2. Cytokine variations in brain induced by IFN- α

In the current study, administration of IFN- α directly into the brain markedly increased IL-1 β , IL-6 and TNF- α expression in brain regions associated with depressive disorders. Previous reports have demonstrated the ability of IFN- α to induce an inflammatory response (Wichers et al., 2006), however, there has been little study regarding the cytokine activation in the CNS, regardless of method of delivery. The

present experiment is consistent with the view that the depressive actions of IFN- α could be a result of induction of proinflammatory cytokines in the CNS.

The current study indicated that acute administration of IFN- α provoked significant increases of proinflammatory cytokine expression in the PFC and hippocampus. Interestingly, with repeated administration, the elevated IL-6 expression was more profound in the hippocampus, whereas acute administration activated IL-6 expression to a greater extent in the PFC. Although both of these brain regions have been implicated in depression, long-term hippocampal inflammation has been associated with depressive behaviour (Fu et al., 2010; Curran & O'Connor, 2001). Furthermore, the cognitive disruptions that accompany IFN- α treatment most likely stem from alterations in the hippocampus, given the role of this region in learning and memory (Squire, 1998; Greene, 2007). As the hippocampus expresses a particularly high density of IL-1 receptors (Parnet et al., 2002), it might also be relatively susceptible to the adverse consequences of neuroinflammation.

The contribution of specific proinflammatory cytokines (IL-1 β , IL-6, or TNF- α) in IFN- α -induced depression is uncertain. Even in the present experiment, in which IFN- α provoked upregulation of each of these cytokines does not aid in differentiating their individual contribution to this illness. However, a specific role for IL-6 has gained increasingly greater support as a contributing factor in depression. Clinical studies have demonstrated elevated circulating IL-6 levels in depressed patients, which are normalized with antidepressants drugs (Basterzi et al., 2005). Further evidence has come from two meta-analyses, one reporting that both IL-1 and IL-6 were positively associated with depression (Howren et al., 2009), and the second that implicated significantly higher

levels of IL-6 in depressed patients compared to controls (Dowlati et al., 2010).

Paralleling these conditions, immunotherapy with IFN- α in patients was also associated with increased IL-6, and patients with elevated plasma IL-6 prior to treatment have been found to be especially vulnerable to subsequent IFN- α -induced depression (Wichers et al., 2006), possibly accounting for the variability that exists concerning the depressogenic effects of IFN- α .

In the present experiment, proinflammatory cytokine mRNA expression was upregulated following repeated administration of IFN- α in both the PFC and hippocampus. Although IL-6 upregulation was significantly more robust, mRNA of other proinflammatory cytokines was also elevated. Proinflammatory cytokines, like IL-1 β and TNF- α , have repeatedly demonstrated their ability to induce depressive behaviour in animal models in that they induce a pattern of behavioural alterations that closely resemble the vegetative symptoms of depression in humans (e.g., anorexia, anxiety, reduced motor activity) (Dantzer et al., 2008). Further, peripheral and central administration of IL-1 β induces the production of proinflammatory cytokines, activates the HPA axis, and induces behavioural depression (Anforth et al., 1998). By contrast, administration of IL-6 induces some similarities with IL-1 β with respect to immune functioning, however, the appearance of behavioural changes have not been reported (Bluthe et al., 2000). It thus seems plausible that the presence of IL-6 potentiates the behavioural effects of IL-1 β , but has little effect on its own. The synergy of IL-1 β and IL-6 is clearly demonstrated in mice that lack IL-6 expression. These mice are less sensitive to the behavioural effects brought on by inoculation with LPS or by IL-1 β injected peripherally or centrally (Bluthe et al., 2000).

Noteworthy, is the finding of upregulation of anti-inflammatory cytokine IL-10 expression following repeated IFN- α administration, which was only present in the PFC. Previous studies report elevated plasma IL-10 levels induced by IFN- α , however, only in conjunction with a social stressor (Anisman et al., 2007). That said, IL-10 may play a fundamental role in depression as analyses of postmortem brains of depressed patients indicated an upregulation of IL-10 and IL-6 in the PFC (Shelton et al., 2010). Elevated plasma IL-10 has also been shown to be a correlative factor in depression in patients undergoing immunotherapy with IFN- α . In particular, plasma concentrations of IL-10 were significantly higher in patients that developed major depressive disorder during IFN- α treatment than in those that did not (Wichers et al., 2006). This does not necessarily suggest that IL-10 is involved in the provocation of depression, as elevated levels of the anti-inflammatory might be indicative of an attempt to counter the mounting proinflammatory immune activation brought about by IFN- α . Indeed, some antidepressants enhance IL-10 production as well as reducing levels of the proinflammatory cytokines TNF- α and IFN- γ (Brustolim et al., 2006). The upregulation of IL-10 in the PFC could be significant indicator of modifications involved in IFN- α -induced depression and further studies in the area might prove valuable.

4.3. Serotonin receptor alterations in the brain following IFN- α administration

Studies in humans have suggested that diminished availability of 5-HT, the 5-HT transporter or variations of particular 5-HT receptors are linked to depression (Albert & François, 2010). As alluded to earlier, disturbances related to 5-HT and several 5-HT receptors have been implicated in major depressive disorders, prompting analysis of 5-

HT receptor mRNA expression in response to IFN- α . For instance, in both human and animal studies, 5-HT_{1A} (Nishi et al., 2009), 5-HT_{1B} (Sari, 2004), and 5-HT_{2A} (Bhagwagar et al., 2006) play an important role in affective disorders, as well as the actions involved in the effectiveness of antidepressants. However, data from animal and human studies has focused on 5-HT levels and its metabolites, and less attention is given to the specific actions of 5-HT receptors. It is for this reason that the present experiment analyzed 5-HT receptor expression in response to IFN- α treatment.

The serotonin changes induced by IFN- α in the present study are in line with other experiments using rats that implicated 5-HT functioning in depression. Previous reports indicate that a single i.c.v injection of IFN- α (200 or 2000 IU) reduced 5-HT and NE levels in the frontal cortex (Kamata et al., 2000). Additionally, repeated systemic administration of IFN- α reduced 5-HT levels in the PFC (Asnis et al., 2003). However, these experiments used recombinant human IFN- α , and the lack of species-specific cytokine could result in varied outcomes, as previously discussed. This is seen in the fact that Kamata et al found no consistent alteration in monoamines in another study (Kamata et al., 1999).

The present study demonstrated 5-HT receptor alterations following administration of murine IFN- α . In the PFC, there was a decrease in 5-HT_{1B} and 5-HT_{2A} receptor mRNA following acute administration of IFN- α , whereas in the hippocampus, IFN- α decreased 5-HT_{1A} receptor mRNA and increased 5-HT_{2A} receptor mRNA (this increase was also observed following repeated administration).

The decreased levels of 5-HT_{1B} mRNA in the PFC observed in this study could be a factor in the depressive behaviours induced by IFN- α . Brain alterations of this receptor

in the PFC have been reported to be involved in depression. Specifically, postmortem studies of brains revealed reduced levels of 5-HT_{1B} mRNA in individuals with major depression (Anisman et al., 2008). Inasmuch as 5-HT_{1B} receptor is present in particularly high density in the PFC may account for its involvement in mood, and for the fact that a similar outcome was not evident in the hippocampus.

Administration of IFN- α decreased hippocampal 5HT_{1A} receptor mRNA and increased 5HT_{2A} receptor mRNA expression. The role of the 5-HT_{1A} receptor in depression is well established, as indicated by the downregulation of 5-HT_{1A} receptors as a necessary action for the SSRIs to be effective (Artigas et al., 1996). The IFN- α - induced reduction in this receptor reported is consistent with other reports. Cells treated with IFN- α showed a significant decrease in the expression 5-HT_{1A} (Cai et al., 2005). Furthermore, a genetic polymorphism for the 5-HT_{1A} receptor has been linked to the onset of IFN- α -induced depression in patients undergoing immunotherapy (Kraus et al., 2007).

Contrary to previous reports, the present experiment showed decreased 5-HT_{2A} expression in PFC and elevated levels in hippocampus following IFN- α administration. The increase in 5-HT_{2A} expression observed could be due to decreased 5-HT following repeated IFN- α administration, since reduced serum 5-HT and its metabolites is highly correlated to the degree of depression during IFN treatment (Horsmans, 2006). It was previously reported that chronic lack of stimulation by 5-HT produces an upregulation of 5-HT₂ receptors in the cortex (Stockmeier & Kellar, 1986). However, the present study reported that an increase in 5-HT_{2A} expression was also present following acute administration, indicating that an upregulation could occur without chronically low levels

of 5-HT. Regardless, 5-HT_{2A} receptors have been shown to have a significant role in the modulation of mood state, consistent with their widespread distribution in brain regions known to modulate mood responses, including the hippocampus (Weisstaub et al., 2006). Parenthetically, it has been hypothesized that acute changes in 5-HT could be due solely to a toxic reaction and may not be due to IFN- α (Sato et al, 2006). However, the doses used in the present experiment were far below what was thought to be toxic levels.

The reduced 5-HT receptor expression in the PFC and hippocampus in mice given a moderate-dose of IFN- α suggests an important role of 5-HT in IFN- α -induced depression. The alterations of the 5-HT system could be induced by elevated cytokine expression, specifically IL-6. In the CSF of patients undergoing IFN- α therapy, increased IL-6 was associated with decreased accumulation of the 5-HT metabolite, 5-HIAA, which, in turn, is correlated with IFN- α -induced depression (Raison et al., 2009). Furthermore, genetic variations in the serotonin transporter gene (SLC6A4) is significantly associated with both increased depressive symptoms and elevated IL-6 plasma levels (Su et al., 2009). Modification in 5-HT and its contribution to IFN- α -induced depression is demonstrated by the fact that IFN- α -induced depression clinically responds to SSRIs (Mussleman et al., 2003).

4.4. Corticosterone variations associated with IFN- α

It is unlikely that actions of proinflammatory (or anti-inflammatory) cytokines standing alone can lead to depressive disorders. The presence of proinflammatory cytokines, especially IL-6, is most likely works in concert with other systems leading to the onset of depressive symptoms. Along with increased proinflammatory cytokine

mRNA, plasma corticosterone levels were significantly elevated following acute administration of IFN- α , and the elevated levels were still evident in animals that received 6 days of infusions.

Corticosterone alteration could potentially be influenced by IL-6 changes elicited by IFN- α . Acute IFN- α administration to healthy volunteers and to hepatitis patients significantly increased ACTH, CRH, as well as circulating IL-6 (Cassidy et al., 2002). Furthermore, IL-6 has an additive effect with CRH, where IL-6 appears to induce the secretion of CRH and, thereby, ACTH (Matta et al., 1992), which could lead to a spiral into depressive symptomatology. In non-human primates, IFN- α treatment increases peripheral ACTH and IL-6 in association with the development of anxiety and depressive symptoms (Felger et al., 2007), supporting the perspective that the grouping of IL-6 and corticoids might be the necessary factor in the onset of depressive behaviour. Of course, the experiments necessary to assess this possibility (e.g., assessing IFN- α effects in the absence of corticosterone changes) have yet to be performed.

Since communication occurs between the immune, endocrine, and central nervous systems, activation of the inflammatory responses can affect neuroendocrine processes, or vice versa. In support of this connection, mice with deletion of the IL-1 receptor lack an HPA neuroendocrine response following mild chronic stress, and the chronic administration of corticosterone or exogenous administration of IL-1 β produced depressive symptoms both in wild type and knockout mice (Goshen et al., 2009). Furthermore, mice lacking the IL-1 receptor exhibited adrenal hypertrophy and elevated serum corticosterone levels in response to a social stressor (Engler et al., 2008). However, the most direct evidence of a role for CRH in IFN-behavioural disturbances

comes from data demonstrating that IFN- α –induced depressive-like behaviour in rodents (as assessed by immobility in the tail suspension test) can be abolished by pretreatment with a CRH receptor antagonist (Yamano et al 2000).

Increased levels of IL-6 receptor mRNA have been found in parvocellular neurons of the hypothalamic paraventricular nucleus following peripheral administration of LPS (Vallières & Rivest, 1997) raising the possibility that IL-6 may have direct actions on hypothalamic CRH neurons. The interaction between inflammatory cytokines and glucocorticoids, and their combined ability to induce depression, is becoming clearer. It appears that IL-1 β and TNF- α can act directly on the hypothalamus to induce CRH gene expression and CRH release (Turnbull & Rivier, 1999; Schmidt et al, 1995). Furthermore, transgenic mice with elevated expression of IL-6 in the CNS exhibit increased glucocorticoid levels after stress (Raber et al., 1997), suggesting an increased stress sensitivity with a potential vulnerability for the development of depression. In fact, a recent study of comorbidity of depression and cancer concluded that depression in cancer patients is associated with increased plasma IL-6 concentrations and dysfunction of the HPA axis (Jehn et al., 2010).

The HPA alteration observed in depressed patients could be the result of biological anomalies that lead to a predisposition to depression. Indeed, IFN- α treatment of a mouse hippocampal cell line disrupted glucocorticoid receptor functioning, a process that may be a factor in the development of depression in patients receiving IFN- α treatment (Hu et al., 2009). In this regard, it was reported that atypical antidepressants as well as SSRIs (e.g., fluoxetine) attenuated glucocorticoid action by lowering hippocampal receptor levels in rats (Szymanska et al., 2009).

4.5. Conclusion

Treatment with IFN- α elevates brain cytokines and plasma corticosterone, and could potentially mediate some of the behavioural effects elicited by this cytokine. As central infusion of IFN- α , at a dose far below that which would induce any effects if administered systemically, suggests that central processes activated by IFN- α are responsible for the behavioural effects provoked. The cytokine response, neuroendocrine upregulation, and serotonergic modifications elicited by IFN- α have been observed in studies that involved systemic administration or directly into the brain. These findings suggest that, 1) systemic IFN- α is able to cross the BBB or activate peripheral processes that activate central processes, and that, 2) systemic exogenous IFN- α is able to induce production of IFN- α in the CNS. Raison et al (2009) reported that pegylated IFN- α administered peripherally leads to increases in CSF IFN- α in patients undergoing immunotherapy, although they were not able to determine if the IFN- α in the CSF was the exogenous IFN- α or if was produced following treatment (Raison et al., 2009). The ability of IFN- α to access the brain following systemic administration indicates that the neurobehavioral actions are most likely centrally mediated, even during systemic treatment.

In conclusion, the increased proinflammatory cytokine production, concomitant elevations in HPA axis activity and serotonergic modifications following treatment with IFN- α , together, are hypothesized to be responsible for the behavioural alterations, as demonstrated by sickness and decreased sucrose consumption.

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