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BEHAVIOURAL, NEUROENDOCRINE AND CENTRAL NEUROTRANSMITTER
CHANGES ASSOCIATED WITH IMMUNE ACTIVATION AND
CYTOKINE CHALLENGE

by

Susan Lacosta

Dissertation presented to
the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
Department of Psychology

Carleton University
Ottawa, Ontario

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Abstract

Multidirectional communication may exist between the immune, neuroendocrine and central nervous systems. In fact, the view was offered that cytokines may act in a signalling capacity to inform the central nervous system (CNS) of immunological challenge. The present investigation assessed the behavioural, neuroendocrine and neurochemical effects of acute and chronic systemic interleukin (IL)-1 β and IL-2 administration, particularly in brain regions sensitive to stressors. Moreover, since IL-1 β and IL-2 promote the release of corticotropin-releasing hormone (CRH), a potent anxiogenic peptide, it was of interest to establish whether these cytokines elicit anxiety. Finally, since IL-2 has been implicated in depression, and IL-2 immunotherapy elicits cognitive disturbances (particularly in spatial tests), the acute and chronic effects of this cytokine were examined in several variants of the Morris water-maze test.

The effects of systemic IL-1 β administration were evident with respect to locomotor activity and exploration of a novel stimulus object, while habituation/exploration in a free-running spontaneous alternation task was unaffected by the cytokine treatment. In addition, systemic IL-1 β administration provoked an anxiogenic-like response in mice tested in an elevated plus-maze. These effects were paralleled by dose-dependent increases of plasma ACTH and corticosterone concentrations, alterations of norepinephrine (NE) levels and/or utilization within the paraventricular nucleus, median eminence plus arcuate nucleus, locus coeruleus and prefrontal cortex, as well as dopamine (DA) turnover in the median eminence plus arcuate nucleus. *In vivo*, systemic administration of IL-1 β provoked a modest

increase of extracellular 5-HIAA from the nucleus accumbens and the hippocampus, and these effects were augmented by a mild stressor. It appears that IL-1 β may influence forebrain monoamines, and may act either additively or synergistically with stressors in promoting these effects.

In contrast to IL-1 β , acute, systemic IL-2 administration did not affect exploratory behaviours, locomotor activity, anxiety, approach to a novel stimulus, habituation measured by spontaneous alternation performance or spatial memory (in a Morris water-maze). However, like the cognitive disturbances associated with chronic IL-2 immunotherapy, chronic treatment with the cytokine impaired Morris water-maze performance when the position of the escape platform varied over days. Neither acute nor chronic IL-2 treatment influenced plasma ACTH or corticosterone levels. However, while acute administration of the cytokine had only modest effects on central monoamine activity, chronic IL-2 provoked widespread, central variations of NE, DA and 5-HT within the locus coeruleus, median eminence plus arcuate nucleus, hippocampus, central nucleus of the amygdala, prefrontal cortex, caudate nucleus, and substantia nigra.

Like IL-1 β , the bacterial endotoxin, lipopolysaccharide (LPS) induced a sickness-like behavioural profile (reduced active behaviours, soporific effects, piloerection, ptosis), which varied with the novelty of the test environment. In addition, systemic LPS provoked an anxiogenic-like response in an elevated plus-maze and light-dark box tests, and decreased contact with a novel stimulus object in an open-field situation. These effects were accompanied by increased plasma ACTH and corticosterone levels, as well as increased turnover of NE, DA and 5-HT in several stress-sensitive brain regions. Although LPS elicits

an anxiogenic-like response, it is unclear whether this effect is secondary to the illness engendered by the endotoxin. Nevertheless, LPS effectively promoted an array of behavioural and neurochemical alterations which are commonly associated with stressful experiences.

Finally, administration of sheep red blood cells (SRBC: 5×10^6) to rats elicited an increase of *in vivo* extracellular DA in the nucleus accumbens, at a time which coincided with the peak immune response (4 days following inoculation). These data are consistent with the supposition that antigenic challenge influences central neurotransmitters, and indicates that such effects are not restricted to the hypothalamus, but are apparent in mesolimbic regions. Moreover, as SRBC does not cause illness, at least some of the neurochemical changes appear to be independent of treatment-induced malaise.

Evidently, cytokine treatments induce a constellation of behavioural, neuroendocrine and central neurochemical changes, some of which are reminiscent of those elicited by stressors. It appears that IL-1 β and IL-2 elicit distinctively different behavioural effects; IL-1 β was associated with anxiogenesis, while IL-2 had no such effect. However, when applied chronically, IL-2 influenced spatial memory. At this juncture, it is premature to ascribe the cytokine-induced behavioural changes to specific amine variations elicited by the treatments; however, these data support the view that cytokines may act as immunotransmitters, and also implicates these cytokines in behavioural pathologies. Further, these data reinforce the position that considerably more attention needs to be devoted to the analysis of behavioural/cognitive consequences of cytokines, particularly in light of their increasing usage within therapeutic settings.

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To my parents and to my husband Fred

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1. General Introduction

It is now well established that multidirectional communication exists between the immune, neuroendocrine and central nervous systems (Anisman et al., 1993; Blalock, 1994; Dantzer & Kelley, 1989; Dunn, 1990; Rabin et al., 1989). Central neurochemical and neuroendocrine manipulations may influence immune functioning (Anisman et al., 1993; Keller et al., 1991), while changes in immune activity influences central neurotransmitter and neuroendocrine processes (Anisman et al., 1993). The view has been expressed that the immune system may act as a sensory organ, informing the brain of immunologic challenge (Blalock, 1994) and that cytokines may act as the messengers between the immune system and CNS (Dunn, 1990,1991). In this respect, several macrophage-derived cytokines such as interleukin (IL)-1 β , IL-6, TNF- α , as well as IL-2 secreted from T helper cells, have received particular attention as possible immunotransmitters (Blalock, 1984; Dunn, 1990; Imura et al., 1991; Koenig, 1991; Rivier, 1995). Essentially, it was suggested that the brain may interpret activation of the immune system (e.g., increased levels of cytokines) as it would a stressor. Indeed, immune activation may elicit neuroendocrine and central neurotransmitter alterations like those provoked by stressors (Anisman et al., 1991; Dunn et al., 1989,1991; Dunn & Welch, 1991). Accordingly, it might be expected that the immune system, like stressors, may contribute to the symptoms of behavioural pathologies (e.g, depression, anxiety related disorders and chronic fatigue syndrome) (Anisman et al., 1993).

Commensurate with the view that IL-1 β may be acting as an immunotransmitter (Dunn, 1990), there have been several reports showing that central or systemic administration of this cytokine, may engender neuroendocrine and central neurochemical

changes. In the main, the assessment of the neurochemical alterations associated with cytokine treatment have been restricted to the hypothalamus, and there is little information available concerning the effects of cytokines on transmitter changes in other brain regions. Furthermore, it is curious that although cytokines have been employed to an ever greater extent in clinical treatments, little data is available concerning the behavioural effects of cytokine treatment. This is all the more surprising when it is considered that in the case of some cytokines (e.g., IL-2), clinical treatment may promote neuropsychiatric symptoms of sufficient severity to require discontinuation of treatment (Caraceni et al., 1993).

In view of the limited data available concerning the behavioural effects of cytokine treatment, one objective of the present investigation was to assess several fundamental behavioural changes associated with systemic IL-1 β administration. Moreover, in the present investigation, an analysis was undertaken concerning the HPA endocrine variations, as well as the central neurochemical consequences elicited by IL-1 β and IL-2 administration. In addition, the behavioural, neuroendocrine and neurochemical consequences of the bacterial endotoxin lipopolysaccharide (LPS) were assessed. This was done since LPS is known to profoundly influence IL-1 β , IL-6 and TNF- α secretion. After all, the possibility needs to be considered that macrophage activation induced by immune challenge provokes release of all three proinflammatory cytokines, as opposed to simply IL-1 β . Thus, the behavioural and biological sequelae of immune challenge might not be accurately ascertained by analysis of the IL-1 β effects alone. By evaluating the effects of both IL-1 β and LPS it can be determined whether congruent activities are induced by these treatments.

The sections that follow provide a broad outline of the effects of immunological

activation (e.g., viral, endotoxin, cytokine) on several aspects of central neurotransmitter, neuroendocrine and behavioural function. In this respect, particular attention will be devoted to the analysis of IL-1 β treatment, as this cytokine has been posited to be the major potential mediator between the neuroendocrine and immune systems.

2. Effects of Immunological Activation

2.1. Central Neurotransmitter Alterations

The position that communication exists between the immune, neuroendocrine and central nervous systems has been supported by the finding that immunological activation influences central neurotransmission. In particular, administration of sheep red blood cells (SRBC) was reported to increase hypothalamic unit activity (Besedovsky et al., 1977; Saphier et al., 1987), increase norepinephrine (NE) turnover (as determined by MHPG accumulation) and reduce NE levels in the hypothalamus. Interestingly, these effects occurred at times which coincided with the peak immune response being established (i.e., 96 hrs post-immunization) (Besedovsky et al., 1983; Dunn et al., 1989; Zalcman et al., 1991). Such alterations of NE activity following SRBC inoculation were not only observed within the whole hypothalamus, but were also noted when assays were restricted to the paraventricular nucleus, which is known to play a prominent role in HPA functioning (Carlson et al., 1987). Moreover, serotonin (5-HT) alterations were detected within the paraventricular and supraoptic nuclei following treatment with SRBC (Carlson et al., 1987).

Although the interrelationship between immune activation and HPA activity has focused on variations of hypothalamic neurotransmitter activity, it has become clear that the

effects of antigenic challenge are evident in other regions as well. In particular, it has been demonstrated that SRBC inoculation influenced NE turnover in both the locus coeruleus and hippocampus (Zalcman et al., 1991). Moreover, 2 days following inoculation with SRBC, reduced hippocampal 5-HT levels were observed, while the levels in the nucleus tractus solitarius were increased (Carlson et al., 1987). As well, SRBC treatment elicited variations of dopamine (DA) turnover and concentrations within the prefrontal cortex and nucleus accumbens at a time corresponding with the immune response being mounted (Zalcman et al., 1991). Of particular interest, however, was the finding that, like stressors, antigenic challenge provoked changes in mesocorticolimbic DA but failed to alter DA within nigrostriatal regions (Zalcman et al., 1991).

Commensurate with these findings, variations in neurotransmitter function have also been demonstrated following mitogen and viral challenge. Indeed, pokeweed mitogen enhanced NE, DA and 5-HT turnover in the hypothalamus, while increased hypothalamic NE, DA, 5-HT and epinephrine turnover was reported 4 hours following administration of the T cell mitogen, Con A (Mefford & Heyes, 1990). Likewise, BALB/c mice infected with the influenza virus, PR8(H1N1), exhibited increased NE turnover in the hypothalamus, as well as tryptophan levels in both the hypothalamus and parietal cortex (Dunn et al., 1989), while exposure to Newcastle disease virus (NDV) enhanced hypothalamic NE and 5-HT concentrations (Dunn & Vickers, 1994).

2.2. Neuroendocrine Variations Following Immunological Challenge

There have been numerous reports indicating that immunological activation

influences HPA functioning. For instance, Besedovsky et al. (1975) demonstrated that SRBC administration provoked a time-dependent enhancement of plasma corticosterone concentrations. Shek & Sabiston (1983) reported an elevation in corticosterone levels on the day of the peak plaque-forming cell (PFC) response to SRBC inoculation; however, this effect was only observed among mice that were high immune responders. Treatment with SRBC also altered normal circadian fluctuations in corticosterone levels on the day of the peak immune response. Specifically, SRBC augmented plasma corticosterone concentrations at 0800 hr, when levels are typically low and diminished corticosterone concentrations at 16:00 hr when levels are normally high (Shek & Sabiston, 1983). However, other investigators failed to detect such an outcome. For instance, Rivier, (1995) indicated that SRBC did not influence HPA functioning, while Shanks et al. (1994) were unable to detect such an outcome under several conditions, even though marked variations of central monoamines were detected. While there has been some question as to the reliability of SRBC effects on HPA functioning, activation of the immune system following viral challenge have been shown to result in endocrine variations. For instance, enhanced plasma corticosterone levels were reported in BALB/c mice exposed to influenza virus, PR8(H1N1) (Dunn et al., 1989), while Newcastle disease virus (NDV) was found to elicit increased plasma adrenocorticotropin hormone (ACTH) and corticosterone concentrations (Dunn & Vickers, 1994).

3. The Role of Cytokines in Central Neurochemical, Neuroendocrine and Behavioural Processes

As mentioned previously, immunological challenge gives rise to variations of CNS activity. It is less certain, however, what factors may be mediating such an immune-CNS interaction. Several factors have been proposed as potential mediators, including activation of the vagal nerve (Watkins et al., 1995a) and endocrine release from activated lymphocytes (Blalock, 1984, 1994). As indicated earlier, however, particular attention has been devoted to the involvement of several macrophage-derived cytokines, such as interleukin (IL)-1 β , IL-6, and TNF- α , as well as IL-2 (a cytokine secreted from T helper cells) in this respect. The majority of the research has focused on the role of IL-1 β as the principal mediator between the immune and central nervous systems, and less data are currently available concerning the roles of IL-2, IL-6 and TNF- α . However, as will be seen later, several of these cytokines may be acting synergistically in affecting physiological functioning, and may be acting in a similar fashion with respect to neuroendocrine and central transmitter processes.

3.1. Interleukin-1

3.1.1. Structure, Synthesis and Secretion

Interleukin-1, a 17 kDa peptide, exists in two structurally related forms (e.g., IL-1 α and IL-1 β) which are produced by separate genes and share approximately 25% homology (Cunningham & De Souza, 1993). Both IL-1 α and IL-1 β are produced as large 31 kDa precursor peptides, which are subsequently cleaved into smaller 17 kDa peptide C-terminal fragments. Generally, IL-1 α remains in its biologically active precursor form in the cytosol

of cells where it functions in an autocrine manner. IL-1 α may also be transported to the cell surface, where it serves as a paracrine messenger to adjacent cells (Dinarello & Wolff, 1993). IL-1 β , on the other hand, is the primary secreted form of this cytokine and unlike IL-1 α , must be intracellularly cleaved into its biologically active form by interleukin-1 β converting enzyme (ICE) before being released into the circulation (Dinarello & Wolff, 1993).

Inasmuch as IL-1 β appears to be more potent than IL-1 α , it has been proposed that IL-1 β may be more important in subserving the neuronal, neuroendocrine and metabolic changes, as well as the behavioural alterations that may follow from immunologic activation (Dunn, 1995). However, it is important to underscore that IL-1 has diverse actions within the brain, and it is not known whether the effects of IL-1 α and IL-1 β have comparable effects across different brain regions. Likewise, it remains to be determined whether the relative potency of these cytokine subtypes are similar across species. Certainly it seems that the differential effects of hIL-1 α and IL-1 β observed in rats (Rivier, 1995) may not be evident in mice (Fukata et al., 1993; Naito et al., 1989).

In addition to IL-1 α and IL-1 β , an endogenous antagonist for the IL-1 receptor (IL-1ra) has recently been identified (Hannum et al., 1990). It appears that IL-1ra is synthesized centrally by neurons and peripherally by macrophages, before being release into the circulation (Eisenberg et al., 1990). The IL-1ra acts as a specific inhibitor of IL-1 activity by blocking the binding of IL-1 to its cell-surface receptor, although its role in neural and neuroendocrine function remains to be fully elucidated (Licinio et al., 1991).

3.1.2. Source of IL-1 in the CNS

In addition to being derived from macrophages, IL-1 is produced centrally by glial cells (astrocytes and microglia) and to a lesser extent by neurons (see review by Dinarello, 1988). A high density of IL-1 has been identified in the hippocampus and several hypothalamic nuclei, with fibre-like projections extending to other brain regions. Specifically, IL-1 immunoreactive fibers have been located in the ventromedial nucleus of the hypothalamus, the posterior hypothalamus, the hypophysial vessels of the median eminence, the stria terminalis and the subfornical organ (Breder et al., 1988).

3.1.3. IL-1 Receptors in the CNS

Interleukin-1 exerts its biological effects by binding to specific high affinity IL-1 receptors. Presently, two distinct IL-1 receptors (e.g., IL-1R1 and IL-1R2) have been identified within both the brain and endocrine organs. The type 1 IL-1 receptor (IL-1R1) appears to bind IL-1 α and IL-1 β with similar affinity while the type II IL-1 receptor (IL-1R2) exhibits a much higher affinity for IL-1 β than for IL-1 α (Benveniste, 1992). Furthermore, radioligand binding studies have suggested that IL-1R1 is the major IL-1 receptor in the mouse CNS (Cunningham & De Souza, 1993).

Centrally, IL-1R1 has been identified in a wide array of brain regions, including the dentate gyrus, choroid plexus, hippocampal CA3 pyramidal neurons, 5-HT neurons in the dorsal raphe and hormone-producing cells in the anterior pituitary (Cunningham & De Souza, 1993). Localization of IL-1R1 mRNA also includes neurons in the anterior dorsal nucleus of the thalamus, sensory neurons of the mesencephalic trigeminal nucleus and

Purkinje cells in the cerebellum, although the functional relevance of these findings needs further clarification (Cunningham et al., 1992).

Curiously, although the hypothalamus is a major site of action for IL-1 in the brain, the existence of IL-1 receptors in this region remains controversial. Indeed, Katsuura et al. (1988a) described an IL-1 receptor on hypothalamic membranes that binds IL-1 β but not IL-1 α , and Takao et al. (1993b) revealed the existence of IL-1R1 on hypothalamic membranes. However, other researchers failed to detect mRNA for IL-1R1 in the hypothalamus (Rothwell, 1991). As more information about IL-1 receptors becomes available, this discrepancy may become resolved.

Although the existence of hypothalamic IL-1 receptors is debatable, Cunningham et al. (1992) suggested that IL-1 may indirectly influence the hypothalamus by way of the raphe and/or hippocampus. Indeed, IL-1R1 produced in the raphe (known to have widespread projections throughout the CNS) is believed to be transported to axon terminals innervating the hypothalamus and cerebral cortex (an area also thought to be void of IL-1 receptors). In addition, IL-1 may influence hypothalamic neurons indirectly by way of hippocampal inputs routed through the bed nucleus of the stria terminalis (BNST). Such pathways might explain the known effects of IL-1 on both hypothalamic activities (e.g., fever, appetite and HPA function) and cortical functions (e.g., sleep) (Cunningham et al., 1992).

In addition to neuronal receptors, IL-1R1 mRNA has also been detected on vascular structures (e.g., in endothelial cells of post-capillary venules and endothelial cells within the choroid plexus) (Cunningham et al., 1992) and astrocytes (Haour et al., 1990). As will be discussed later, the presence of IL-1 receptors on vascular structures may provide further

insight as to the mechanisms by which circulating IL-1 gains access to the CNS.

3.1.4. Central Actions of IL-1 β

The presence of endogenous IL-1 and IL-1 receptors in the brain, suggests a role for this cytokine in modulating CNS functioning (Schobitz et al., 1994). For instance, IL-1 β has been shown to play a pivotal role in modulating HPA activity following immune challenge, primarily by acting at the level of the hypothalamus. Systemic IL-1 β administration readily induces the release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus (PVN), which promotes pituitary ACTH release and hence adrenal glucocorticoid secretion (Berkenbosch et al., 1990; Dunn, 1990; Rothwell, 1991). Central neurotransmitter turnover is also influenced by IL-1 β treatment, although, as will be discussed shortly, most of the research has focused on the hypothalamus and less information is available concerning changes in extrahypothalamic sites. In addition to influencing neuroendocrine and neurotransmitter function, IL-1 β induces an array of behavioural symptoms typically referred to as sickness behaviour (Kent et al., 1992b). Indeed, apart from inducing fever, IL-1 β has been reported to provoke anorexia, sleep and reduce both locomotor activity and exploratory behaviour (Kent et al., 1992b). Many of the symptoms of sickness behaviour can be induced by both peripheral or central administration of IL-1 β . Moreover, central administration of IL-1ra antagonizes the effects of peripheral IL-1 β treatment, suggesting that IL-1 β -induced sickness behaviour likely involves central processes (Kent et al., 1992a; Rothwell, 1991).

In the sections that follow, a description of the role of IL-1 β in modulating HPA

activity will be provided, along with a discussion of the possible mechanisms by which circulating IL-1 β is believed to enter the brain. Moreover, the synergistic actions of IL-1 β with other cytokines and their role in modulating HPA activity will be outlined. Next, a brief description of the central effects of IL-1 β on immune function, as well as the role of this cytokine in neuropathology will be given. This will be followed by a review of the neurotransmitter and behavioural effects elicited by IL-1 β treatment.

3.1.5. Role of IL-1 β in Modulating the HPA Axis

As indicated earlier, the immune system is capable of modulating neuroendocrine responses through various messengers which are released by activated immune cells. In fact, Blalock and his colleagues proposed that activated lymphocytes secrete an ACTH-like substance which stimulated the CNS either directly or indirectly. It was shown for example, that following hypophysectomy, immune challenge was able to increase plasma ACTH levels, possibly reflecting the release of ACTH from activated lymphocytes (Blalock, 1984). However, Dunn (1990) indicated that Blalock's findings may have been due to incomplete pituitary ablations (e.g., an ACTH source distinct from lymphocyte-ACTH secretion). Thus, while lymphocytes might well have been the source for ACTH in Blalock's studies, it is equally possible that ACTH released from remnants of pituitary were responsible for the observed endocrine variations. While the possibility still exists that ACTH may play some role in mediating the immune-CNS communication, more recent views concerning activation of the HPA axis during immune responses have largely focused on the contribution of IL-1 β . However, IL-6 and TNF- α also appear to play a role in this respect (Berkenbosch et al.,

1987; Besedovsky et al., 1986; Blalock, 1994; Sapolsky et al., 1987). It seems that IL-1 β modulates the HPA axis by acting primarily at the level of the hypothalamus (Gaillard, 1994), although direct effects on the pituitary and adrenal cells have been proposed (Andreis et al., 1991; Blalock, 1994).

Following an immune challenge, activated immune cells (e.g., macrophages) secrete IL-1 β into circulation. It has been proposed that the circulating IL-1 β may gain access to the brain, albeit in very limited amounts. Indeed, IL-1 β is a large molecular weight, water-soluble protein which does not readily pass the blood brain barrier mechanism. However, IL-1 β may enter the brain at sites where the blood-brain barrier is absent or leaky, such as in the area of the circumventricular organs (Blatteis, 1992; Dantzer & Kelley, 1989; Koenig, 1991; Rothwell, 1991). In accordance with the suggestion that IL-1 β may directly or indirectly influence hypothalamic function, it has been demonstrated that administration of IL-1 β increases c-fos expression within this region. While Ericsson et al. (1994) reported time and dose-dependent increases in c-fos expression in the hypothalamus following intravenous injection of IL-1 β , Rivest et al. (1992) reported that a single i.v. injection of IL-1 β failed to increase c-fos immunoreactivity. However, Rivest et al. (1992) suggested that a constant elevation of peripheral IL-1 β may be needed in order to promote the activation of hypothalamic neurons. When c-fos was assessed in individual nuclei of the hypothalamus, it was found that continuous i.v. infusion of IL-1 β increased c-fos expression in the PVN and supraoptic nucleus (SON) of the hypothalamus, as well as in the central amygdaloid nucleus (CeA) (Niimi et al., 1996).

Administration of IL-1 β appears to induce c-fos in hypothalamic nuclei containing

CRH neurons, as well as in neurons containing AVP and oxytocin. Indeed, Ericsson et al. (1994) reported that following IL-1 β administration, the majority of c-fos immunoreactive cells in the parvocellular portion of the PVN also expressed CRH mRNA. Similarly, IL-1 β -induced c-fos expression was found in the CeA, a structure known to include CRH-containing neurons (Ericsson et al., 1994). Furthermore, Niimi et al. (1996) recently found IL-1 β -induced c-fos immunoreactivity in both AVP and oxytocin cells in the PVN and SON.

It has been demonstrated that even very small amounts of IL-1 β (<0.1%) may come to provoke the release of CRH from the PVN (Hopkins & Rothwell, 1995). Theoretically, the infiltration of peripheral IL-1 β may be sufficient to stimulate CRH containing nuclei of the PVN. The CRH release provokes the secretion of ACTH from the pituitary, which in turn, results in the release of glucocorticoids from the adrenal glands (Blalock, 1994). In accordance with this view, systemic and intraventricular administration of IL-1 β have been shown to promote the secretion of hypothalamic CRH, as well as ACTH and corticosterone (Berkenbosch et al., 1990; Dunn, 1990; Rothwell, 1991; Sapolsky et al., 1987; Wehrenberg et al., 1994). Moreover, the IL-1 β -induced secretion of ACTH and corticosterone can be blocked by immunization with antisera to CRH or by a CRH antagonist, such as α -helical CRF (Berkenbosch et al., 1987; Navarra et al., 1990; Spinedi et al., 1992).

Although it is generally accepted that IL-1 β is a potent stimulator of CRH neurons, this does not preclude the possibility that IL-1 β acts directly on the pituitary to promote ACTH release. In fact, when applied to pituitary cells in culture, IL-1 β was found to influence POMC expression and ACTH secretion (Berkenbosch et al., 1990; Brown et al., 1987). In this respect, however, several studies indicated that an extended incubation time

of anterior pituitary cell cultures with IL-1 β (greater than 24h) was necessary in order for IL-1 β to influence POMC expression and ACTH secretion (Kehrer et al., 1988; Suda et al., 1989). It has been argued that such an effect may not be the direct result of IL-1 β per se, but may be due to factors related to the extended period of exposure to the cytokine. Further to the same issue, Jessop & Lightman (1994) recently failed to demonstrate a direct effect of IL-1 β on ACTH release from the anterior pituitary *in vitro*. Specifically, priming the anterior pituitary with low doses of CRH (e.g., doses insufficient to cause the release of ACTH) did not influence anterior pituitary ACTH release in response to IL-1 β . It remains possible, however, that other hypothalamic hormones may play a role in this respect.

In addition to changes in CRH following administration of IL-1 β , attention has recently focused on the potential role of arginine vasopressin (AVP) in IL-1 β -induced HPA activity. Systemic administration of IL-1 β has been shown to increase central and peripheral concentrations of AVP (Schmidt et al., 1995). Inasmuch as CRH and AVP act synergistically to provoke ACTH secretion, the pronounced effects of IL-1 β on pituitary ACTH release may stem from the conjoint action of both these peptides. It is particularly significant that like chronic stressors, acute administration of IL-1 β may result in a delayed, but long lasting phenotypic change in CRH neurons within the PVN (Schmidt et al., 1995). These CRH neurons come to coexpress AVP, so that their activation results in greater ACTH and corticosterone secretion. In effect, it is argued that systemic IL-1 β administration produces a time-dependent sensitization-like effect so that subsequent treatments that ordinarily stimulate HPA activity (e.g., stressors or IL-1 β administration) will induce more profound HPA effects (Schmidt et al., 1995).

3.1.6. Penetration of IL-1 β into the Hypothalamus

It is presently unknown how circulating IL-1 β gains access into the brain in order to stimulate the release of hypothalamic CRH. However, a number of possibilities exist in this respect. For instance, it has been suggested that IL-1 β crosses the blood-brain barrier only with the help of a specific carrier transport system, although such a system has not been identified (Banks, 1989). The possibility has also been entertained that IL-1 β binding to cerebral blood vessel endothelium might stimulate either an increase in vascular permeability, diapedesis of mononuclear cells or activate some carrier-mediated transport system, resulting in the release of IL-1 β into the brain (Cunningham & De Souza, 1993). In addition, the peripheral production of IL-1 β may activate the CNS to induce its own synthesis in the brain. Indeed, it has been suggested that binding of IL-1 β to cerebral blood vessel endothelium may activate a second messenger system, which indirectly stimulates the production of IL-1 β by astroglia. This second messenger system has yet to be identified but prostaglandins and IL-6 have been mentioned in this respect (Cunningham & De Souza, 1993; Rothwell, 1991). Alternatively, it is possible that products stimulated by circulating IL-1 β might gain entry into the brain at the organum vasculosum laminae terminalis (OVLT) (Blatteis, 1992; Dantzer & Kelley, 1989; Koenig, 1991; Rothwell, 1991), but these also remain to be identified.

There is reason to suspect that access of IL-1 β into brain tissue is not only stimulated as a result of immune activation, but may also increase under trauma conditions (e.g., following seizure, concussive injury or brain cell damage) (Dunn, 1990; Hopkins & Rothwell, 1995; Imura et al., 1991). In addition to its secretion from activated immune cells,

it will be recalled that IL-1 β is also released from both neurons and microglia within the CNS (Rothwell, 1991). It is not known, however, whether central changes in IL-1 β concentrations associated with immune challenge, stress or brain trauma reflect de novo synthesis of IL-1 β or altered accessibility of the cytokine to the brain. Furthermore, there is reason to believe that both microglia and neurons secrete IL-1 β but only the former are involved in the response to tissue damage. Indeed, Berkenbosch et al. (1990) proposed that IL-1 β derived from neurons may be involved in the transfer of information from the periphery, while that produced by glial cells play a role in neuronal growth and repair. The latter role may function in mediating neuropathological changes associated with neurodegenerative diseases (Berkenbosch et al., 1990; Rothwell, 1991).

Although current hypotheses have focused on the entry of IL-1 β into the brain or IL-1 β -induced signalling at the blood-brain interface, an alternative hypothesis of immune-brain communication has recently been put forth. It has been suggested that the local release of IL-1 β by activated immune cells might be able to activate peripheral vagal afferent nerves, (see Watkins et al., 1995a,b). As predicted, systemic administration of IL-1 β resulted in activation of the nucleus tractus solitarius, the principal projection site of vagal afferents (Watkins et al., 1995a). As well, subdiaphragmatic section of the vagus nerve (e.g., the main afferent nerve from the peritoneal cavity to the brain) blocked the hyperalgesia (Maier et al., 1993), hyperthermia (Watkins et al., 1995b) and the hypothalamic depletion of NE induced by peripheral IL-1 β administration (Fleshner et al., 1995). This surgical procedure also attenuated the increase in plasma corticosterone levels provoked by the cytokine treatment (Fleshner et al., 1995). Similarly, subdiaphragmatic vagotomy diminished the depressing

effects of peripheral IL-1 β and LPS administration on food-motivated behaviour in mice (Bret-Dibat et al., 1995) and LPS-induced disturbance of social exploration in rats (Bluthe et al., 1994). It is important to underscore at this time that a vagal route for cytokine-brain communication does not exclude the possible existence of multiple routes by which cytokines may influence brain function, resulting in different behavioural and physiological outcomes.

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3.1.7. IL-1 β Synergisms

Cytokines such as IL-1 β , IL-6 and TNF- α have been found to act synergistically in modulating HPA activity. Specifically, although IL-6 alone is a poor stimulator of the HPA axis, this cytokine appears to potentiate or sustain HPA activation induced by IL-1 β or TNF- α . In particular, increases in plasma ACTH were reported to be greater in animals receiving systemic coadministration of IL-6 with either IL-1 β or TNF- α , than in animals receiving a single cytokine treatment (Matta et al., 1992; Perlstein et al., 1991, 1993; Zhou et al., 1996). Interestingly, Neta et al. (1992) reported that pretreatment with monoclonal anti-IL-6 receptor antibody antagonized the ACTH response induced by IL-1 β administration. Thus, these data suggest that either IL-1 stimulates IL-6 to promote ACTH secretion or alternatively, that exogenously administered IL-1 β acts synergistically with endogenous IL-6. Commensurate with the latter possibility, Zhou et al. (1996) found that systemic administration of IL-6 concurrently with either exposure to novelty stress or coadministration of systemic IL-1 β , potentiated HPA activation.

The role of synergistic interactions between IL-6 and other cytokines or stressors on

the HPA axis may have important implications in disease outcomes. Indeed, during infection, stimulation of the HPA axis by increased IL-1 β , TNF- α and IL-6 production may act in a feedback manner to prevent the immune system from overreacting. However, if infection occurs in conjunction with stress, IL-6 may potentiate HPA activation, possibly leading to excessive glucocorticoid secretion and subsequent immunosuppression (Zhou et al., 1996).

3.1.8. Mediators of IL-1 β Actions on HPA Activity

3.1.8a. Nitric oxide

As already indicated, the exact mechanisms by which cytokines modulate HPA activity have yet to be fully elucidated. Recently, however, the roles of several potential mediators (e.g., nitric oxide, prostaglandins) have received particular attention in this respect. Nitric oxide (NO), a gaseous free radical, has been identified as an intercellular messenger in the CNS, as well as in the neuroendocrine system (Dawson & Snyder, 1994). Controversy exists, however, with respect to the role of NO in IL-1 β -induced HPA activation. For example, NO has been reported to have both a stimulatory (Brunetti et al., 1993, 1996; Sandi & Guaza, 1995) and an inhibitory effect (Costa et al., 1993) on IL-1 β -induced hypothalamic CRH release. In addition, while NO was found to potentiate IL-1 β -induced release of ACTH from the anterior pituitary (Brunetti et al., 1993), other researchers suggest that NO is not involved in pituitary ACTH release induced by either IL-1 β , CRH or AVP administration (Hashimoto et al., 1995; Ohta et al., 1993). Furthermore, Rivier & Shen, (1994) reported an inhibitory role for NO *in vivo*, but speculated that the median eminence may be the major site of action of NO and not the PVN or anterior pituitary.

3.1.8b. Prostaglandins

In addition to the well documented role of prostaglandins (PGs) in fever production, PGs have recently been posited to play a role in IL-1 β -induced activation of the HPA axis. Indeed, increased hypothalamic PG production has been reported following IL-1 β or endotoxin administration (Komaki et al., 1992, Watanabe et al., 1994, Weidenfeld et al., 1993). More importantly, enhanced secretion of ACTH following i.c.v. injection of IL-1 β was blocked by indomethacin (Katsuura et al., 1988b, Watanabe et al., 1991) or ibuprofen (Rivier, 1995) injected either systemically or centrally. Rivier, (1995) further demonstrated that central ibuprofen administration failed to attenuate ACTH secretion induced by peripherally injected IL-1 β . These results suggest that hypothalamic PG mediates the variation of HPA functioning induced by central IL-1 β administration, but that other factors account for the effects elicited by peripheral IL-1 β treatment (Rivier, 1995).

More recently, a role for prostaglandins has been reported with respect to the activation of specific hypothalamic nuclei associated with peripheral or central IL-1 β administration. Niimi et al. (1996) showed that the induction of c-fos immunoreactivity by either continuous intravenous or central administration of IL-1 β was blocked by indomethacin in the PVN, SON and ARC nuclei of the hypothalamus. Of course, while suggesting that IL-1 β has effects on PG functioning in these particular brain regions, the functional implications of these changes (i.e., with respect to behaviour or HPA activity) can not be deduced from these data.

3.1.9. Role of Corticosterone

Although IL-1 β is a potent stimulator of HPA activity, the effects of this cytokine do not go unchecked. Indeed, glucocorticoids are believed to play a protective role in HPA functioning by acting in a negative feedback manner. Recently, Goujon et al. (1995) demonstrated a regulatory role for corticosterone in the behavioural effects induced by IL-1 β . Adrenalectomy was found to exacerbate the decrease in social exploration induced by systemic injection of IL-1 β . Similarly, this effect was reported following acute administration of RU-38486 (a glucocorticoid receptor antagonist) and attenuated by chronic replacement with corticosterone (Goujon et al., 1995). These findings suggest that the enhance susceptibility of adrenalectomized mice to the behaviourally depressing effects of IL-1 β may be the result of a reduction in glucocorticoid concentration (Goujon et al., 1995). Furthermore, it has been suggested that excessive synthesis and release of IL-1 β in response to glucocorticoid release may sensitize adrenalectomized animals to the lethal effects of cytokines (Bertini et al., 1988). At the molecular level, glucocorticoids downregulate the synthesis and secretion of IL-1 β (as well as IL-6 and TNF- α) by activated monocytes and macrophages (Knudsen et al., 1987; Morrow et al., 1993; Zuckerman et al., 1989). In addition, glucocorticoids have been found to inhibit transcriptional expression of the IL-1 β gene and decrease the stability of IL-1 β mRNA (Knudsen et al., 1987; Lee et al., 1988).

3.1.10. Central Effects of IL-1 β on Immune Function

Traditionally, IL-1 β was considered solely in terms of its actions on immune functioning. As indicated earlier, it has been known for some time that IL-1 β , (as well as IL-

6 and $\text{TNF-}\alpha$) is released from activated macrophages and is essential for the excitation of lymphocytes, notably the T helper cells (Blalock, 1984; Dunn, 1990; Imura et al., 1991; Koenig, 1991; Rivier, 1995). In addition, it appears that variations of central IL-1 β , at least within the hypothalamus, may have pronounced effects on immune functioning. For example, central administration of IL-1 β has been reported to provoke peripheral IL-2 production and natural killer cell activity (Sundar et al., 1989) and has been shown to induce the synthesis and release of IL-6 in a variety of cells (e.g., macrophages, endothelial cells, pituitary cells) (Sironi et al., 1989; Spangelo et al., 1991). In addition, it has been suggested that central IL-1 β may influence immune activity in an endogenous negative feedback manner. Specifically, glucocorticoids released in response to IL-1 β -induced CRH and ACTH secretion might act directly on immune cells to inhibit further release of IL-1 β into the circulation, thus resulting in immunosuppression (Saperstein et al., 1992).

3.1.11. Role of IL-1 β in Neuropathology

While some of the effects of IL-1 β are associated with immunological challenge, other actions have been related to injuries that do not involve immune mechanisms. In particular, given that elevated levels of the cytokine have been detected following closed head injury and seizure (Rothwell, 1991), it has been proposed that IL-1 β may play a role in responses to brain injury. In addition, IL-1 β has been reported to contribute to neurotoxicity, in that central infusion of IL-1 β promotes glial cell activation and neuronal cell loss (Rothwell & Hopkins, 1995). Central injection of IL-1ra also markedly inhibited neurodegeneration induced by focal cerebral ischaemia (see Rothwell & Hopkins, 1995),

suggesting that *in vivo*, endogenous IL-1 modulates neurodegenerative processes. In contrast, however, the possibility has been suggested that IL-1 β may be neuroprotective at low concentrations (Rothwell & Hopkins, 1995), although the relationship between neuronal damage and cytokine level needs further examination.

3.1.12. Effect of IL-1 β on Neurotransmitter Function

The presence of IL-1 and its receptors in the brain coupled with the finding that this cytokine can influence CNS functioning, suggests the possibility that IL-1 β produces many central actions including the modulation of various neuronal systems. For instance, it is well documented that IL-1 β affects catecholaminergic activities in the hypothalamus. For instance, Mefford et al. (1991) reported an increase in hypothalamic NE and DA turnover following systemic injection of IL-1 β , while TNF- α increased NE utilization (Mefford et al., 1991). Similarly, Masana et al. (1990) demonstrated an increase in hypothalamic NE and DA utilization following peripheral IL-1 β administration, while Palazzolo & Quadri (1990) reported an increase in IL-1 β stimulated release of hypothalamic NE and DA *in vitro*.

Variations of NE in more discrete hypothalamic regions have been documented as well. Systemic administration of IL-1 β was reported to stimulate the release of NE within the medial basal, lateral and paraventricular nuclei of the hypothalamus (Dunn, 1988; Mohankumar & Quadri, 1993). Similarly, Kabiersch et al. (1988) reported changes in central NE metabolism (e.g., decreased NE and increased MHPG levels) within the arcuate nucleus and median eminence of the hypothalamus following peripheral injection of IL-1 β , although no changes in DA, 5-HIAA or 5-HT were detected in these brain regions.

In accordance with IL-1 β -induced changes of NE activity, the integrity of central catecholaminergic fibers appears to be important for IL-1 β modulation of HPA function. In particular, peripheral IL-1 β is believed to communicate with the hypothalamus through an afferent pathway (e.g., to the PVN) from catecholaminergic neurons in the brain stem. Indeed, 6-OHDA lesions of the ventral noradrenergic ascending bundle (Chuluyan et al., 1992), as well as i.c.v. administration of prazosin (an α_1 -adrenergic antagonist) (Weidenfeld et al., 1989), blocked both ACTH and corticosterone responses induced by central IL-1 β administration.

In addition to the NE variations associated with IL-1 β treatment, it is clear that administration of this cytokine profoundly influences 5-HT activity. In particular, systemic administration of IL-1 β elevated tryptophan concentrations, suggesting an increased release of 5-HT (Dunn & Welch, 1991). In addition, peripheral administration of IL-1 β increased 5-HT turnover in the hypothalamus, as well as in the prefrontal cortex and brainstem (Dunn, 1988) and was found to increase 5-HIAA concentrations in both the prefrontal cortex and hippocampus (Zalcman et al., 1994). Commensurate with the effects of systemically administered IL-1 β , central injection of the cytokine has been reported to influence anterior hypothalamic unit activity (Bartholomew & Hoffman, 1993).

Further to the same issue, several studies have focused on the effect of central IL-1 β administration on neurotransmitter activity. Increased levels of 5-HIAA in the anterior hypothalamus have been reported following i.c.v. injection of IL-1 β (Carmelina et al., 1991; Gemma et al., 1991). Moreover, pretreatment with IL-1ra blocked this effect, suggesting that IL-1 modulates serotonergic function in the hypothalamus and that this system is mediated

by the IL-1 receptor. In a further study, Shintani et al. (1993) reported that a small dose of IL-1 β (1 ng) injected directly into the anterior hypothalamus enhanced the *in vivo* release of NE, DA and 5-HT, and their respective metabolites. Similarly, central injection of IL-1 β directly into the medial basal hypothalamus promoted the release of DA and DOPAC, as well as 5-HIAA (Mohankumar et al., 1991,1993) from this brain region. Curiously, IL-1 β was found to inhibit the *in vitro* release of 5-HT from the hypothalamus (Palazzolo & Quadri, 1992). It should be underscored at this time that the differential effects of IL-1 β on hypothalamic activity *in vivo* and *in vitro* may be due to the fact that in the latter situation, the hypothalamus exists in isolation, void of inputs from other parts of the brain.

While most of the research assessing the neurochemical consequences of IL-1 β treatment have focused on the hypothalamus, it appears that IL-1 β influences neurotransmitter activity at extrahypothalamic sites as well. Indeed, Linthorst et al. (1995) recently reported an increase in hippocampal 5-HT and 5-HIAA levels following central injection of this cytokine *in vivo*. These neurochemical effects of IL-1 β were antagonized following central injection of IL-1ra, suggesting a role for IL-1 receptors in hippocampal serotonergic activity. Furthermore, systemic IL-1 β administration influenced *in vivo* 5-HT turnover in the nucleus accumbens and hippocampus, while having only modest effects on biogenic amine release in the prefrontal cortex (Merali et al., 1997). As well, it appears that systemic IL-1 β treatment decreases acetylcholine (ACh) levels in hippocampal dialysates (Rada et al., 1991).

As mentioned previously, IL-1 β plays a role in body temperature regulation and fever production (Dinarello, 1988; Rothwell, 1991). It has been suggested, however, that the

neurochemical effects of IL-1 β may simply reflect its thermoregulatory properties. Indeed, in a study by Masana et al. (1990), indomethacin (a prostaglandin inhibitor) blocked NE and DA changes induced by relatively high doses of IL-1 β (e.g., 10 μ g/rat vs. 1 μ g/rat employed by Kabiersch et al., 1988), suggesting that prostaglandins may play a role in the central amine changes induced by this cytokine (Masana et al., 1990). However, Dunn (1988) and Kabiersch et al. (1988) reported amine changes at subpyrogenic doses of IL-1 β . More recently, pretreatment with indomethacin failed to antagonize both the increase in hypothalamic MHPG following systemic IL-1 β administration (Dunn & Chuluyan, 1992) and the increase in NE release following central injection of IL-1 β into the anterior hypothalamus (Shintani et al., 1993). Evidently, IL-1 β may elicit central amine changes through mechanisms independent of temperature regulation. In addition to IL-1 β -induced alterations in neurochemical activity, it should be noted at this time that the behavioural effects of IL-1 β also appear to be independent of their pyrogenic effects. Indeed, central administration of IL-1ra blocked the behavioural effects of IL-1 β injected i.c.v., but failed to influence the temperature effects accrued by the cytokine (Kent et al., 1992a).

3.1.13. Effect of IL-1 β on Behaviour

While the behavioural consequences of stressors have been studied extensively, limited research exists regarding the impact of immunological activation on behavioural or psychological processes. However, it has been repeatedly suggested that the behavioural alterations resulting from IL-1 β administration are in many ways reminiscent of those engendered by psychological or physical stressors (Anisman et al., 1991). Administration

of IL-1 β , like LPS, has been shown to induce an array of behavioural symptoms which are usually referred to as sickness behaviour (Kent et al., 1992b). In addition to the fever induced by these treatments, animals display piloerection, curled body posture, decreased exploration, reduced feeding and possibly anhedonia (Hart, 1988; Kent et al., 1992b). It is generally assumed that these behaviours are of adaptive significance in that they all favour increased functioning of the immune system. For instance, the piloerection and curled body posture maintain body temperatures which results in enhanced immune cytotoxicity and decreased viral proliferation (Hart, 1988). It must be underscored however, that these behaviours are not necessarily secondary to the peripheral actions associated with immune activation or viral insult (e.g., feelings of illness) but likely involve central processes. In fact, central administration of cytokines have been shown to induce a behavioural profile similar to that elicited by peripheral cytokine treatment, while central administration of IL-1ra antagonizes the effects of peripheral administered IL-1 β (Kent et al., 1992a; Rothwell, 1991). Furthermore, recent experiments in our laboratory have suggested that administration of cytokines may engender an anhedonia (quite apart from the anorexic effects) and may also provoke an anxiogenic effect (Lacosta et al., 1996; Song et al., 1996). Inasmuch as IL-1 β is a potent activator of CRH and CRH administration is known to produce anxiogenic effects (Gray, 1991; Heilig et al., 1994), it comes as no surprise that IL-1 β may effectively elicit anxiety; however, the anxiogenic effects of IL-1 β are not readily discernable as they may be masked by the profound sickness behaviours induced by the treatment.

Many of the studies examining the effects of IL-1 β have focused on variations of feeding or related behaviours. Acute treatment with IL-1 β has been reported to provoke

anorexia (Bluthe et al., 1989; Hellerstein et al., 1989; Kent et al., 1994; McCarthy et al., 1985; Plata-Salaman, 1989,1994), taste aversion (Tazi et al., 1988) and disruptions of responding for food reward (Bluthe et al., 1989). Likewise, chronic infusion of IL-1 α over 5 consecutive days was also shown to decrease food consumption in rats (Otternus et al., 1988).

Anorexia induced by IL-1 β administration appears to be the result of the cytokine's action on the hypothalamus. Reductions in food and water intake were demonstrated following direct injection of IL-1 β into the lateral ventricles (Plata-Salaman, 1994) or into the ventromedial hypothalamus (Kent et al., 1994) of the rat. Furthermore, Laviano et al. (1995) reported that intra-VMH injections of IL-1 α significantly enhanced food intake in anorexic tumour-bearing rats. The later finding suggests that IL-1 is involved in the pathogenesis of cancer anorexia and that the VMH may be a major site of action of IL-1 in mediating the anorexia seen during tumour development.

In addition to its effects on feeding, both systemic and central administration of IL-1 β alter the exploratory behaviour of mice (Dunn et al., 1991; Spadaro & Dunn, 1990). Intraventricular administration of IL-1 β reduced the time of contact with novel stimuli in a novel multicompartiment chamber, without influencing locomotor activity (Spadaro & Dunn, 1990). Moreover, no other behavioural measures examined (e.g., rears, grooming or scratching) were influenced by the cytokine treatment. The variations of exploratory behaviour elicited by IL-1 β did not appear to involve CRH or prostaglandins, since neither central administration of α -helical CRF nor indomethacin significantly altered the IL-1 β -induced behavioural changes (Spadaro & Dunn, 1990). Although these results do not rule

out a possible role for CRH or prostaglandins, they suggest that these peptides may not be the only mediators of cytokine-provoked alterations in exploration. Indeed, these behaviours may involve opioid and dopaminergic systems since both naloxone (an opiate antagonist) and sulpiride (a selective D₂-receptor antagonist) completely blocked the behavioural effects of IL-1 β (Spadaro & Dunn, 1990).

At present, conflicting reports exist as to the effect of IL-1 β on locomotor activity (as distinct from exploration of novel objects). Intraventricular administration of IL-1 β failed to influence locomotor activity in mice during exploration of a novel multicompartiment chamber (Spadaro & Dunn, 1990). However, a decrease in locomotor activity has been reported in rats following chronic infusion of IL-1 α over 5 consecutive days (Otternus et al., 1988). Moreover, treatment with IL-1 β disrupted swim performance in a "behavioural despair" paradigm (Hellerstein et al., 1989), although it is unclear whether this effect was the result of disturbances in motoric, motivational or affective processes. In addition, Linthorst et al. (1995) recently showed that central injection of IL-1 β attenuated behavioural activity in rats, while central injection of IL-1 α antagonized this effect. This finding suggests a role for IL-1 receptors in the behaviourally depressing effects of IL-1. However, in none of these studies were systematic behavioural assessments conducted which examined dose-response curves of behavioural variations occurring over time following cytokine treatment. Thus, even the most fundamental aspects of the behavioural actions of IL-1 β remain to be determined.

3.2. Interleukin-2

3.2.1. Introduction

Interleukin-2 is a small (15.5 kDa), single-chain peptide, composed of 133 amino acids (Nistico, 1993) which is known to possess potent immunostimulatory properties. IL-2 is synthesized and secreted by T-helper cells in response to antigenic challenge. In turn, IL-2 stimulates T-cytotoxic cell proliferation, differentiation and activation, and promotes B-cell proliferation and differentiation into antibody-secreting cells. Moreover, IL-2 induces the growth and activation of antigen-independent natural killer cells and lymphokine-activated killer cells (Kintzel & Calis, 1991; Nistico & De Sarro, 1991a; Waldmann, 1993).

In addition to its potent stimulatory effects on the immune system, IL-2 appears to be involved in regulating CNS functioning (see review Nistico, 1993). In contrast to IL-1 β , however, the effects of IL-2 have been less thoroughly documented. As will be discussed later, IL-2 has been found to influence HPA activity, as well as neuroendocrine and neurotransmitter processes. Moreover, IL-2 also appears to induce a variety of behavioural effects. Given the clinical use of IL-2 in cancer immunotherapy, as well as the neuropsychiatric sequelae of IL-2 treatment, it is curious that few studies have been reported concerning the behavioural effects of IL-2 in animals (Caraceni et al., 1992; Denicoff et al., 1987; Fent & Zbinden, 1987; Meyer & Valentine, 1995).

The following sections will provide a brief description of the presence and distribution of IL-2 within the CNS. This will be followed by a discussion of the potential modulatory role of IL-2 on HPA functioning, and the influence of IL-2 on neuroendocrine and neurotransmitter processes. Finally, an overview will be provided of the limited literature

concerning the behavioural effects induced by this cytokine.

3.2.2. Distribution of IL-2 in the CNS

Although produced by activated T-helper cells, IL-2 production may also occur within the brain. High densities of IL-2 immunoreactivity and IL-2-binding sites have been reported in the hypothalamus (particularly the median eminence-arcuate nucleus complex), while moderate densities were found in the hippocampus, caudate nucleus, locus coeruleus and amygdala (Araujo et al., 1989; Lapchak et al., 1991; Villemain et al., 1990). In addition, IL-2 mRNA expression has been reported in several discrete brain regions (Farrar, 1987; Koenig, 1991; Nistico & De Sarro, 1991a), although it is presently uncertain whether this cytokine is released exclusively from astrocytes or microglia or whether some of the IL-2 is derived neuronally.

3.2.3. IL-2 Receptors in the CNS

IL-2 binds to specific membrane-associated receptors which are composed of 3 functional subunits (IL-2R α , IL-2R β , IL-2R γ) (Nistico, 1993, Petitto & Huang, 1994). The signal transduction mechanisms that occur following the binding of IL-2 to its receptor are presently unclear, although a possible role for intracellular second messengers has been reported in this respect (Nistico, 1993). The distribution of IL-2 binding sites and IL-2 immunoreactivity appears to be positively correlated, suggesting that this cytokine is both synthesized and/or stored in the same region where it interacts with its receptor (Nistico, 1993). Indeed, the presence of an IL-2R α protein antigen (Lapchak et al. 1991), as well as

IL-2R β mRNA have been reported in the rodent forebrain (frontal cortex, striatum) and hippocampus (Petitto & Huang, 1994). These data, coupled with the finding of IL-2R α gene expression from primary neocortical neurons (Shimojo et al. 1993), suggests that centrally, IL-2 receptors may be located on neurons (Petitto & Huang, 1994).

3.2.4. Neuromodulatory Role of IL-2 in the CNS

The presence of endogenous IL-2 receptors and IL-2 immunoreactivity in normal brains suggests a role for this cytokine in modulating CNS functioning (Nistico, 1993). Within the CNS, IL-2 plays a role in both CNS development and homeostatic repair mechanisms in response to injury (Merrill, 1990; Nistico & De Sarro, 1991a). Indeed, IL-2 has been shown to modulate the growth and differentiation of glial and neuronal cells (Merrill, 1990). The neurotropic effects of IL-2 are also gleaned from the cytokine's ability to influence oligodendrocyte proliferation (Benveniste & Merrill, 1986) and the enhanced viability of neocortical neurons in culture (Shimojo et al., 1993). As will be discussed shortly, IL-2 also plays a role in neuronal activity in the hippocampus, locus coeruleus and discrete hypothalamic nuclei (Merrill, 1990, Nistico & De Sarro, 1991b) and acts as a modulator of the neuroendocrine system by directly influencing the release of pituitary hormones (e.g., ACTH) (Karanth & McCann, 1991).

Apart from its neurotropic effects, IL-2 is influenced by CNS trauma and disease (Merrill, 1990; Nistico & De Sarro, 1991a). Increased central IL-2 immunoreactivity and IL-2 binding sites have been reported following injury to the brain (Lapchak & Araujo, 1993; Merrill, 1990; Nieto-Sampedro & Chandy, 1987). For instance, the expression of IL-2

receptors in the hippocampus was elevated following lesioning with kainic acid (Araujo et al., 1989). Likewise, elevations of both IL-2 and IL-2 receptors were reported in brain tissue, plasma, and CSF of patients suffering from certain pathologies involving abnormal immune function (e.g., multiple sclerosis, systemic lupus erythematosus, Alzheimer's disease and acquired immunodeficiency syndrome) (Hoffman et al., 1986; Lapchak & Araujo, 1993; Luber-Narod & Rogers, 1988; McGeer & Rogers, 1992; Merrill, 1990; Nistico, 1993; Waldmann, 1993). As well, elevated levels of IL-2 have also been reported in the CSF of schizophrenic patients (Licinio et al., 1993), leading to the view that schizophrenia may result from the excessive production of endogenous IL-2 (Smith, 1990).

3.2.5. Penetration of IL-2 into the CNS

Although it is generally believed that IL-2 is endogenous to the brain, this does not preclude the possibility that some of the cytokine detected centrally may originate in the periphery. In addition to an endogenous source of IL-2, blood-borne IL-2 has been reported to readily cross the blood-brain barrier (Waguespack et al., 1994). Indeed, acute systemic administration of IL-2 appears to alter cerebrovascular permeability without altering the integrity of the blood-brain barrier, allowing peripheral IL-2 to access the brain (Banks & Kastin, 1992; Ellison et al. 1990). However, damage to the blood-brain barrier was reported following chronic administration of the cytokine (Ellison et al., 1990). Thus, one can imagine still greater entry of IL-2 into brain tissue following sustained insults that promote IL-2 production (e.g., viral challenges).

3.2.6. Role of IL-2 in Modulating HPA Activity

While it is well established that IL-1 β is a potent modulator of the HPA axis, less is known about the ability of IL-2 to influence HPA functioning. Administration of IL-2 has been shown to dose-dependently increase POMC mRNA in pituitary cells *in vitro* (Brown et al., 1987), as well as in the anterior pituitary *in vivo* (Chesnokova, 1997; Harbuz et al., 1992). Similarly, IL-2 stimulated the *in vitro* release of ACTH from pituitary cells (Karanth & McCann, 1991), as well as corticosterone from adrenocortical cell cultures (Tominaga et al., 1991). In contrast, however, Zalcman et al. (1994) failed to report changes in either plasma ACTH or corticosterone concentrations following acute systemic administration of IL-2, although levels were enhanced following repeated central administration of the cytokine (Hanisch et al., 1994, 1997). Moreover, while plasma corticosterone levels were increased throughout the diurnal cycle, plasma ACTH levels were enhanced only during the active period among rats (e.g., dark phase), suggesting an IL-2 mediated increase in the adrenal sensitivity to ACTH (Hanisch et al., 1994). In addition, repeated treatment with IL-2 failed to alter elevations of plasma ACTH and corticosterone induced by exposure to restraint stress (Hanisch et al., 1994). It would appear that while IL-2 excites some aspects of the HPA axis, it seems that this cytokine is not a particularly potent stimulator of pituitary ACTH and adrenal glucocorticoid release, at least when applied on an acute basis. Yet, as will be described in the ensuing section, several studies have made it clear that IL-2 may have profound effects on central neuropeptides, which ought to culminate in variations of HPA activity.

3.2.7. Role of CRH and AVP

It has been suggested that the modulating effect of IL-2 on HPA activity may be mediated by the hypothalamic release of CRH (Cambroner et al., 1992), AVP (Bindoni et al., 1988; Pardy et al., 1993; Raber & Bloom, 1994), or the synergistic actions of these peptides (Raber et al., 1995). Indeed, IL-2 has been shown to stimulate the release of CRH from hypothalamic slices *in vitro* (Cambroner et al., 1992; Raber et al., 1995). Moreover, IL-1 β and IL-2 act in a synergistic manner to potentiate the *in vitro* hypothalamic release of CRH (Cambroner et al., 1992). Commensurate with these findings, i.c.v. administration of IL-2 increased the *in vivo* firing activity of neurons in several hypothalamic nuclei (e.g., ventromedial, supraoptic and PVN), where the majority of hypothalamic CRH neurons are located (Bindoni et al., 1988). Curiously, however, IL-2 failed to alter CRH mRNA content in the PVN (Harbuz et al., 1992).

The mechanisms by which IL-2 stimulates hypothalamic CRH secretion remain to be elucidated. Recently, NO has been implicated as a possible mediator in this respect (Raber et al., 1995). Indeed, administration of an NO antagonist (e.g., LNMA) blocked the *in vitro* release of CRH from hypothalamic slices induced by IL-2 administration (Raber et al., 1995). Moreover, administration of the synthetic corticoid, dexamethasone, blocked the stimulatory effect of IL-2 on CRH secretion in perfused hypothalami, suggesting that the action of IL-2 on HPA functioning is subject to glucocorticoid feedback regulation. However, the CRH response to IL-2 was not altered by either adrenalectomy or administration of indomethacin (Cambroner et al., 1992).

Recent studies have reported that IL-2 not only modulates CRH activity but also

influences both AVP expression and secretion from the hypothalamus. For instance, acute systemic administration of IL-2 enhanced the expression of AVP mRNA in the hypothalamus of mice (Pardy et al., 1993). In a further study, administration of IL-2 induced a dose-dependent increase in the release of AVP from hypothalamic slices *in vitro* (Raber & Bloom, 1994). In addition to alterations in AVP expression and secretion, i.c.v. administration of IL-2 enhanced the *in vivo* firing of neurons in both the supraoptic and paraventricular nuclei of the hypothalamus, regions known to secrete AVP (Bindoni et al., 1988). Although the available data are limited, it appears that the effects of IL-2 on CRH and AVP are not restricted to the hypothalamus. A dose-dependent increase in the release of both amygdaloid CRH (Raber et al., 1995) and AVP (Raber & Bloom, 1994) *in vitro* is induced by IL-2 administration. Thus, it was suggested that the changes of amygdala peptide activity elicited by IL-2 contributes to neuro-immune interactions (Raber & Bloom, 1994).

3.2.8. Role of Corticosterone

In addition to its negative feedback control of HPA activity, exerted in part by an inhibitory effects on IL-1 β , glucocorticoids have also been shown to exert a modulatory effect on IL-2 (Boumpas et al., 1991; Gillis et al., 1979). In particular, administration of dexamethasone inhibits the expression of IL-2 and IL-2R located on lymphocytes (Boumpas et al., 1991; Gillis et al., 1979; Reed et al., 1986). Clinical studies have also reported an inhibitory effect of glucocorticoids on circulating levels of sIL-2R. Indeed, patients with severe juvenile rheumatoid arthritis showed an elevation of sIL-2R, compared to patients suffering from either a mild form of the disease or those undergoing glucocorticoid therapy

(Tanaka et al., 1991). Likewise, patients with chronic progressive multiple sclerosis were found to have lower serum sIL-2R levels following long-term glucocorticoid treatment compared to untreated patients (Bansil et al., 1991). Recently, Sauer et al. (1993) reported that the influence of glucocorticoids on circulating sIL-2R levels is dependent on both the state of immune activation and on the duration of exposure to glucocorticoids. Specifically, chronic (but not acute) glucocorticoid administration attenuated the elevated circulating sIL-2R levels in patients with atopic dermatitis, while treatment with this steroid failed to alter sIL-2R levels in controls. Moreover, patients suffering from hypercortisolism (e.g., Cushing's disease) and hypocortisolism, showing a significant decrease and increase in circulating sIL-2R levels, respectively, supported the proposition that abnormal glucocorticoid function influences circulating levels of sIL-2R.

3.2.9. Role of IL-2 on Neurotransmitter Function

Although the data concerning IL-2 are limited compared to those of IL-1 β , it has been shown that in addition to its effects on neuroendocrine processes, IL-2 influences central neurochemical functioning. For instance, IL-2 has been reported to stimulate anterior hypothalamic and locus coeruleus unit activity (Bartholomew & Hoffman, 1993). Systemic administration of IL-2 was found to increase NE turnover in the hypothalamus and DA turnover in the prefrontal cortex (Zalcman et al., 1994).

Treatment with IL-2 has also been reported to enhance the release of DA from striatal slices (Lapchak, 1992). Similarly, IL-2 elicited a biphasic pattern of veratrine-evoked DA release from striatal slices (Petitto et al., 1997), as well as K⁺-evoked DA release from

mesencephalic cells in culture (Alonso et al., 1993). In each case, the evoked release of DA was enhanced at low concentrations of IL-2 but inhibited at high concentrations of the cytokine. Interestingly, peripheral IL-2 injection reduced the *in vivo* release of DA from the nucleus accumbens (Anisman et al., 1996), and such an effect was exacerbated by presentation of a moderately noxious environmental stimulus (e.g., air puff) (Song et al., 1996).

It has recently been shown that IL-2 exerts pronounced effects on hippocampal neurotransmitter functioning. Paralleling the inhibition of K⁺-evoked release of ACh from hippocampal slices *in vitro* (Araujo et al., 1989), it was demonstrated that IL-2 blocks long term potentiation in the hippocampus (Tancredi et al., 1990). Interestingly, as in the case of the DA changes seen in the striatal tissues assessed *in vitro*, Hanisch et al. (1993) reported that IL-2 elicited a biphasic pattern of K⁺-evoked ACh release from rat hippocampal neurons (e.g., inhibiting ACh release at high concentrations while stimulating ACh release at lower doses of the cytokine). However, in contrast to the effects in the hippocampus, as well as the striatal DA changes, IL-2 failed to influence evoked-ACh release in either the striatum or frontal cortex (Araujo et al., 1989).

It is clear that IL-2 induces region-specific effects on ACh release from cholinergic nerve terminals. It seems that IL-2 exerts its effects in those regions where IL-2 and IL-2 receptors are localized along with terminals of far-projecting ACh neurons (e.g., the hippocampus). No such effect is evident, however, in regions comprising ACh interneurons (e.g., the striatum) (Hanisch et al., 1993). It is also interesting to note that ACh neurons of the striatum, which appear to be insensitive to the modulatory effects of IL-2, are also spared

in Alzheimer's disease (Hanisch et al., 1993). Thus, the view has been suggested that the increased levels of IL-2 reported under certain pathological conditions may be responsible, in part, for the diminished release of ACh from terminals located in regions affected in Alzheimer's disease (e.g., hippocampus, frontal cortex) (Hanisch et al., 1993). However, the possibility also exists that other mediators induced by IL-2 (e.g., TNF- α) may play a role in the modulatory actions of this cytokine on ACh nerve terminals (Ellison & Merchant, 1991).

3.9.10. Effect of IL-2 on Behaviour

Given the potent immunostimulatory properties of IL-2, the use of this cytokine in cancer treatment was initially met with great optimism (Rosenberg, 1990). Recently, however, IL-2 immunotherapy has been shown to be accompanied by a variety of adverse behavioural effects, including cognitive and memory impairments, depression, drowsiness, confusion, hallucinations and psychosis (Caraceni et al., 1992; Denicoff et al., 1987; Meyer & Valentine, 1995). The neuropsychiatric disturbances associated with the clinical use of IL-2 are often severe enough to require discontinuation of the treatment (Caraceni et al., 1992; Denicoff et al., 1987; Meyer & Valentine, 1995).

3.2.10a. Acute IL-2 administration

It is curious that although IL-2 has been used clinically in cancer immunotherapy and that treatment with the cytokine is associated with severe neuropsychiatric consequences, only limited data are available regarding the behavioural effects of this treatment in rodents. Microinfusion of IL-2 into either the cerebral ventricle or directly into the locus coeruleus

increased sleep in rats (De Sarro et al., 1989, 1990; Nistico & De Sarro, 1991b). Moreover, these effects were antagonized by either systemic pretreatment with the opioid receptor antagonist, naloxone, or by application of monoclonal antibodies against IL-2 receptors directly into the locus coeruleus (De Sarro et al., 1990; Nistico & De Sarro, 1991b). In contrast, yohimbine (a specific α -2-adrenergic antagonist) was without effect in this respect. These findings suggest that the soporific effects of IL-2 may involve the interaction of this cytokine with opioid receptors within the locus coeruleus (De Sarro et al., 1990).

In addition to its influence on sleep, unilateral injection of IL-2 into the striatum or substantia nigra has been shown to elicit ipsilateral turning and circling (De Sarro et al., 1990), and microinjection of the cytokine into the caudate or substantia nigra induced ipsilateral turning (Nistico & De Sarro, 1991b). These findings suggest that some of the motoric effects of IL-2 involve the inhibition of nigrostriatal DA mechanisms and that this cytokine may inhibit nigrostriatal DA neurons (De Sarro et al., 1990; Lapchak, 1992). As well, it seems that IL-2 may also interact with opioid or catecholamine receptors, since opioids and DA agonists applied to nigrostriatal structures induce circling (De Sarro et al., 1990).

In conjunction with neuronal activation promoting unilateral circling, IL-2 has been shown to influence locomotor activity. In fact, central administration of the cytokine into either the hippocampus or ventro-medial hypothalamus increased both locomotor activity, as well as exploratory behaviour in rats (De Sarro et al., 1990; Nistico & De Sarro, 1991b). In a recent study, Petitto et al. (1997) investigated the motoric effects induced by the cytokine, as well as the potential role of IL-2 in anxiety. Although acute systemic

administration of IL-2 enhanced the locomotor activity of mice in an elevated plus-maze (Petitto et al., 1997), treatment with the cytokine failed to influence anxiety reactions in either an elevated plus-maze test (altered entries onto the open arms) or in an acoustic startle response test (Petitto et al., 1997).

It has recently been suggested that apart from the motoric effects associated with IL-2, this cytokine may affect motivational or hedonic processes. For instance, it was recently reported that IL-2 influenced drug-induced responding for food reinforcement. In a rewarded discrimination task in which animals were trained to distinguish *d*-amphetamine from saline or ethylketocyclazocine (EKC) from saline, systemic injection of IL-2 potentiated responding elicited by both *d*-amphetamine and EKC. Moreover, this effect was antagonized by naloxone (e.g., an opioid receptor antagonist) (Ho et al., 1994). Since amphetamine and EKC act via dopaminergic and opioid mechanisms, respectively, these data suggest that the potentiating effects of IL-2 may involve an interaction between opioid and DA mechanisms (Ho et al., 1994).

In addition to modulating drug-induced responding for food reward, acute, systemic IL-2 administration has been reported to attenuate responding for rewarding stimulation from the lateral hypothalamus. Interestingly, while the effects of IL-2 were initially limited, they became progressively more pronounced over several days of repeated testing in the absence of further IL-2 treatment (Anisman et al., 1996). In contrast, however, nonreinforced behaviour was not influenced by the cytokine treatment, suggesting that IL-2 does not interfere with perceptual or cognitive processes. Moreover, since the cytokine-provoked disturbances in self-stimulation were protracted, the effects of IL-2 did not appear to be

readily attributable to either motoric factors or general malaise (Anisman et al., 1996). Inasmuch as DA activity in the nucleus accumbens has been implicated in responding to positive reinforcement (Fibiger & Phillips, 1988), and IL-2 has been found to alter accumbal DA release, the view was suggested that the effects of IL-2 on brain self-stimulation may be related to an anhedonia, possibly resulting from a disruption of mesolimbic DA activity (Anisman et al., 1996).

3.2.10b. Chronic IL-2 administration

Commensurate with the neuropsychiatric disturbances associated with clinical IL-2 immunotherapy, behavioural repercussions have been reported in rodents following chronic IL-2 administration. Memory impairments were seen in a passive avoidance task in aged mice that received long-term systemic IL-2 treatment, while the performance of either similarly treated young adult mice or aged mice not exposed to the cytokine were unimpaired (Nemni et al., 1992). Likewise, disruptions in Morris water-maze performance were observed following chronic i.c.v. administration of IL-2 (Hanisch et al., 1997).

In parallel with the memory disturbances induced by IL-2, neurotoxic effects have recently been reported following prolonged treatment with the cytokine. Nemni et al. (1992) found marked hippocampal neuronal cell loss and neuronal degeneration in aged mice exposed to chronic peripheral IL-2 treatment. Since memory impairments and neuropathological changes were evident only in aged mice chronically treated with IL-2, it was suggested that aging may be a crucial factor for the neurotoxic effects of the cytokine. Moreover, since specific binding sites for IL-2 have been reported in the hippocampus and

IL-2 blocks ACh release within this region, IL-2 may influence memory function through its effects on cholinergic hippocampal cells (Nemni et al., 1992).

As mentioned previously, changes in blood-brain barrier viability have been observed following long-term peripheral treatment with IL-2 (Ellison et al., 1990). Similarly, Hanisch et al. (1994) reported periventricular tissue damage and gliosis following chronic central administration of the cytokine. In a further study, chronic i.c.v. IL-2 treatment was shown to induce astrogliosis, microglial activation and infiltration of T cells into the brain region surrounding the infused ventricle. Moreover, neuronal loss and myelin damage also occurred close to the location of IL-2 infusion (Hanisch et al., 1997). These findings suggest that in addition to inducing neuronal damage on its own, chronically elevated central levels of IL-2 may also facilitate the induction of other mechanisms (e.g., microglial activation, T-cell infiltration, neurotoxic cytokine production), which ultimately result in both myelin damage and neuronal loss. Indeed, T-cell infiltration into the brain provoked by the cytokine may be a major source of IL-2-induced cytotoxicity. Specifically, following entry into the brain, stimulated T-cells may produce additional IL-2, as well as other cytokines known to possess neurotoxic properties (e.g., TNF- α , IFN- γ).

Chronic central administration of IL-2 has also been reported to alter central receptor regulatory processes. Specifically, central infusion of IL-2 over 2 weeks was associated with a reduction in the binding levels of ACh in the frontoparietal cortex and hippocampus (e.g., CA1 region), and DA binding in the frontoparietal cortex and nucleus accumbens were reported (Hanisch et al., 1997). As mentioned previously, these brain areas are not only rich in IL-2R expression, but are regions where IL-2 has been shown to influence ACh and DA

neurotransmission (Alonso et al., 1993; Hanisch et al., 1993; Lapchak et al., 1992). In addition, it has been suggested that changes in IL-2-induced receptor binding levels may have resulted from either damage to cholinergic and dopaminergic neurons or alternatively, from a disruption in the release of ACh and DA induced by the long-term cytokine treatment (Hanisch et al., 1997).

4. Bacterial Lipopolysaccharide (LPS)

4.1. Introduction

Lipopolysaccharide, an endotoxin produced from the cell walls of gram-negative bacteria, has been employed in evaluating the effects of immune activation on HPA function and neuroendocrine processes (Rivier et al., 1989). Indeed, LPS acts as a nonspecific activator of the immune system by inducing the secretion of several macrophage-derived cytokines (e.g., IL-1 β , IL-6 and TNF- α) (Zuckerman et al., 1989). During pathological conditions, the entry of endotoxins into the bloodstream results in neuroendocrine and behavioural changes reminiscent of those seen during the acute phase response to infection (Kent et al., 1992b). Although the mechanisms responsible for LPS-induced effects are not well understood, the view has been suggested that cytokines secreted in response to LPS challenge, may function as potential mediators in this respect (Zuckerman et al., 1989). In the sections that follow, a brief description of the possible mechanisms by which LPS is believed to modulate HPA function will be provided. This will be followed by a review of the neuroendocrine and behavioural effects of LPS administration, together with a description of the roles played by cytokines in subserving these effects.

4.2. Penetration of LPS into the CNS

The mechanisms by which circulating LPS enters the brain and subsequently influences HPA activity are presently unknown. It is generally believed that LPS does not cross the blood-brain barrier (Lavicky & Dunn, 1995), although it has been suggested that endotoxins, like IL-1 β , may enter the CNS at areas where the blood-brain barrier is weak (e.g., at the circumventricular organs) (Saper & Breder, 1994). In addition, it has been reported that peripheral injection of LPS may enhance cerebral vascular permeability (Eckman et al., 1958), thus allowing the entry of the endotoxin into the brain (Van Dam et al., 1993). In fact, LPS has been shown to increase blood-brain barrier permeability, although this effect was not evident until 3 hours following peripheral injection with the endotoxin (Wispelwey et al., 1988).

In addition to the possibility of direct action on the CNS, LPS may exert an indirect effect on the brain through the modulatory actions of IL-1 β . Indeed, systemic administration of LPS has been shown to stimulate the synthesis and release of macrophage-derived cytokines (e.g., IL-1 β , IL-6, TNF- α) from activated immune cells (Zuckerman et al., 1989). Furthermore, the view has been proposed that LPS-induced increase in blood-brain barrier permeability may be a possible route by which circulating IL-1 β penetrates the brain. Indeed, peripheral LPS administration has recently been reported to stimulate central IL-1 β production (Takao et al., 1993a; Quan et al., 1994). As will be discussed shortly, it is possible that this centrally derived IL-1 β may also function in modulating LPS-induced HPA activity.

4.3. Role of LPS in IL-1 β Activity

Recent studies have shown that peripheral administration of LPS induces the central expression of IL-1 transcripts. Ban et al. (1992) revealed increases in IL-1 mRNA expression in the hippocampus following i.v. injection of LPS in mice. In a similar fashion, Reinisch et al. (1994) reported an increase of IL-1R1 mRNA in the whole mouse brain following systemic administration of LPS, while Gabellec et al. (1996) reported an increase in IL-1R1 and IL-1R2 mRNAs in the whole brain in response to systemic endotoxin injection. Interestingly, IL-1R1 mRNA expression was increased predominantly in the brain and pituitary (Zuckerman et al., 1989), whereas IL-1R2 mRNAs were more abundant in the spleen as compared to brain tissues (Gabellec et al., 1996).

In addition to the increased expression of IL-1 receptors in the brain, LPS is associated with IL-1 receptor down regulation in both the hippocampus (Haour et al., 1990; Takao et al., 1993a, 1994) and pituitary (Takao et al., 1993a) following peripheral LPS administration. Interestingly, this down regulation in central IL-1 receptors is apparently not due to a lack of mRNA expression, since the expression of transcripts for IL-1 receptors was up-regulated following LPS administration (Gabellec et al., 1996). As well, peripheral administration of LPS results in the appearance of IL-1 immunoreactivity in both macrophages and microglia in the brain (Van Dam et al., 1992).

Parallelling such effects, systemic LPS administration has been found to stimulate central IL-1 β production. Koenig et al. (1990) reported enhanced synthesis of IL-1 β in the hypothalamus and pituitary following peripheral LPS administration. In a similar fashion, the production of IL-1 β has been reported in the hippocampus of both mice and rats

following systemic injection of LPS (Takao et al., 1993a; Quan et al., 1994). However, although present in plasma 2 hours following peripheral LPS administration, IL-1 β was not found in the brain until 6 hours post injection (Quan et al., 1994). Moreover, like peripheral LPS treatment, central injection of the endotoxin has also been found to enhance IL-1 β levels in the brain (Coceani et al., 1988; Lisanby et al., 1990; Quan et al., 1994; Sundar et al., 1990).

4.4. Effect of LPS on HPA Activity

It has been widely demonstrated that LPS is a potent stimulator of the HPA axis. Indeed, administration of LPS either intraperitoneally (Linthorst et al., 1995; Mazzocchi et al., 1995) or intravenously (Ebisui et al., 1994) provokes the release of CRH from the PVN, leading to increased plasma ACTH and corticosterone concentrations (Kakucska et al., 1993). In contrast to the acute effects of LPS, attenuation of neuroendocrine changes has been reported following chronic LPS treatment. Wehrenberg et al. (1994) found that chronic systemic LPS administration (e.g., 4 days of endotoxin treatment) failed to alter CRH or ACTH concentrations. This contrasts with the effects of chronic IL-1 β administration, which results in sensitization of HPA activity (Schmidt et al., 1995). Discrepancies in the effects of LPS and IL-1 β may simply reflect differences in the specificities in their effects on immune function. Specifically, unlike IL-1 β , administration of LPS activates immune processes in a nonspecific manner by stimulating the secretion of IL-1 β , as well as IL-6 and TNF- α . In addition, although the synergistic action of CRH and AVP prevents desensitized of HPA activity in response to chronic IL-1 β administration (Schmidt et al., 1995), this

mechanism maybe less effective following long-term LPS treatment.

Interestingly, the mechanisms mediating HPA activation following high (e.g., fever producing) doses of endotoxin appear to be distinct from those mediating low doses of the endotoxin. Indeed, depletion of macrophages blocked the plasma ACTH and corticosterone responses to sub-pyrogenic doses of LPS, but not in response to pyrogenic doses of LPS (Derijk et al., 1991). This finding suggests that the subpyrogenic effects of LPS on HPA activity are mediated peripherally, while the pyrogenic effects of the endotoxin may involve central mechanisms. In addition, the varying dose-dependent effects of LPS suggest that the pyrogenic actions of this endotoxin can be disassociated from its effects on HPA activity.

4.5. Role of Cytokines in LPS-Induced HPA Activation

As previously mentioned, IL-1 β is believed to play a pivotal role in ACTH and corticosterone responses induced by peripheral LPS administration (Rivier et al., 1989; Schotanus et al., 1993). Indeed, administration of a monoclonal anti-IL-1 receptor antibody partially antagonized the LPS-induced HPA response in mice (Rivier et al., 1989), while the depletion of IL-1 by chemically destroying macrophages, blocked this response in rats (Derijk et al., 1991). However, it has recently become apparent that IL-1 β does not act alone in mediating the effects of LPS on HPA activity, but that other cytokines (e.g., IL-6 and TNF- α) play a role in this respect. Furthermore, these cytokines appear to play distinct roles in response to immune activation. For instance, kinetic differences in cytokines have been reported following peripheral endotoxin administration. Levels of TNF- α were found to peak one hour following LPS administration, while IL-1 β and IL-6 levels peaked within 2-4 hours

(Derijk et al., 1993; Schotanus et al., 1993; Zanetti et al., 1992). Moreover, levels of TNF- α declined more rapidly than those of IL-1 β and IL-6 (De Simoni et al., 1995). These findings suggest that following LPS administration, TNF- α plays a role in initiating HPA activation, while IL-1 β and IL-6 may act to sustain HPA functioning. It appears likely that such differences reflect LPS-induced variations of de novo synthesis of the cytokines within the brain. In particular, Gatti & Bartfai (1993) reported differences in the pattern of central mRNA induction for these cytokines following peripheral LPS administration.

In addition to playing distinct roles on endocrine secretion, the synergistic action of cytokines have also been shown to influence LPS-induced HPA function. Perlstein et al. (1993), for instance, reported that LPS-induced activation of the HPA axis is mediated by IL-6, as well as by the synergistic interaction of IL-1 and TNF- α (Perlstein et al., 1993). Pretreatment with IL-6 antibody completely antagonized LPS-induced ACTH release both 2 and 4 hours after injection, while pretreatment with either IL-1 receptor antibody or TNF- α antibody blocked LPS-induced ACTH release 4 hours following endotoxin administration. Conversely, the ACTH response 2 hours after LPS challenge was not antagonized by pretreatment with IL-1 receptor antibody or TNF- α antibody given separately, but was attenuated when the antibodies were administered in combination. Given that both TNF- α and IL-1 stimulate IL-6 release (Shalaby et al., 1989), this finding suggests that LPS directly stimulates IL-6 release 2 hours after LPS injection while TNF- α and IL-1 act synergistically to induced IL-6 release 4 hour following endotoxin administration.

Contrary to this assertion, the findings of Schotanus et al., (1993) failed to support a fundamental role for IL-6 in LPS-induced activation of HPA function. Indeed, systemic

administration of LPS resulted in a dose-dependent increase of ACTH, corticosterone and IL-6 concentrations. However, the dose of endotoxin needed to produce a significant increase in both ACTH and corticosterone appears to be at least 4 times lower than that necessary to induce an increase in plasma IL-6 concentrations. It might be noted as well, that while systemic administration of IL-1ra blocks LPS-induced release of ACTH and corticosterone, it does not influence plasma IL-6 levels (Schotanus et al., 1993; Smith & Kluger, 1992). Inasmuch as IL-1ra failed to inhibit increases in plasma IL-6 concentrations in response to low doses of endotoxin in rats, the actions of IL-1ra probably do not reflect a direct effect on IL-6 activity. As a result, it appears that activation of the HPA axis in response to a low dose of endotoxin are dependent on the endogenous production of IL-1 by macrophages, while the rise in plasma IL-6 concentrations following endotoxin injection may be mediated through IL-1-independent mechanisms (Schotanus et al., 1993).

The relative contributions of various cytokines to LPS-induced HPA activity appears to vary as a function of the endotoxin dose employed. Ebisui et al. (1994) reported that at low doses of LPS, plasma ACTH and corticosterone levels were completely antagonized by anti-TNF- α , while IL-1ra was without effect. Conversely, at high doses of LPS, both anti-TNF- α and IL-1ra significantly inhibited LPS-induced enhancement of HPA activity. These findings suggest that at low doses of LPS, TNF- α exclusively mediates LPS-induced activation of the HPA axis, while at higher doses, both IL-1 and TNF- α participate in this respect (Ebisui et al., 1994). A comparable analysis is not available concerning the dose dependent effects of LPS on IL-6 activity and its subsequent influence (direct and synergistic) on HPA activity. Thus, while the data of Schotanus et al. (1993) and Smith &

Kluger (1992) discussed earlier, seem to suggest dose dependence of IL-6 with respect to LPS activation of HPA activity, such a conclusion ought to be held in abeyance pending more detailed dose-effect analysis.

4.6. Sites of Action of LPS on HPA Axis

4.6.1. Humoral mechanisms

Without question, the bulk of the evidence suggests that LPS modulates HPA function by acting indirectly on the hypothalamus. As already indicated, systemic LPS administration provokes the release of CRH from the PVN, leading to increased plasma levels of ACTH and corticosterone (Kakucska et al., 1993). In addition, enhanced paraventricular CRH mRNA induced by systemic injection of LPS was abolished by intraventricular IL-1ra administration (Kakucska et al., 1993). Thus, it is likely that CRH is released as the result of LPS-induced IL-1 activation of the PVN. In addition, the view has been proposed that peripheral LPS may act through a non-paraventricular pathway to influence HPA function. Specifically, LPS has been shown to exert a direct influence at the level of the pituitary or adrenals (Ban et al., 1993; Elenkov et al., 1992; Schreiber et al., 1993). Elenkov et al. (1992) reported that LPS increased plasma levels of ACTH and glucocorticoids in animals with lesions of the PVN. In addition, Mazzocchi et al. (1995) argued that LPS may act directly at the level of the adrenals by stimulating the local production of cytokines. However, this action does not appear to involve the secretion of blood-borne IL-1, since the selective depletion of macrophages failed to prevent HPA activation in response to pyrogenic doses of the endotoxin (Derijk et al., 1991).

4.6.2. Vagal route

Although humoral mechanisms (e.g., the direct effect of LPS or the indirect modulatory role of IL-1 β) have been suggested as possible routes by which peripheral LPS influences HPA function, an alternative mechanism has recently been proposed in this respect. Specifically, the view has been offered that endotoxin-induced HPA activation may involve vagal afferents that relay peripheral information to the brain. Gaykema et al. (1995) reported that subdiaphragmatic vagotomy blocked Fos expression in CRH neurosecretory cells in the PVN and ACTH secretion in response to low doses of endotoxin, but only attenuated these responses following high doses of LPS. This finding suggests that at low doses of LPS, activation of HPA function may be mediated by vagal afferents, while at high doses of endotoxin, additional pathways (e.g., possibly humoral) may be involved in stimulating CRH neurons and ACTH secretion. Furthermore, it has been postulated that the local secretion of IL-1 β by peripherally administered endotoxin may activate vagal afferents that terminate in the NTS either directly or through production of other mediators (e.g., prostaglandins). The resulting activation of noradrenergic neurons within the NTS that project to the PVN, may ultimately stimulate the release of CRH from this region into the circulation (Gaykema et al., 1995).

4.7. Effect of LPS on Neurotransmitter Function

In addition to the effects of LPS on neuroendocrine processes, systemic endotoxin administration provokes alterations in central neurotransmitter activity. In this respect, particular attention has focused on the neurotransmitter variations within the hypothalamus,

although LPS appears to induce amine changes at extrahypothalamic sites as well. For instance, Elmquist et al. (1993) reported a dramatic increase in the number of Fos-IR cells in the paraventricular nucleus, supraoptic nucleus, central nucleus of the amygdala and the nucleus of the solitary tract, 2 hours following peripheral LPS treatment. The presence of Fos-IR cells in these brain regions following LPS administration supports the involvement of immune activation in a wide array of both endocrine and autonomic functions. Paralleling such findings, peripheral LPS administration has been shown to provoke NE turnover in the hypothalamus (Dunn & Welch, 1991; Massana et al., 1990; Mefford et al., 1991; Mefford & Heyes, 1990), as well as in both the prefrontal cortex and brain stem (Dunn & Welch, 1991; Massana et al., 1990). In addition, the LPS-induced increase in hypothalamic NE turnover was completely blocked following coadministration of indomethacin (Masana et al., 1990; Mefford & Heyes, 1990), suggesting that prostaglandins may play a pivotal role in central catecholamine responses induced by peripheral LPS administration.

In addition to NE changes, systemic LPS treatment also increased hypothalamic DA turnover (Mefford et al., 1991; Mefford & Heyes, 1990) and DA utilization in the prefrontal cortex (Masana et al., 1990). Contrary to these findings, however, Dunn & Welch, (1991) reported only a minimal effect of LPS on DA turnover within the hypothalamus, as well as in the prefrontal cortex and brain stem. It is unclear whether these differential effects are related to the time following LPS treatment when brain tissue was taken or whether the differences stemmed from the particular nuclei (within the hypothalamus) that were included in the analysis. After all, as in the case of stressors, DA alterations might have been detectable in the arcuate nucleus, but might have been obfuscated when other regions were

included.

Repeated treatment with LPS (e.g., for 7 consecutive days) has been reported to result in desensitization (e.g., tolerance) to the acute neurochemical effects of the endotoxin on NE and DA activity (Mefford et al., 1991). However, chronic administration of LPS failed to alter NE and DA utilization elicited by subsequent treatment with IL-1 β (Mefford et al., 1991). These data suggest that although LPS provokes the release of IL-1 β , this action is probably not involved in the tolerance to acute LPS administration which occurs following chronic exposure to the endotoxin. Treatment with LPS has also been found to influence central serotonergic activity. For instance, Dunn & Welch (1991) reported an increase in 5-HT turnover and tryptophan levels in both the hypothalamus and prefrontal cortex following systemic LPS injection. Moreover, these effects were attenuated by pretreatment with propranolol, suggesting the involvement of the sympathetic nervous system in this respect (Dunn & Welch, 1991).

Recently, Dunn & Chuluyan (1994) compared noradrenergic and serotonergic responses to LPS in endotoxin resistant mice, where the interaction of LPS with macrophages is known to be defective. As compared with controls, endotoxin-resistant mice showed reductions in cerebral noradrenergic and HPA activity in response to peripheral LPS administration. Contrary to this finding, no deficits in serotonergic responses were reported. These findings suggested that while LPS activates cerebral catecholamines and HPA function by similar mechanisms, different processes appear to be involved in serotonergic responses to endotoxin (Dunn & Chuluyan, 1994). Moreover, paralleling such effects, Dunn (1992) reported that following peripheral LPS injection, the peak catecholamine response

occurred much earlier than that of 5-HT (e.g., 2 hours after peripheral LPS injection compared to 8 hours for 5-HT) and coincided with the peak ACTH and corticosterone responses.

In a recent *in vivo* study, Linthorst et al. (1995) reported an increase in 5-HIAA and 5-HT concentrations in the hippocampus following systemic LPS administration. Moreover, central injection of IL-1ra attenuated the endotoxin-induced increase in hippocampal 5-HT, suggesting the involvement of central IL-1 mechanisms. However, other factors may also be important in the response to peripheral LPS treatment. Indeed, although central IL-1ra minimized hippocampal serotonergic changes, central administration of the antagonist failed to alter either free corticosterone levels or behavioural activity following endotoxin treatment. It is important to remember that LPS administration results in the release of IL-1 β , as well as IL-6 and TNF- α , and each of these cytokines alone has been reported to stimulate HPA axis with varying efficacy (Schobiz et al., 1994; Tilders et al., 1993a). Thus, the unhindered effects of LPS (following IL-1ra) may have involved these other cytokines.

Consistent with the post-mortem neurochemical studies, peripheral LPS administration was found to increase *in vivo* dialysate concentrations of NE, DA and their catabolites, as well as 5-HIAA levels in both the medial hypothalamus and medial prefrontal cortex (Lavicky & Dunn, 1995). Thus, it seems that systemic endotoxin treatment enhanced the release of NE, DA and 5-HT in both regions. In addition, following systemic injection of the same dose of LPS, greater neurochemical increases were shown in the hypothalamus relative to the prefrontal cortex (Lavicky & Dunn, 1995). This finding suggests that the effects of LPS may be regionally selective and that the hypothalamus may be particularly

sensitive to the effects of endotoxin treatment. Moreover, pretreatment with indomethacin was found to completely block LPS-induced catecholamine and indolamine changes in both the medial hypothalamus and medial prefrontal cortex, suggesting a role for prostaglandins in this respect (Lavicky & Dunn, 1995).

4.8. LPS-Induced Sickness Behaviour: Pyrogenic Effects of LPS

4.8.1. Role of IL-1 β

Endotoxin-induced activation of the immune system results in a variety of behavioural responses collectively termed sickness behaviour (e.g., increased fever, aphagia, hyperalgesia, hypersomnia and decreased activity) (Kent et al., 1992b). In particular, peripheral, as well as central administration of LPS have been shown to induce potent pyrogenic effects (Derijk et al., 1993; Klir et al., 1992,1994; Kluger, 1991; Long et al., 1990). Although the mechanisms responsible for LPS-induced fever are not well understood, they appear to occur secondary to the release of endogenous pyrogens (e.g., PGE stimulation by IL-1 β and TNF- α) by activated immune cells (Dinarello, 1984a,b; Dinarello et al., 1986).

As mentioned previously, IL-1 β plays a fundamental role in LPS-induced fever. For instance, elimination of peripheral macrophages was found to attenuate the LPS-induced rise in plasma IL-1 β levels and block the fever response to pyrogenic doses of peripheral LPS treatment (Derijk et al., 1993; Marquette et al., 1994). Parallelling this effect, peripheral administration of antibodies directed against IL-1 β attenuated fever in response to systemic LPS injection, (Kluger, 1991; Long et al., 1990; Rothwell, 1990). These findings suggests that the pyrogenic effects of LPS are dependent on mechanisms involving peripheral

macrophages and that peripherally produced IL-1 β may be an important mediator in the febrile response to LPS treatment.

4.8.2. Synergistic actions of cytokines

In addition to IL-1 β , other cytokines have been reported to play a role in the pyrogenic effects of LPS administration. In particular, IL-6 appears to be a potent mediator of LPS-induced fever (Klir et al., 1992,1994; LeMay et al., 1990b; Long et al., 1990). For instance, systemic treatment of pyrogenic doses of LPS enhanced the concentration of IL-6 (but not IL-1 β) within the anterior hypothalamus (Klir et al., 1992; Roth et al., 1992), while microinfusion of IL-6 into the anterior hypothalamus has been found to stimulate the production of fever (Klir et al., 1992). Although these results suggest that elevated levels of IL-6 in the anterior hypothalamus are involved in the induction of LPS-induced febrile response, circulating IL-6 does not appear to directly mediate LPS-induced fever. Indeed, Derijk et al. (1993) showed that elimination of peripheral macrophages resulted in a significant reduction in the pyrogenic response to LPS but did not influence plasma IL-6 levels in response to endotoxin administration.

It has recently been shown that the febrile response to LPS administration does not involve the direct action of IL-1 β on the CNS, but that IL-1 β may act indirectly through effects on central IL-6 function. For instance, systemic administration of antiserum to IL-1 β was found to attenuate levels of IL-6 within the CSF, as well as fever induced by LPS treatment (LeMay et al., 1990a). In a more recent study, Klir et al. (1994) reported that microinjection of anti-IL-1 β into the anterior hypothalamus significantly attenuated LPS-

induced fever and reduced central IL-6 levels in this region. These findings support the view that systemic LPS administration stimulates the peripheral production of IL-1 β (as well as other cytokines), which in turn, induce the central production and release of IL-6 within the anterior hypothalamus. Moreover, since IL-6-induced fever can be attenuated by indomethacin (LeMay et al., 1990b), it is believed that the enhanced levels of hypothalamic IL-6 induced by LPS, provokes an increase in PGE synthesis, resulting in fever (Klir et al., 1992). As mentioned previously, however, the source of IL-1 β in the induction of LPS-induced fever remains speculative.

4.8.3. Role of corticosterone

Recently, corticosterone has been reported to mediate the febrile response to LPS administration. Indeed, the view has been offered that secretion of glucocorticoids in response to LPS-induced HPA activation play an important role in regulating the host response to infection. For instance, adrenalectomized rats showed an increased fever response to LPS which was antagonized by either dexamethasone treatment (Coelho et al., 1992) or corticosterone implants (Johnson et al., 1996). In a similar fashion, pretreatment with the glucocorticoid antagonist, RU-38486 has been reported to mimic the effects of adrenalectomy on the pyrogenic effects of systemic LPS administration (McClellan et al., 1994; Morrow et al., 1993) while glucocorticoid treatment attenuated endotoxin-induced fever response in these animals (Coelho et al., 1992, Morrow et al., 1993). Commensurate with these findings, it appears that glucocorticoids act in a negative feedback manner to prevent the excessive synthesis and release of IL-1 β and TNF- α by activated immune cells

in response to LPS. As a result, glucocorticoids may function in a protective manner to reduce the susceptibility of organisms to the pyrogenic effects of endotoxin exposure (Zuckerman et al., 1989).

4.8.4. Dose dependent effects of LPS

As mentioned previously, the mechanisms mediating the pyrogenic effects of LPS appear to be distinct from those mediating responses to low doses of the endotoxin. Indeed, while the fever-producing effects of LPS may involve central mechanisms, the subpyrogenic effects of the endotoxin (e.g., effects on HPA activity) are mediated peripherally (Derijk et al., 1991). Thus, it appears that the pyrogenic and subpyrogenic actions of LPS can be disassociated from one another. For example, the behavioural effects of LPS appear to be dose-dependent. Johnson et al., (1996) reported that subpyrogenic doses of systemically injected LPS increased plasma corticosterone levels but failed to influence social behaviour, while pyrogenic doses of the endotoxin increased HPA activity and diminished social behaviour. This finding suggests that while low doses of LPS were sufficient to activate HPA activity, higher doses of the endotoxin were needed to influence behaviour.

4.9. Feeding Behaviour

In addition to its effects on fever production, LPS has been reported to influence feeding behaviour. Peripheral and central administration of LPS have been shown to result in dose-dependent reductions in food intake (O'Reilly et al., 1988; Plata-Salaman & Borkoski, 1993). Furthermore, cytokines (e.g., IL-1 β , IL-6, TNF- α) released in response to

LPS administration may play a pivotal role in mediating this effect (Plata-Salaman, 1995). The view has been also been suggested that LPS-induced reduction in food intake may be centrally mediated, since both peripheral and central IL-1 β administration have been shown to induce anorexia (Plata-Salaman & Borkoski, 1993).

It seems likely that LPS and IL-1 β -induced anorexia are mediated through different mechanisms. For example, chronic administration of IL-1 β results in sensitization to the anorexic effects of the cytokine, while tolerance develops in response to repeated exposure to LPS (Langhans et al., 1993). In a further study, pretreatment with paracetamol (a cyclooxygenase inhibitor) attenuated IL-1 β -induced anorexia but failed to influence food intake following LPS administration. This finding suggests that while prostaglandins may play a role in IL-1 β -induced anorexia, LPS-induced reductions in feeding may involve other mechanisms.

In addition to its effect on food consumption, LPS has recently been shown to influence feeding preferences. Yirmiya (1996) reported that systemic administration of LPS significantly diminished saccharin preference in a dose dependent manner, without affecting water intake. Thus, it was suggested that LPS may be inducing an anhedonic effect, much like that induced by stressors. In effect, the "sickness behaviour" associated with LPS treatment may involve anhedonia mediated by central mechanisms, as well as anorexia.

4.10. Exploratory Behaviour

As previously mentioned, LPS has been reported to decrease social exploration (Bluthe et al., 1992; Johnson et al., 1996; Yirmiya, 1996). Interestingly, this effect was

attenuated by peripheral, but not central administration, of IL-1ra (Bluthe et al., 1992). In a similar fashion, centrally injected IL-1ra failed to influence the reduction in general activity reported following systemic LPS administration in rats (Linthorst et al., 1995). Thus, these findings implicate peripheral IL-1 receptors in modulating these behaviours (Bluthe et al., 1992).

Systemic LPS administration has recently been shown to reduce both the locomotor activity and exploratory behaviour of rats in an open-field (Yirmiya, 1992). In a more discrete behavioural analysis, Dunn et al. (1992) reported that systemic and central administration of LPS attenuated the exploratory behaviour of mice in a multicompartiment chamber (e.g., reduced the contact time with the novel stimuli). This reduction of exploratory behaviour was accompanied by nonspecific behavioural effects, including a decline in the number of stimulus contacts, compartmental entries, number of rears, locomotor activity, as well as the presence of intermittent pausing (Dunn et al., 1992).

It is interesting that peripheral and central administration of LPS mimicked the ability of IL-1 β to suppress the exploratory behaviour of mice in the multicompartiment chamber (Dunn et al., 1991, 1992; Spadaro & Dunn, 1990). However, distinct behavioural differences existed. For instance, the reduction in stimulus contact time following LPS treatment resulted from a decline in the number of contacts with the novel stimuli, whereas treatment with IL-1 β increased the number of contacts made by mice (Dunn et al., 1992). In addition, indomethacin reduced the time of stimulus contact ordinarily induced by LPS, but not in response to IL-1 β administration (Dunn et al., 1992). Furthermore, administration of LPS induced nonspecific behavioural effects which were not evident following treatment

with IL-1 β (Dunn et al., 1992). These results suggest that IL-1 β may not be solely responsible for mediating the behavioural actions of LPS. Rather, some of the effects of LPS may simply be a reflection of general malaise induced by the endotoxin treatment (Dunn et al., 1992).

In a recent study, Yirmiya (1996) investigated the effect of chronic antidepressant treatment on several LPS-induced sickness behaviours. Chronic treatment with imipramine reduced the suppressive effects of LPS on food consumption, body weight, social interaction, as well as locomotor activity and exploratory behaviour in an open-field (Yirmiya, 1996). As a result of these findings, Yirmiya (1996) suggested that the LPS-induced effects were not simply the result of sickness behaviour, but that these effects were indicative of anhedonia (e.g., depressive-like symptoms). Indeed, evidence exists suggesting that immune activation and cytokine release may play a contributory role in the provocation of clinical depression (Maes et al., 1995). In illnesses associated with high levels of immune activation (e.g., autoimmune diseases, multiple sclerosis, stroke), the incidence of depression were reported to exceed 50% (Minden & Schiffer, 1990; Parker et al., 1992; Schwartz et al., 1993). However, these depressive states may be secondary to the illness rather than reflecting immune-induced affective states.

As in the case of the febrile response to LPS, it seems that glucocorticoids play a role in the behavioural components of sickness. For instance, adrenalectomy enhanced the reduction in social exploration of juvenile conspecific mice induced by peripheral LPS treatment (Goujon et al., 1995), while chronic replacement of corticosterone antagonized this effect (Johnson et al., 1996). In a similar fashion, peripheral administration of the

glucocorticoid antagonist, RU-38486 augmented LPS-induced reduction in social exploration (Goujon et al., 1995). In addition, Pezeshki et al. (1996) reported an enhanced reduction in locomotor activity in adrenalectomized rats administered LPS when compared to intact controls. Commensurate with these findings, it has been suggested that in adrenalectomized animals, LPS-induced synthesis and release of IL-1 β and TNF- α by activated immune cells may increase susceptibility to the behaviourally depressing effects of endotoxin exposure (Zuckerman et al., 1989).

5. Experimental Overview

It is clear from the preceding discussion that IL-1 β and IL-2 may influence neuroendocrine processes, and like stressors, may have effects on hypothalamic and extrahypothalamic biogenic amine activity. However, the data concerning the extrahypothalamic alterations are sparse. One purpose of the present investigation was to assess the effects of these cytokines on central biogenic amines, particularly in brain regions sensitive to stressors. In this respect, several studies were conducted in which post-mortem tissue of mice were assessed following cytokine challenge. In addition, *in vivo* studies (by microdialysis) were conducted to examine the interstitial release of monoamines from limbic sites following IL-1 β treatment in rats. Moreover, it was of interest to establish whether these cytokines elicit anxiogenic effects. This proposition was predicated on the finding that cytokines promote the release of CRH, a potent anxiogenic peptide (Gray, 1991; Heilig et al., 1994). Finally, several experiments were conducted to assess the effects of immune challenge (as opposed to cytokine administration) on CNS neurotransmitters and/or

behaviour. In particular, we examined the effects of systemic SRBC administration on *in vivo* DA changes, as well as the effects of systemic LPS on both post-mortem biogenic amine concentrations, and behaviour in several tests of anxiety.

Experiment 1. Effects of Systemic IL-1 β Administration on Exploratory Behaviours, Neuroendocrine Levels and Central Biogenic Amines in Mice

Few studies have systematically evaluated the behavioural effects of IL-1 β across a wide range of doses, and as a result, even the most fundamental behavioural actions of this cytokine remain to be fully described. Inasmuch as IL-1 β is a potent stimulator of CRH, a neuropeptide which has been shown to induce anxiogenic effects, the possibility was considered that IL-1 β might likewise induce anxiety, particularly in a novel situation. As indicated earlier, the central actions of this cytokine have not been examined extensively. In the main, the neurochemical alterations associated with IL-1 β treatment have been restricted to the hypothalamus and relatively little information is currently available concerning the extrahypothalamic effects of this cytokine. Accordingly, one purpose of the initial experiment was to examine the effects of IL-1 β on monoamine activity in the hypothalamus, as well as in several extrahypothalamic sites. Inasmuch as IL-1 β has been proposed to have central actions much like those elicited by stressors (Dunn, 1988,1990; Shintani et al., 1993; Zalcman et al., 1994), it was of particular interest to determine whether amine changes elicited by IL-1 β are most pronounced in brain regions (e.g., locus coeruleus, prefrontal cortex, nucleus accumbens, central nucleus of the amygdala) where stressors have previously been found to affect NE, DA and 5-HT activity (Anisman et al., 1993; Deutch & Roth, 1990; Stanford, 1995).

1.1. Interleukin-1 β : A Dose-Response Study

It will be recalled that treatment with IL-1 β has been reported to influence motor activity (possibly as part of the illness engendered by the cytokine), and has been found to affect exploratory behaviour such as nose-poke toward a novel object (Dunn et al., 1991; Spadaro & Dunn, 1990). The initial experiment was undertaken to evaluate in greater detail some of the behavioural effects of systemically administered IL-1 β on exploratory and locomotor behaviours across a wide range of doses, with particular attention being devoted to the tendency of an animal to approach a novel stimulus object. In addition to open-field exploration, spontaneous alternation was evaluated in a free-running Y-maze. In this task, rodents permitted to explore a free-running Y-maze in the absence of reinforcement, tend to enter the least recently visited arm of the maze (i.e., spontaneous alternation). Spontaneous alternation requires the animal to distinguish between the arm of the maze most recently visited from that least recently entered. This behaviour is modifiable by treatments thought to influence attentional, habituation and short-term memory processes, but appears to be independent of the motoric actions of this treatment (Kokkinidis & Anisman, 1981). Accordingly, the possibility was considered that this paradigm might be ideally suited to detect cognitive types of changes induced by IL-1 β , regardless of whether the cytokine influenced locomotor activity. In parallel with the behavioural effects exerted by IL-1 β , in this experiment we also assessed the central amine alterations induced by the cytokine, as well as the dose-related effects of IL-1 β on plasma ACTH and corticosterone levels.

Materials and Methods

Subjects

All experimental procedures described in this dissertation were approved by the Carleton University Animal Care Committee and met the guidelines set out by the Canadian Council on Animal Care (CCAC). Experimentally-naive, male, CD-1 mice (Charles Rivers, Canada Inc.) were employed in this and in each of the ensuing experiments. Mice were obtained at 9 weeks of age and were acclimatized to the laboratory conditions for 3 weeks prior to testing. Unless otherwise specified, mice were housed in groups of 5 in standard polypropylene cages, permitted free access to food and water and maintained on a 12-h light-dark cycle (light phase: 0700-1900 h). To avoid the influence of diurnal factors, all testing was conducted between 0800-1200 h.

Apparatus and procedure

Open-field behavioural assessment

Mice ($n = 12/\text{group}$) received intraperitoneal (i.p.) injection with either carrier-free hIL-1 β (0.2, 0.4, 0.8, 1.6 μg) or vehicle, in a volume of 0.25 ml of phosphate buffered saline. The IL-1 β which was generously provided by Dr. C. Reynolds (Biological Research Branch, National Cancer Institute, Frederick, Maryland, USA), had a specific activity of 1.8×10^4 IU/ μg . Immediately following injection, mice were individually placed in a clear polypropylene open-field (36.5 x 31.0 x 16.5 cm), which was located in a darkened room. A cylindrical shaped (silver-coloured) container (length = 9.8 cm, diameter = 7.0 cm) was located in the centre of the open-field. Over the course of a 40 min period, the behaviour of

the mice in the open-field was recorded by a video camera mounted directly above the testing apparatus. The videotapes were subsequently analyzed using a time-sampling procedure. In particular, 2 min epochs in each 7 min period were evaluated (i.e., 0-2, 7-9, 14-16, 21-23, 28-30, 35-37 min) with respect to (a) time spent in the container, (b) frequency and time of contacts with the outer portion of the container, (c) number of entries and exits from the container, (d) frequency of rearing responses, (e) time spent in locomotor activity, (f) time spent sitting or resting, and (g) time spent digging. As well, the latency to make initial contact with the container and the latency for the initial entry into the container were recorded.

Y-maze behavioural assessment

Immediately following the open-field test, mice were individually placed in a symmetrical Y-maze to evaluate free-running spontaneous alternation for a 10 min period. The black Plexiglas Y-maze had arms measuring 19.0 x 9.0 x 17.0 cm, and a floor made up of 0.32 cm stainless-steel rods spaced 1.0 cm apart. The maze was situated in a darkened room, and the behaviour of the mice was recorded by an overhead video camera. The sequence of arm entries (an arm entry being defined as all four paws placed in an arm of the maze) was subsequently determined, and the number of alternation responses calculated. An alternation response was defined as an animal visiting the least recently entered arm, while a non-alternation (perseveration) response was defined as an animal entering the most recently visited arm. Thus, a sequence of responses consisting of entries into arm 1, 2, 3, 1, 2, 1, 3, 2, 1, 2 was considered to comprise 6 alternation responses (underlined) and two

perseverative responses (non-underlined) and the alternation score was .75 (calculated as alternation responses/alternation responses plus perseveration responses: note that the initial two arm entries were not included in the alternation score).

Neurochemical and neuroendocrine determinations

Following behavioural testing, mice were decapitated, trunk blood collected, and brains were removed. The hypothalamus was removed in its entirety while other brain regions were micropunched from coronal brain slices obtained using a dissecting block fitted with slots, which served as guides for single edged razor blades. Brain sections were placed on glass slides and frozen on dry ice. The prefrontal cortex, nucleus accumbens, caudate, hippocampus, substantia nigra and locus coeruleus were obtained by micropunch using hollow microdissection needles and the tissue was frozen at -80°C for determination of amine and metabolite concentrations. Brain punches were taken using the mouse brain atlas of Franklin and Paxinos (1997), together with that of Palkovits and Brownstein (1988). Plasma obtained from the trunk blood was collected and stored at -80°C for subsequent ACTH and corticosterone determinations.

The plasma ACTH and corticosterone levels were assayed, in duplicate, by commercial RIA kits (ICN Biomedicals Inc., CA, USA), and had been found to have inter- and intra-assay variability of less than 10%. In order to eliminate inter-assay variability, the samples were run in a single assay. Levels of NE, DA and 5-HT, and their respective metabolites, 4-hydroxy-3-methoxyphenylglycol (MHPG), dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA) were determined by high

performance liquid chromatography (HPLC) using a modification of the method of Seegal et al. (1986). Tissue punches were sonicated in a homogenizing solution which was comprised of 14.17 g monochloroacetic acid, 0.0186 g disodium ethylenediamine tetraacetate (EDTA), 5.0 ml methanol and 500 ml H₂O. Following centrifugation, the supernatants were used for the HPLC analysis. Using a waters M-6000 pump, guard column, radial compression column (5 μ , C₁₈ reverse phase, 8mm x 10cm), and a three cell coulometric electrochemical detector (ESA Model 5100A), 20 μ l of the supernatant was passed through the system at a flow rate of 1.5 ml/min (1400-1600 PSI). The mobile phase used for the separation was a modification of that used by Chiueh et al. (1983); each liter consisted of 1.3 g of heptane sulfonic acid, 0.1 g disodium EDTA, 6.5 ml triethylamine and 35 ml acetonitrile. The mobile phase was then filtered (0.22 μ m filter paper) and degassed following which the pH was adjusted to 2.5 with phosphoric acid. The area and height of the peaks was determined using a Hewlett-Packard integrator. The protein content of each sample was determined using bicinchoninic acid with a protein analysis kit (Pierce Scientific, Brockville, Ontario) and a spectrophotometer (Brinkman, PC800 colorimeter).

Data analysis

The open-field behavioural data were subjected to analysis of variance (ANOVA) (5 Drug doses) with repeated measures (Time following treatment). The analysis of variance was performed with df adjusted using *Hyundtfeldt epsilon*. Newman-Keuls multiple comparisons were employed to compare the means of the simple effects of the significant interactions. As the number of subjects relative to the number of variables was too small for

MANOVA, the α level for the multiple ANOVAs was set at .01 for the behavioural measures. Moreover, the findings were considered meaningful only when effect sizes (e^2) indicated that the treatment accounted for more than 10% of the variance. The spontaneous alternation behaviour and locomotor activity (number of arm entries) in the Y-maze, as well as each of the neuroendocrine and neurochemical measures were analyzed using a one-way ANOVA. Post-hoc tests were conducted using Newman-Keuls multiple comparisons ($\alpha = 0.05$).

1.2. Effect of IL-1 β on Spontaneous Alternation in the Absence of a Prior Stressor

In the initial experiment, performance of mice on the Y-maze may have been confounded or obfuscated by the prior stress associated with being placed in the open-field. Thus, it was of interest to evaluate the behavioural effects of acute systemic IL-1 β treatment on spontaneous alternation performance in the Y-maze in the absence of prior exposure to the open-field.

Material and Methods

The apparatus and the subject characteristics were the same as in Experiment 1.1. Mice received either i.p. administration of IL-1 β (0.4 μ g) or saline ($n = 8$ /group). Following injection, mice were returned to their home cages for 40 minutes, after which they were individually tested in the Y-maze for a 10 min period (as described in the initial experiment).

1.3. Effect of Repeated Systemic IL-1 β Administration on Y-Maze Performance

Inasmuch as repeated administration of IL-1 β may have effects distinguishable from those of acute administration, a further study assessed the effect of repeated systemic IL-1 β treatment on spontaneous alternation performance in the Y-maze.

Materials and Methods

Naive CD-1 mice received either i.p. administration of IL-1 β (0.5 μ g) or saline (n = 6/group) on each of 5 consecutive days. At 40 min following cytokine treatment on each day, animals were individually placed in the Y-maze for 10 min and the frequency and sequence of arm entries recorded (as described in the initial experiment).

1.4. Systemic IL-1 β Administration Influences Biogenic Amines in Naive Mice

In the initial experiment mice were exposed to a novel environment, and amine changes were examined in the hypothalamus as a whole. As prior behavioural testing may have influenced amine and neuroendocrine activity, an unadulterated index of the IL-1 β actions may not have been obtained. Accordingly, in Experiment 1.4 brain tissue samples and plasma were taken from IL-1 β and vehicle-treated mice in the absence of any other treatments. As the actions of the cytokine may have been restricted to only selected nuclei, in Experiment 1.4 the effects of IL-1 β were assessed in specific hypothalamic nuclei (e.g., the paraventricular nucleus and in the median eminence plus arcuate nucleus), as well as in the central nucleus of the amygdala, a region thought to play a pivotal role in mediating anxiety.

Materials and Methods

Mice ($n = 60$) received i.p. injection of either saline or IL-1 β (0.1, 0.5, 1.0 μg) and were immediately returned to their home cage for 50 minutes. Mice were then sacrificed, trunk blood collected and brains were removed/dissected (as described in Experiment 1.1), except that instead of removing the hypothalamus as a whole, discrete hypothalamic nuclei (e.g., the paraventricular nucleus and median eminence plus arcuate nucleus), as well as the central nucleus of the amygdala, were micropunched using the mouse brain atlas of Franklin and Paxinos (1997). Tissue samples and plasma obtained from the trunk blood were stored at -80°C for subsequent central amine, ACTH and corticosterone determinations, respectively, as described in the initial experiment.

Results

Active behaviours: locomotion, digging, rearing

Analysis of variance of the time spent locomoting yielded a significant Cytokine treatment $\times$ Sampling period interaction, $F(20, 255) = 3.40$, $p < 0.01$. The multiple comparisons of the simple effects comprising this interaction indicated that during the initial 0-2 min period, the locomotor activity of mice that received either the 0.4 or 0.8 μg doses of IL-1 β exceeded that of saline-treated animals (see Figure 1). Among mice treated with the cytokine, locomotor activity declined markedly over the course of the test session, such that during the last three sampling periods the activity of each of the IL-1 β groups was significantly lower than that of vehicle-treated animals. As will be discussed shortly, since mice in the various groups spent different amounts of time in the novel container, and over

Figure 1. Mean ($\pm$ S.E.M.) of several behaviours (locomotor activity, digging, rearing, time in novel container, entries into container, contacts with container) over time following treatment with several doses of IL-1 β . Each point denotes the behavioural change that occurred during a 2 min period.

the course of the test session, evaluation of locomotor activity alone does not provide an unadulterated description of the animal's behavioural profile in response to the cytokine.

Like locomotor activity, both the time spent digging and the frequency of rearing responses varied as a function of the Cytokine treatment x Sampling period interaction, $F(20, 255) = 2.93$ and 3.37 , $p < 0.01$. The multiple comparisons indicated that the frequency of digging was very low and did not differ between groups during the initial sampling period. However, over sampling periods the time spent digging increased in saline-treated animals, so much so that by the last two periods mice spent approximately 10% of their time engaged in this behaviour. In the IL-1 β -treated animals, digging appeared infrequently and for brief periods of time. As early as the second sampling period, the time spent digging in the IL-1 β mice (0.4 - 1.6 μ g) was lower than that of saline-treated animals, while at the fourth sampling period the vehicle-treated animals could also be distinguished from mice that received the 0.2 μ g dosage. The frequency of rearing responses in saline-treated animals did not vary over the course of the test session. In mice that received IL-1 β , however, rearing increased between the initial two sampling periods, and then declined precipitously. As a result, the frequency of rearing responses throughout the second half of the test session was significantly below that of saline-treated mice.

Entry and contact with the novel container

The time of first contact with the novel container, as well as the first entry into the container, did not vary as a function of the cytokine treatment. In contrast, the number of entries and the time spent in the container, as well as the time spent in contact with the outer

portion of the container, varied as a function of the Cytokine treatment x Sampling period interaction, F 's (20, 255) = 2.05, 3.69 and 2.39, p 's < 0.01, respectively. The multiple comparisons indicated that the time spent in the container increased over sampling periods in each of the groups. However, in saline-treated mice the increase was noted only between the first and second sampling periods, and remained stable thereafter. In mice that received the 0.2 - 0.8 μ g doses of IL-1 β , the time spent in the container continued to increase throughout the test session. As a result, the time in the container in these groups exceeded that of vehicle animals during the last three sampling periods. Among mice that received the highest IL-1 β dosage (1.6 μ g), the increase was not apparent over the latter part of the test session. Instead, these animals remained outside the container, where they tended to be inactive. Indeed, the time spent sitting motionless outside the container revealed a significant effect of the Cytokine treatment, $F(4, 50) = 5.18$, $p < 0.01$ and Sampling periods, $F(5, 250) = 6.63$, $p < 0.01$. The multiple comparisons confirmed that the 1.6 μ g dose of IL-1 β lead to the inactive posture being adopted to a greater extent (40% of the time) than among any of the remaining groups, who adopted this posture between 8-16% of the time.

The multiple comparisons of the number of entries into the container indicated that this behaviour occurred infrequently during the initial sampling period, but increased thereafter. The increase was most pronounced in animals that received low doses of IL-1 β (0.2 and 0.4 μ g). While the frequency of entries declined over time among the cytokine-treated mice, the frequency of entries continued to increase over the course of the test session in vehicle-treated animals. Accordingly, during the latter half of the test session, the vehicle animals exhibited a greater number of entries than did mice that received IL-1 β .

Essentially, during the latter half of the test session, IL-1 β -treated mice tended to remain within the container without making repeated entrances and exits. In fact, as seen in Figure 1 and confirmed by multiple comparisons, during the latter part of the test session, the saline-treated animals made relatively frequent contacts with the outer part of the container as compared to mice that received IL-1 β .

Spontaneous alternation

In mice permitted to explore the novel open-field prior to Y-maze exposure, the number of arm entries varied significantly with the Cytokine treatment, $F(4, 51) = 10.02$, $p < 0.01$. The multiple comparisons confirmed that at each of the doses IL-1 β -treated mice emitted fewer arm entries as compared to controls. As well, mice that received the 1.6 μg dose made fewer arm entries than did mice that received either 0.2 or 0.8 μg doses (see Table 1). In contrast to the motor activity, the groups were found not to differ with respect to spontaneous alternation performance (see Table 1). Mice of each group exhibited high rates of alternation, emitting approximately twice as many alternation as opposed to non-alternation responses, yielding alternation scores that ranged between 65 and 70%.

Paralleling the findings seen in mice previously placed in a novel environment, naive animals (i.e., those not exposed to the novel open-field prior to Y-maze testing) treated with an intermediate dose (0.4 μg) of IL-1 β showed a reduction of total arm entries relative to saline-treated mice, $F(1, 14) = 56.27$, $p < 0.01$ ($\bar{x} \pm \text{SEM} = 33.87 \pm 2.45$ vs 45.12 ± 2.84 , respectively). However, once again spontaneous alternation performance was unaffected by the treatment ($\bar{x} \pm \text{SEM} = 65.21 \pm 2.97$ and 66.24 ± 1.99 , for IL-1 β and saline-treated mice,

Table 1. Effect of IL-1 β on Spontaneous Alternation Performance over a 10 min period in a Free-Running Y-Maze

Treatment	% Alternation	Total Arm Entries
Saline	68.25 $\pm$ 2.07	42.20 $\pm$ 1.65
0.2 μ g	67.55 $\pm$ 2.59	33.73 $\pm$ 2.72**
0.4 μ g	65.68 $\pm$ 2.43	26.92 $\pm$ 1.73**
0.8 μ g	69.91 $\pm$ 1.95	31.09 $\pm$ 2.51**
1.6 μ g	66.14 $\pm$ 2.32	22.83 $\pm$ 2.50**

** p < 0.01 relative to saline-treated mice

respectively).

Likewise, repeated exposure to IL-1 β (0.5 μ g) on each of 5 consecutive days failed to alter spontaneous alternation performance in the Y-maze among naive animals. Analysis of variance of the spontaneous alternations scores on Day 1 vs Day 5 confirmed that performance was unaffected by the repeated IL-1 β treatment. As seen in Table 2, the IL-1 β -treated mice actually scored slightly higher alternation levels than did the saline-treated mice. Analysis of the number of arm entries revealed a significant Cytokine treatment x Days interaction, $F(1, 10) = 13.28$, $p < 0.01$. Newman-Keuls multiple comparisons of the simple effects indicated that on the first test day the number of arm entries in mice that received IL-1 β was significantly lower than among saline-treated animals. Activity declined over days in the saline-treated animals, but not in mice that received IL-1 β .

Plasma ACTH and corticosterone

In mice exposed to the novel environment, plasma corticosterone levels varied significantly with the Cytokine treatment, $F(4, 50) = 8.86$, $p < 0.01$. The multiple comparisons indicated that each of the IL-1 β doses increased plasma corticosterone levels relative to that of control (saline-treated) animals, while the differences between the various IL-1 β groups did not differ from one another (see Figure 2). Parenthetically, the levels of corticosterone were about 3-fold higher in saline-treated mice relative to that typically observed in non-stressed controls, an effect likely attributable to exposure to the novel environment (i.e., being placed in the open-field, followed by the Y-maze test). Thus, the

Table 2. Mean ($\pm$ S.E.M) Spontaneous Alternation and Number of Arm Entries after 1 or 5 Days of IL-1 β or Saline Treatment

	Day 1	Day 5
<u>Spontaneous Alternation</u>		
Saline	65.33 $\pm$ 3.65	65.83 $\pm$ 2.81
IL-1 β	69.67 $\pm$ 2.51	71.17 $\pm$ 2.66
<u>Arm Entries</u>		
Saline	32.67 $\pm$ 2.76	24.17 $\pm$ 1.23
IL-1 β	21.50 $\pm$ 1.57*	26.50 $\pm$ 2.01

* p < 0.05 relative to saline-treated mice

Figure 2. Mean ($\pm$ S.E.M.) plasma ACTH and corticosterone 50 min following administration of IL-1 β (* $p < 0.05$ relative to saline-treated animals).

plasma corticosterone variations ought to be considered as a reflection of the conjoint effects of mild stressor exposure coupled with IL-1 β treatment. The effects of the cytokine treatment on ACTH concentrations failed to reach statistical significance. However, as *a priori* predictions had been made concerning the effects of IL-1 β , multiple comparisons were conducted to assess the effects of the cytokine treatment relative to that of saline controls. In fact, the cytokine increased plasma ACTH concentrations, at least at the highest dosage. Once again, however, it is possible that the ACTH changes reflected the effects of the cytokine treatment coupled with exposure to a novel environment.

Paralleling the effects observed in the initial experiment, in naive animals (e.g., those not exposed to the novel environment), IL-1 β treatment greatly influenced plasma corticosterone levels, $F(3, 44) = 86.12, p < 0.01$. Newman-Keuls multiple comparisons confirmed that relative to saline-treated animals, each of the IL-1 β doses increased circulating corticosterone (see Table 3). Moreover, the increase was greater with the higher doses (0.5 and 1.0 μg) than with the lower doses (0.1 μg). It will be recalled that the between-group differences, while significant, were less marked in the initial experiment, likely owing to the confounding influence of exposure to the novel environment. Like the variations of corticosterone, the analysis revealed that ACTH varied as a function of the Cytokine treatment, $F(3, 51) = 2.91, p < 0.05$. Although each of the doses of IL-1 β increased ACTH concentrations, the multiple comparisons indicated that this increase was only significant at the 0.5 μg dosage (see Table 3). Of course, ACTH variations in response to environmental stimuli occur sooner and are more transient than the corticosterone alterations. As blood samples were taken at only a single time following IL-1 β treatment, it is possible

Table 3. Effect of IL-1 β on Plasma ACTH and Corticosterone Concentrations

Treatment	ACTH (pg/ml)	Corticosterone (μ g/dl)
Saline	154.48 $\pm$ 23.59	3.20 $\pm$ 0.98
0.1 μ g	262.29 $\pm$ 63.83	23.67 $\pm$ 2.44*
0.5 μ g	387.18 $\pm$ 71.25*	36.89 $\pm$ 3.29*
1.0 μ g	284.90 $\pm$ 34.61	32.26 $\pm$ 2.96*

* p < 0.01 relative to saline-treated mice.

that detection of the between-group differences as a function of cytokine treatment were limited by the narrow window during which the blood was sampled.

Biogenic amines

In mice exposed to the novel environment, administration of IL-1 β did not influence NE or MHPG concentrations within the hypothalamus, hippocampus or prefrontal cortex, although a modest rise of MHPG was evident in the prefrontal cortex following the 0.8 and 1.6 μ g doses of IL-1 β ($\bar{x} \pm \text{SEM} = 3.22 \pm 0.39$ and 3.51 ± 0.32 ng/mg protein, respectively) relative to vehicle-treated mice ($\bar{x} \pm \text{SEM} = 2.77 \pm 0.18$ ng/mg protein). In the locus coeruleus the MHPG concentrations were altered by the Cytokine treatment, $F(4, 46) = 2.57$, $p < 0.05$, whereas the NE concentrations were unaffected (see Figure 3). Newman-Keuls multiple comparisons indicated that relative to vehicle animals, MHPG concentrations were elevated in mice that received 0.2, 0.4 and 1.6 μ g of IL-1 β . In contrast to the MHPG variations, concentrations of DA and DOPAC were not affected by the IL-1 β treatment in any of the brain regions examined, notably the nucleus accumbens, prefrontal cortex, substantia nigra or caudate. Likewise, neither 5-HT nor 5-HIAA were altered by the cytokine treatment in the hypothalamus, hippocampus or prefrontal cortex.

Biogenic amine effects in the absence of prior behavioural testing

In contrast to the limited amine variations observed in the entire hypothalamus, NE levels within the PVN were significantly affected by the IL-1 β treatment, $F(3, 29) = 4.90$, $p < 0.01$ (see Figure 4). The multiple comparisons indicated that the 0.5 μ g dose reduced NE

Figure 3. Mean ($\pm$ S.E.M.) NE and MHPG concentrations in the locus coeruleus (LC) 50 min following administration of IL-1 β (* $p < 0.05$ relative to saline-treated animals).

Figure 4. Mean ($\pm$ S.E.M.) NE and MHPG concentrations in the paraventricular nucleus (PVN) 50 min following administration of IL-1 β (* $p < 0.05$ relative to saline-treated animals).

levels, while this effect approached significance with the 1.0 μg dose ($p < 0.1$). The concentrations of NE within the locus coeruleus, median eminence plus arcuate nucleus, and prefrontal cortex were unaffected by the cytokine treatment. However, MHPG concentrations in the locus coeruleus and in the median eminence plus arcuate nucleus were significantly affected, F 's (3, 41 and 3, 38) = 2.91 and 4.35 p 's < 0.05 . Multiple comparisons indicated that while MHPG levels were somewhat increased by each dose of IL-1 β , the increase was only significant at the 0.5 μg dosage (see Figure 5). Furthermore, the levels of MHPG within the prefrontal cortex were influenced by the cytokine, $F(3, 38) = 3.48$, $p < 0.05$. The multiple comparisons indicated that only the 1.0 μg dose of IL-1 β increased MHPG concentrations relative to saline-treated mice. Finally, treatment with IL-1 β did not alter NE or MHPG concentrations within the hippocampus or the amygdala.

The concentrations of DA within the median eminence plus the arcuate nucleus did not vary significantly as a function of the IL-1 β treatment, whereas DOPAC concentrations were affected by the cytokine, $F(3, 35) = 71.88$, $p < 0.05$. Newman-Keuls multiple comparisons indicated that relative to saline-treated mice ($\bar{x} \pm \text{SEM} = 6.07 \pm 1.66$ ng/mg protein), the 0.1 and 0.5 μg doses of IL-1 β increased the accumulation of the metabolite ($\bar{x} \pm \text{SEM} = 18.92 \pm 4.50$ and 14.14 ± 1.69 ng/mg protein, respectively), whereas the difference was not statistically significant with respect to the 1.0 μg dose ($\bar{x} \pm \text{SEM} = 11.58 \pm 2.45$ ng/mg protein). Thus, it seems that the cytokine increased the utilization of DA within the median eminence plus arcuate nucleus, but this effect was only evident at lower cytokine doses.

Levels of DA and DOPAC in the prefrontal cortex, nucleus accumbens, substantia

Figure 5. Mean ($\pm$ S.E.M.) MHPG concentrations in the locus coeruleus (LC), the median eminence plus arcuate nucleus (ME/ARC) and the prefrontal cortex (PFC) 50 min following administration of IL-1 β (* $p < 0.05$ relative to saline-treated animals).

nigra and amygdala were not found to vary as a function of the IL-1 β treatment. Within the caudate, DA levels were not affected by the IL-1 β treatment, while DOPAC levels were significantly reduced at each of the cytokine doses, $F(3, 36) = 4.21$, $p < 0.05$ ($\bar{x} \pm \text{SEM} = 24.86 \pm 1.39$, 19.04 ± 1.88 , 18.10 ± 1.41 , 17.81 ± 1.89 ng/mg protein for the saline, 0.1, 0.5 and 1.0 μg doses of IL-1 β , respectively). The failure to observe a significant effect of IL-1 β on DOPAC within the caudate in the first experiment may have been related to the exposure of the mice to the novel environment.

Administration of IL-1 β failed to alter 5-HT or 5-HIAA levels in either the prefrontal cortex, hippocampus, amygdala or the median eminence plus arcuate nucleus. In contrast, the concentrations of 5-HIAA within the PVN were influenced by the IL-1 β treatment, $F(3, 28) = 4.14$, $p < 0.05$. Multiple comparisons indicated that the 1.0 μg dose significantly increased 5-HIAA levels ($\bar{x} \pm \text{SEM} = 15.38 \pm 1.96$ ng/mg protein) in comparison with that of saline-treated mice ($\bar{x} \pm \text{SEM} = 8.64 \pm 1.01$ ng/mg protein).

Discussion

Paralleling the effects of IL-1 β on sickness behaviours (Kent et al. 1992b), systemic IL-1 β treatment, dose-dependently influenced open-field behaviour. In general, the IL-1 β -treated animals spent progressively less time involved in active behaviours, such as exploration and contact with the novel container. However, as will be discussed later, the various doses of this cytokine produced subtle differences in the behavioural profiles that animals exhibited. These characteristic differences could not be detected simply by assessing locomotor activity, but required a more detailed behavioural analysis.

The behavioural profile in the open-field, particularly in relation to the number of entries into the novel container, as well as the time spent within this container, varied appreciably across the various doses of IL-1 β . Moreover, while the peak effects of IL-1 β are usually thought to occur approximately 1 hr after systemic administration, the present data suggest that the effects are apparent much earlier. At low doses (0.2 and 0.4 μ g), IL-1 β -treated mice initially made numerous entries and exits into and from the novel container, after which they tended to remain in the container. At the mid range dose (0.8 μ g), mice did not show the early tendency to enter and exit the container, but tended to enter and remain in the container earlier. Unfortunately, since observation of the animals within the container was restricted, detailed dose-related comparisons of their behaviour within the container could not be made. At the highest dosage (1.6 μ g) mice exhibited clear signs of illness (piloerection, ptosis); entries into the container were few, and mice tended to remain outside the container in an immobile state (e.g., curled body posture). Assessment of locomotor activity alone, does not allow for the differentiation of the dose-dependent effects of the cytokine. In contrast, when coupled with entries into the novel container, the dose-dependent effects of IL-1 β treatment are evident. Thus, a behavioural profile comprised of locomotor activity and exploratory behaviours may provide a valid, reliable and quantifiable method of assessing illness characteristics. Interestingly, despite the profound alterations of locomotor activity and the apparent sickness induced by IL-1 β , there were no apparent effects on spontaneous alternation performance after acute or repeated IL-1 β treatment. As will be discussed later, these data suggest that the cytokine did not affect the attentional processes necessary for adequate spontaneous alternation performance to progress.

As previously reported (Bereknbosch et al., 1990; Dunn, 1990; Rothwell, 1991), treatment with IL-1 β increased plasma ACTH and corticosterone concentrations. Likewise, IL-1 β induced several central amine alterations, including decreased NE levels within the paraventricular nucleus, and increased NE turnover within the median eminence plus arcuate nucleus, locus coeruleus and prefrontal cortex. Moreover, treatment with the cytokine augmented the turnover of DA in the median eminence plus arcuate nucleus. Thus, these data suggest that while IL-1 β indeed influences hypothalamic monoamine activity, substantial extrahypothalamic amine variations are also elicited by the cytokine treatment.

Experiment 2. Effects of Systemic IL-1 β Administration on Several Tests of Anxiety in Mice

As indicated earlier, since IL-1 β stimulates CRH release, which has been shown to induce anxiogenic effects, the possibility was considered that this cytokine might likewise induce anxiety. Accordingly, in this series of experiments the effects of IL-1 β on anxiety-related behaviours were assessed.

2.1. Systemic IL-1 β Effects on Behaviour in a Plus-Maze Test of Anxiety

The initial experiment of this series examined the effects of systemic IL-1 β on performance in an elevated plus-maze test of anxiety. In this test, animals ordinarily spend a greater proportion of time exploring the closed arms of the plus-maze compared to the open arms (which are assumed to be more fear-provoking). This effect has been reported to be antagonized by anxiolytic agents (Hogg, 1996; Lister, 1987), while anxiogenic compounds reduce open-arm entries, without necessarily affecting entries into the closed arms of the maze (Hogg, 1996; Lister, 1987).

Materials and Methods

Mice received i.p. injection of either IL-1 β (0.5 μ g), or vehicle, in a volume of 0.25 ml of phosphate buffered saline. The characteristics of the cytokine was the same as that described previously. Immediately following injection, mice of each group were placed in their home cages for 40 min, after which they were either behaviourally tested or left

undisturbed in their home cages ($n = 12-13/\text{group}$). The behavioural test consisted of mice being individually placed in a small, novel open-field, (for a 5 min period), a procedure previously found to stimulate motor activity. Immediately thereafter, animals were tested in the elevated plus-maze for 5 minutes. The elevated plus-maze had two arms enclosed by 21 cm high walls, while the remaining two arms were open (all arms measured 24.8 cm long x 7.7 cm wide). The maze was situated in a dimly lit room, such that the open arms were darkened, while the closed arms were illuminated by a 40w light. For testing purposes, mice were placed in the center portion of the maze and the behaviour of the animals was recorded over a 5 min period by a ceiling-mounted video camera. The sequence and timing of arm entries (an arm entry defined as all four paws being placed in an arm of the plus-maze), as well as the number of stretch-attend postures emitted (defined as a gesture in which an animal pokes at least its entire head all the way into an open arm of the maze, without actually entering that arm) were subsequently determined from the videotapes.

Statistical analysis

The effects of IL-1 β on exploratory behaviours in the elevated plus-maze were analyzed by a one-way ANOVA.

2.2. Systemic IL-1 β Effects on Behaviour in a Light-Dark Box Test of Anxiety

It has been proposed that different behavioural tests may be tapping into different aspects of anxiety. Moreover, the mechanisms underlying these diverse elements of anxiety may differ from one another. For instance, Lee and Davis (1997) suggested that the central

amygdala plays a fundamental role in subserving fear-elicited anxiety, whereas the bed nucleus of the stria terminalis may be more important in subserving generalized anxiety (i.e., cue independent). This study was conducted to further assess whether IL-1 β induces anxiogenesis by employing the light-dark box test of anxiety. In this test, animals are placed in one compartment of a box, which is illuminated on one side and dark on the other. Generally, the nocturnal mouse or rat spends more time in the dark than in the light side of the apparatus (Costall et al., 1989; Onaivi & Martin, 1989). Treatments which increase anxiety provoke an increase in the time an animal spends in the dark side of the apparatus, while anxiolytics have the opposite effect (Costall et al., 1989; Onaivi & Martin, 1989). Thus, it was expected that systemic IL-1 β administration would increase the time spent in the dark portion of the chamber.

Materials and Methods

Mice (n = 9/group) received either i.p. IL-1 β (0.1 or 0.5 μ g) or saline injection and were then placed individually in holding cages in a darkened testing room for 40 min. After this time, animals were placed in the dark side of a light-dark box and permitted to explore the apparatus for a 10 min period. The light-dark box (45.5 x 24 x 20.5 cm), which was located in a darkened room, was composed of a light side (31.5 cm in length and illuminated with a 40 w bulb), and a smaller dark side (14 cm in length), separated by a divider. A gap (12.5 x 5 cm) at the bottom of the divider permitted mice to move between the light and dark sides of the apparatus. The number of transitions between the light and dark compartments, the latency to first enter the light compartment, the time spent in each compartment, as well

as the number of stretch-attend postures emitted (defined as a gesture to enter from the dark to the light side, in which an animal pokes at very least its entire head all the way into the light without actually entering the light side of the box), were recorded using a ceiling-mounted video camera for the ensuing 10 min period.

Data analysis

The light-dark box behavioural data were analyzed by a one-way ANOVA, and Newman-Keuls multiple comparisons were used for post-hoc comparisons between individual means ($\alpha = 0.05$).

2.3. Systemic IL-1 β Effects on Behaviour in a Light-Dark Box Test of Anxiety:

Effect of Prehabitation

It will be recalled that systemic IL-1 β administration elicits marked reductions of locomotor activity and exploration when mice are tested in a relatively familiar environment or one that is not particularly stressful (see Experiment 1.1; see also Anisman & Merali, 1998). However, when mice are placed in a somewhat threatening situation, a reduction of motor activity is not evident (see Experiment 2.1 and 2.2). In effect, when placed in a threatening environment, animals do not have the luxury of adopting a sickness posture, and instead exploratory behaviours important for the animal's survival are expressed. By the same token, the possibility exists that the avoidance tendencies mice display in a light-dark box test of anxiety may be influenced by the animal's previous experience in this situation. Thus, an additional study was conducted in which mice were tested either with or without

prior habituation to the test apparatus.

Materials and Methods

The subject characteristics and apparatus were the same as in the preceding experiment. Half the mice ($n = 24$) were prehabituated to the light-dark box, while the remaining ($n = 24$) mice were not. The habituation procedure comprised of placing mice individually in the dark side of a light-dark box and permitting them to freely explore the entire apparatus for a 10 min period on each of 3 consecutive days. The control mice did not receive exposure to the testing apparatus but remained in their home cages. The following day, animals from both groups received an i.p. injection of either IL-1 β (0.5 μ g) or saline, were immediately returned to their home cages for 40 min, and then placed in the light-dark box as previously described. The latency to enter the illuminated compartment, the number of transitions between the light and dark compartments, the time spent in each compartment, as well as the number of stretch-attend postures (as defined previously), were recorded for a 10 min period using a ceiling-mounted video camera.

Data analysis

Performance in the light-dark box was analyzed by a 2 (Prior habituation) $\times$ 2 (Cytokine Treatment) ANOVA. Post-hoc tests were conducted using Newman-Keuls multiple comparisons ($\alpha = 0.05$).

Results

Elevated plus-maze test

Administration of IL-1 β appeared to induce an anxiogenic-like effect in the elevated plus-maze. Specifically, mice that received IL-1 β spent significantly less time in the open arms of the maze, F 's (1, 24) = 10.692, $p < 0.01$ and made significantly fewer open-arm entries relative to saline-treated animals, F 's (1, 24) = 7.854, $p < 0.05$ (see Figure 6). Paralleling the time spent in the open arms of the maze, IL-1 β -treated mice took significantly longer to make an initial entry into an open arm of the plus-maze, $F(1, 24) = 6.076$, $p < 0.05$, and tended to exhibit a significantly greater number of stretch-attend postures than did their saline-injected counterparts, $F(1, 24) = 3.997$, $p < 0.05$ (see Figure 6). In contrast, IL-1 β did not alter the number of closed-arm entries emitted by mice, suggesting that the effects of the cytokine were not related to motor impairments.

Light-dark box test

Table 4 shows the effects of IL-1 β on various behaviours in the light-dark box. Contrary to prediction, performance was not affected by the cytokine treatment with respect to time spent in the light, latency to first enter the light, total number of transitions, or stretch-attend postures emitted. In each case, however, there was some indication of an anxiogenic-like effect of the cytokine treatment. The absence of any significant effects was not surprising given the marked variability seen in this particular behavioural paradigm.

Figure 6. Mean ($\pm$ S.E.M.) time mice spent on the open arms of an elevated plus-maze, the number of open and closed-arm entries emitted, as well as the latency to initially enter an open arm of the maze, during a 5 min test following treatment with several doses of IL-1 β (* $p < 0.05$ relative to saline-treated animals).

Table 4. Effect of IL-1 β on the Behaviour of Mice in a Light-Dark Box Test

Treatment	Saline	0.1 μ g	0.5 μ g
Time in Light	151.55 $\pm$ 44.39	172.78 $\pm$ 32.59	114.66 $\pm$ 38.63
Latency	159.66 $\pm$ 85.04	88.66 $\pm$ 47.60	165.67 $\pm$ 83.65
Transitions	23.44 $\pm$ 7.19	19.00 $\pm$ 4.27	17.33 $\pm$ 5.80
Stretch-Attends	17.67 $\pm$ 1.45	18.67 $\pm$ 2.60	11.33 $\pm$ 3.52

Light-dark box test: Effect of prehabitation

The number of transitions animals emitted was significantly reduced by the Cytokine treatment irrespective of prior exposure to the apparatus, $F(1, 44) = 6.67$, $p < 0.05$ (see Table 5). Likewise, time spent in the light was reduced by prior habituation to the box, $F(1, 44) = 6.08$, $p < 0.05$, while the effect of the drug treatment did not reach statistical significance. Although the Prehabitation x Cytokine Treatment interaction did not reach statistical significance, post hoc comparisons of the simple effects were conducted since a prior predictions had been made concerning this interaction. These comparisons revealed that in the absence of prior habituation, IL-1 β treatment was without effect. However, in prehabituated animals the time in the light was significantly reduced by the cytokine treatment and differed from that of saline-treated animals.

Discussion

It appears that systemic IL-1 β provoked an anxiogenic-like response in mice tested in an elevated plus-maze. The anxiety was characterized by a) a reduction in the time animals spent in the open arms of the maze, and the number of open-arm entries emitted, b) an increase in the time animals took to initially enter an open arm of the maze, as well as c) an increase in the number of stretch-attend postures emitted. Although IL-1 β was found to reduce locomotor behaviour in an open-field (Experiment 1.1), the behavioural changes observed in the elevated plus-maze did not appear to be secondary to motor effects, since the number of closed-arm entries was not altered by the cytokine treatment. In fact, as will be discussed later regarding LPS, the motoric consequences of cytokine treatments may be

Table 5. Effect of Prehabituation and IL-1 β Treatment on Behaviour in a Light-Dark Box Test

	Number of Transitions	Time in Light
Saline/ No Habituation	37.33 $\pm$ 3.99	189.50 $\pm$ 17.07
Saline/ Habituation	37.75 $\pm$ 6.32	158.33 $\pm$ 24.75
IL-1 β / No Habituation	25.83 $\pm$ 3.35	181.00 $\pm$ 15.84
IL-1 β / Habituation	22.58 $\pm$ 6.27	94.83 $\pm$ 16.50

dependent upon whether the test situation was a novel or familiar one. Suffice it at this juncture, that within the elevated plus-maze test, IL-1 β did not appreciably influence motor activity.

It is important to mention at this time that the anxiogenic-like effects of IL-1 β should be interpreted with caution. As will be discussed shortly, attempts to replicate the findings of this study have yielded inconsistent results. It remains to be established whether the anxiogenic-like effects of IL-1 β are modest, or whether the observed variability between experiments reflects the insensitivity of this behavioural paradigm.

Contrary to prediction, IL-1 β did not provoke an anxiogenic-like response in the light-dark box test. However, when animals were prehabituated to the maze, it was found that the cytokine reduced the time mice spent in the illuminated portion of the apparatus. On the one hand, such an outcome may reflect increased anxiety provoked by IL-1 β that might not have been evident under more stressful conditions (i.e., in the absence of prior habituation). Alternatively, it can be argued that following prehabitation to the apparatus, the sickness response provoked by IL-1 β was more likely to be expressed in the form of animals leaving the illuminated compartment and spending more time in a curled body posture (a characteristic posture of sick animals) within the dark compartment. A more detailed discussion of this issue will be presented later.

Experiment 3. Effects of Systemic IL-2 Administration on Exploratory Behaviours, Neuroendocrine Levels and Central Biogenic Amine Activity in Mice

There have been very few studies that assessed the behavioural effects of IL-2. Indeed, even the most fundamental behavioural actions of this cytokine remain to be described. This is particularly surprising given that IL-2 is being employed in immunotherapy and as part of a cocktail in the treatment of AIDS (Licinio et al., 1993). Moreover, among cancer patients, IL-2 therapy has frequently been associated with neuropsychiatric disturbances sufficiently severe enough to require discontinuation of treatment (Meyer & Valentine, 1995). Interestingly, in humans, the most pronounced cognitive disturbances associated with this therapy involve deficits with respect to spatial abilities (Capuron et al., 1998; Caraceni et al., 1992).

As in the case of the behavioural effects, there is scant information regarding the central neurochemical consequences of IL-2 treatment. To be sure, as mentioned earlier, there have been reports indicating that IL-2 may affect hormonal processes, particularly those involving hypothalamic mechanisms (Hanisch & Quirion, 1996; Hanisch et al., 1994, 1997). Yet, remarkably, only a handful of reports have investigated the effects of this cytokine on neurotransmitter functioning at extrahypothalamic sites, and most of these have assessed IL-2 effects in slice preparations.

The purpose of the ensuing series of experiments was to describe (a) some of the behavioural effects of acute and chronic IL-2 treatment, particularly the exploratory changes, anxiogenic-like effects, and alterations of spatial learning ability, and (b) the

neurochemical consequences of these cytokine treatments, with particular reference to the effects observed at extrahypothalamic regions.

3.1. Interleukin-2: A Dose-Response Study

The initial experiment assessed some of the fundamental behavioural consequences of acute IL-2 across a range of doses. Since cytokines, such as IL-1 β and TNF- α , have been associated with anxiogenic-like effects (Connor et al., 1998), it was of interest to establish whether IL-2 would likewise promote behavioural changes reminiscent of those typically associated with anxiety. Accordingly, open-field exploration and locomotor activity were examined, as was the influence of IL-2 on the tendency of an animal to approach a novel stimulus object. The effects of this cytokine were also evaluated with respect to exploratory behaviour in a free-running Y-maze spontaneous alternation test. In addition, the present investigation assessed the dose-related effects of IL-2 on plasma ACTH and corticosterone levels, and on brain region-specific concentrations of biogenic amines and their respective metabolites. The rationale for the choices of behaviours examined and the specific procedures employed, are described in the ensuing sections.

Materials and Methods

Behavioural assessments

The subject characteristics were the same as in the initial experiment. Mice (n = 10/group) received i.p. injection of either recombinant human IL-2 (0.05, 0.1, 0.2, 0.4, 0.8,

1.6 µg) or vehicle, in a volume of 0.25 ml of phosphate buffered saline. The carrier-free IL-2 was generously provided by Dr. C. Reynolds (Biological Research Branch, National Cancer Institute, Frederick, Maryland, USA) and had a specific activity of 1.1×10^4 IU/µg and nondetectable levels of endotoxin. Immediately following injection, mice were individually placed in a clear polypropylene open-field containing a small, cylindrical shaped (silver-coloured) container, as described in Experiment 1.1. The open-field behaviours which were the same as that described previously, were analyzed using a time-sampling procedure in which 2 min epochs in each 6 min period were evaluated commencing 7 min following cytokine administration (i.e., 7-9, 14-16, 21-23, 28-30, 35-37 min).

Spontaneous alternation performance

As mentioned previously, when permitted to explore a symmetrical Y-maze, mice and rats exhibit a characteristic pattern of exploration wherein they tend to enter the least recently visited arm of the maze (spontaneous alternation). This behaviour, which reflects the animal's ability to attend to environmental stimuli, is influenced by habituation processes. Treatments that disrupt habituation (e.g., anticholinergics) reduce alternations to chance levels. Moreover, unlike the effects of acute d-amphetamine treatment which elicits perseverative responses, chronic d-amphetamine administration promotes random exploration of the maze (Kokkinidis & Anisman, 1980). Inasmuch as IL-2 may engender neuropsychiatric symptoms involving attentional processes, it was of interest in the present investigation to assess the effects of IL-2 on spontaneous alternation performance. Accordingly, immediately following the open-field test, mice were individually placed in a

symmetrical Y-maze for 10 min in order to evaluate free-running spontaneous alternation, as described in Experiment 1.1.

Neurochemical and neuroendocrine determinations

Immediately following behavioural testing, mice were decapitated, trunk blood collected and brains removed and sectioned as described previously. Tissue samples and plasma obtained from the trunk blood were stored at -80°C for subsequent central amine, ACTH and corticosterone determinations.

3.2. Effects of Acute IL-2 on Behaviour in an Elevated Plus-Maze Test of Anxiety

Since IL-2 was shown to stimulate the release of CRH (Hanish & Quirion, 1996), an anxiogenic peptide (Gray, 1991; Heilig et al., 1994), the possibility was considered that IL-2, like IL-1 β , might induce anxiety (Lacosta et al., 1996). Accordingly, the effect of acute systemic IL-2 administration was assessed on performance in an elevated plus-maze test of anxiety under conditions where IL-1 β was found to affect performance (Lacosta et al., 1996).

Materials and Methods

Mice (n = 14/group) received i.p. injection of either IL-2 (0.5 μ g), or vehicle (0.25 ml) and were immediately placed in their home cages for 40 min, after which time they were behaviourally tested. The behavioural test consisted of a 5 min exposure to a small novel open-field (a procedure previously found to stimulate motor activity). Immediately after the open-field test, mice were placed in the center portion of an elevated plus-maze, and the

behaviour of the animals was recorded over a 5 min period by a ceiling-mounted video camera, as described previously.

3.3. Acute IL-2 Effects on Biogenic Amines Within the PVN, Median Eminence/Arcuate Nucleus and Central Amygdala of Naive Mice

In Experiment 3.1, animals treated with IL-2 were exposed to a novel environment (i.e., open-field and Y-maze), which itself could potentially influence central amine processes. In addition, the amine variations elicited by IL-2 were examined within the hypothalamus as a whole. It was of interest, however, to ascertain whether IL-2 would influence amine variations within discrete hypothalamic and amygdaloid nuclei. Thus, an additional experiment was conducted in naive mice, to assess the neurochemical consequences of acute IL-2 administration on biogenic amine variations within the PVN, the median eminence plus arcuate nucleus, as well as the central nucleus of the amygdala.

Mice ($n = 8/\text{group}$) received i.p. injection of either IL-2 (0.5 or 1.6 μg) or saline (0.25 ml), and were immediately returned to their home cages for 50 minutes. Mice were then sacrificed and brains were removed/dissected (as described previously) and stored at -80°C for subsequent central amine determinations.

3.4. Effects of Chronic IL-2 on Behaviour

Given that chronic administration of IL-2 in human cancer immunotherapy may elicit cognitive disturbances (Capuron et al., 1998), as well as depressive illness and schizophrenic-like symptoms (Caraceni et al., 1992; Denicoff et al., 1987; Meyer &

Valentine, 1995), it was of interest to evaluate the effects of such an IL-2 regimen on behavioural and neurochemical processes in mice.

3.4.1. Open-Field Exploration and Approach to a Novel Stimulus

Mice ($n = 12/\text{group}$) received i.p. injection of either IL-2 ($1.0 \mu\text{g}$) or saline (0.25 ml) on each of 6 consecutive days, and were returned to their home cages where they remained undisturbed. Following injection on Day 7, animals were maintained in their home cages for 30 min and then individually placed (for a 30 min period), in a clear polypropylene open-field which was located in a dimly lit room. A cylindrical shaped opaque plastic object (height = 13.5 cm, diameter = 5.5 cm) was vertically positioned in the center of the open-field. Once again, the behaviour of the mice was videotaped and subsequently analyzed with respect to (a) latency to make initial contact with the stimulus object, (b) time spent in contact with the object, (c) time engaged in locomotor activity and (d) frequency of rearing responses, using a time-sampling procedure. In this instance, 2 min epochs in each 6 min period were evaluated from 32 to 58 min following cytokine administration (i.e., 32-34, 38-40, 44-46, 50-52, 56-58).

Neurochemical determinations

Following open-field testing, the IL-2 and vehicle-treated mice were used to determine central biogenic amine levels and turnover, as well as HPA hormones. Accordingly, immediately after behavioural evaluation in the open-field test (i.e., 1 hr after administration of IL-2 or saline), mice were decapitated, trunk blood collected and brains

removed and sectioned. Tissue samples and plasma were stored at -80°C for subsequent biogenic amine, ACTH and corticosterone determinations as described earlier.

3.4.2. Elevated Plus-Maze

In a separate set of animals, anxiety in an elevated plus-maze test was assessed following repeated IL-2 or saline treatment. Mice (n = 12/group) received i.p. administration of either IL-2 (1.0 µg) or saline (0.25 ml) on 7 successive days. At 1 hr following the last treatment, performance on the elevated plus-maze was assessed for 5 min, as previously described.

3.4.3. Spontaneous Alternation

To further evaluate the potential chronic effects of IL-2 administration on habituation/exploration, spontaneous alternation performance was assessed in mice following repeated IL-2 treatment. Mice (n = 6/group) received i.p. administration of either IL-2 (1.0 or 2.0 µg) or saline (0.25 ml) on 7 consecutive days. One hr following the last treatment, animals were tested in a free-running spontaneous alternation test, as described earlier.

3.5. Effects of Acute and Chronic IL-2 Treatment on Morris Water-Maze Performance

Repeated central injections of IL-2 were found to disrupt performance in a Morris-water maze (Hanisch & Quirion, 1996). Moreover, as mentioned previously, the clinical use of IL-2 immunotherapy among cancer patients has been associated with pronounced cognitive disturbances, particularly in tests involving spatial learning and

memory (Capuron et al., 1998; Caraceni et al., 1992). Thus, several experiments were conducted to assess the effects of acute and chronic systemic IL-2 treatment on performance in a Morris water-maze test of spatial memory.

Two variants of the Morris water-maze test were employed. In one paradigm (fixed position), the platform remained in the same location throughout the entire experiment, while in the second version of this test, the position of the platform varied across days. In the latter paradigm, animals are naive as to the platform's location on the first trial of each day. Over days, the latencies to find the platform decline, suggesting that animals learn the concept that a platform is located somewhere within the pool (i.e., reference memory). Once an animal has located the platform on any given day, subsequent trials evaluate the animal's ability to recall the position of the platform (i.e., working memory) (Whishaw, 1985).

3.5.1. Effects of Acute IL-2 on Performance in a Fixed Platform Position Paradigm

Mice were tested in a white, circular, polypropylene Morris water-maze (1.2 m in diameter, 28 cm height), filled with water (21°C) to a depth of 22.5 cm. A white Plexiglas platform (22 cm height, 15 cm wide), was submerged 0.5 cm below the water surface. The Morris water-maze was situated in a laboratory that contained assorted extramaze cues and the experimenter remained in the same position in the room throughout all testing trials. Mice ($n = 12/\text{group}$) received systemic injection of either IL-2 (1.0 μg ; i.p.) or saline (0.25 ml), on each of 5 consecutive days. One hour following cytokine treatment on each day, mice received 5 test trials in the Morris water-maze. The behaviour of the animals was recorded by a video camera mounted directly above the testing apparatus. During testing, the platform

remained stationary in the northwest quadrant of the maze and the latency to find the platform was determined. Each trial consisted of an individual mouse being carefully placed into the water, facing the outer edge of the pool, at one of 4 possible starting points (e.g., north, south, east, west). The starting location for each trial was randomly determined, with the provision that two successive trials never began from the same site and that all start locations were used in a given day. A trial was terminated and the latency recorded when the mouse reached the platform and remained on it for 10 sec. If an escape response was not made within 60 sec, the trial was terminated, and the mouse was placed on the platform for 10 sec. Thereafter, mice were transferred to a dry holding cage, where they remained for a 60 sec period before the next trial was given.

3.5.2. Effects of Acute IL-2 on Performance in a Variable Platform Position Paradigm

The effects of IL-2 were evaluated in a variable platform paradigm that permitted evaluation of both working and reference memory. The procedure was the same as that described in the fixed position paradigm with several exceptions. As this task is more difficult than the fixed position paradigm, mice received 8 trials/day on each of 8 successive days. On each day, mice ($n = 10/\text{group}$) were tested 1 hr after either IL-2 (1.0 or 2.0 μg ; i.p.) or saline (0.25 ml) administration. On each day the platform was located in 1 of 4 different positions (north, south, east or west quadrants), with the provision that each location was represented once during the first 4 days and once during the next 4 days. Each trial was presented at 1 min intervals between trials, and commenced from one of the four starting locations, such that all four starting positions were represented in the first four trials and

again in the last four trials.

3.5.3. Effects of Chronic IL-2 on Performance in a Fixed Platform Position Paradigm

Mice ($n = 11-12/\text{group}$) received injection of either IL-2 (1.0 or 2.0 μg ; i.p.) or saline (0.25 ml) on each of 12 successive days. On the last 5 days, mice were tested in the Morris water-maze fixed position paradigm, as described earlier.

3.5.4. Effects of Chronic IL-2 on Performance in a Variable Platform Position Paradigm

Two experiments were conducted to assess the effects of chronic IL-2 administration on Morris water-maze performance employing a variable platform position paradigm. In the first experiment, mice ($n = 6/\text{group}$) received systemic injection of either IL-2 (1.0 or 2.0 μg ; i.p.) or saline (0.25 ml) on each of 15 successive days. During the first 7 days following injection, animals were returned to their home cages and left undisturbed. On the remaining 8 days, Morris water-maze testing was conducted 1 hr after cytokine administration. The procedure employed in the variable position paradigm was identical to that described earlier (i.e., where the platform was always located in the center of one of the 4 quadrants).

When the position of the platform is consistently located within the central portion of a quadrant, it is difficult to identify non-memory characteristics that may have led to performance deficits. Accordingly, an additional experiment was conducted to determine whether the performance deficit associated with chronic IL-2 treatment was related to specific response styles (e.g., thigmotaxis, perseveration). To this end, the procedure described by Whishaw (1985) was adopted in which the platform was positioned at different

locations relative to the edge of the pool. In addition, as suggested by Whishaw (1985), in this experiment trial presentations were given in pairs from a given start location at a relatively brief interval (10 sec). Thus, mice ($n = 12/\text{group}$) received i.p. injection of either IL-2 (1.0 μg) or saline (0.25 ml), on 15 successive days. At 1 hr following injection on days 8-15, mice were tested in the Morris water-maze as described in the preceding experiment, except that (a) the platform was located in either the center of one of two quadrants (as in the preceding study), in the center of the pool (to detect whether animals avoid swimming away from the edge and towards the midsection of the apparatus), or several cm from the edge of the pool (to determine whether the treatment elicits a thigmotaxic response), and (b) animals received 4 pairs of trials (i.e., 8 trials per day) on each day, wherein each pair commenced from the same starting location and the second trial of the pair was given immediately following the completion of the first trial (i.e., after a 10 sec rest on the platform). Following each pair of trials, mice were returned to a dry holding cage for 2 min before the next pair of trials was given.

3.6. Effects of Chronic IL-1 β on Performance in a Variable Platform Position Paradigm

Although mice chronically treated with IL-2 did not exhibit obvious signs of illness, the possibility could not be dismissed that variations of performance stemmed from subtle malaise engendered by the chronic cytokine treatment. Accordingly, an additional experiment was conducted to evaluate Morris water-maze performance under conditions known to induce profound illness. In particular, we have previously observed that systemic administration of IL-1 β (0.2 - 1.6 μg) elicits marked HPA activation and sickness

characterized by reduced locomotor activity and exploration, soporific effects, ptosis, piloerection and anorexia (Brebner et al., 1998; Lacosta et al., 1998). Thus, if the effects of IL-2 on Morris water-maze performance were secondary to illness, then such a deficit would be expected following chronic IL-1 β treatment.

Mice (n = 7 - 8/group) received systemic injection of either IL-1 β (1.0 μ g; i.p.) or vehicle (0.25 ml) over 15 successive days. The human recombinant, carrier-free IL-1 β (specific activity of 1.8×10^4 IU/ μ g), which was generously provided by Dr. C. Reynolds (Biological Research Branch, National Cancer Institute, Frederick, Maryland, USA), had negligible levels of endotoxin (< 2.5 EU/mg protein). The test procedure was identical to that of the preceding experiment, with Morris water-maze testing taking place 1 hr following injection on the last 8 days of the cytokine regimen.

Statistical analysis

The behavioural and neurochemical data were analyzed by analysis of variance (ANOVA). Where necessary, repeated measures ANOVA was employed (e.g., the open-field behavioural data that were sampled at several times following treatment). As well, when analyses involved Days and Trials effects, the ANOVA included 2 within measures. Comparisons between the mean of significant main effects or the simple effects of significant interactions were conducted using Newman-Keuls multiple comparisons ($\alpha = 0.05$).

Results

Dose-dependent effects of acute IL-2: Active exploratory behaviours, response to a novel stimulus, and spontaneous alternation

Acute systemic IL-2 had little effect on any of the behaviours examined. As seen in Table 6, IL-2 did not influence open-field behaviours including locomotor activity, digging, and rearing. Likewise, the time of first contact with the novel stimulus (container), as well as the first entry into the container, did not vary as a function of acute IL-2 administration. Further, the time spent within the novel container, the number of entries into the container, the time spent in contact with the outer portion of the container, as well as the frequency of stretch-attend postures emitted were unaffected by the cytokine treatment (see Table 6). While the behaviours were found to decline over the course of the sampling period (habituation), the change was comparable in the IL-2 and saline-treated mice. Consistent with the lack of effect of IL-2 in an open-field habituation test, exploratory behaviour in the free-running Y-maze was not affected by the cytokine treatment in terms of either spontaneous alternation responses or the total number of arm entries emitted (see Table 7). In effect, the habituation patterns were unaltered by IL-2, even though this cytokine has been reported to influence central ACh (Hanish & Quirion, 1996), a transmitter known to affect habituation processes (Bignami & Michalek, 1978).

Effects of acute IL-2 on behaviour in a plus-maze test of anxiety

Acute, systemic IL-2 administration failed to influence behaviour in an elevated plus-maze test of anxiety, including the total time mice spent in the open arms, the number

Table 6. Active Behaviours in an Open-Field and Exploration of a Novel Stimulus Following Acute IL-2 Treatment

Treatment	Saline	0.05 ug	0.1 ug	0.2 ug	0.4 ug	0.8 ug	1.6 ug
<u>Active Behaviors (sec)</u>							
Locomotion	8.02 ± 0.87	9.96 ± 1.00	11.68 ± 1.11	7.84 ± 0.90	7.82 ± 0.71	9.69 ± 0.81	6.93 ± 1.12
Digging	5.00 ± 0.88	4.78 ± 0.90	7.22 ± 1.09	5.89 ± 1.12	7.34 ± 1.57	8.30 ± 2.01	6.98 ± 1.48
Rearing	9.60 ± 1.09	11.38 ± 1.89	12.22 ± 1.19	8.96 ± 1.52	9.20 ± 1.57	11.16 ± 1.11	10.51 ± 1.61
<u>Response to Novel Stimulus</u>							
Time of First Contact	24.3 ± 5.52	53.1 ± 25.9	42.9 ± 9.42	19.0 ± 4.76	23.7 ± 8.00	20.4 ± 3.98	24.4 ± 7.31
Time of First Entry	344.3 ± 122.6	335.8 ± 59.2	365.0 ± 120.1	252.0 ± 59.1	241.9 ± 48.0	360.4 ± 90.4	178.0 ± 54.6
Time in Novel Container	42.6 ± 5.39	54.9 ± 6.00	33.1 ± 4.59	40.8 ± 5.65	49.5 ± 4.97	39.7 ± 5.59	44.4 ± 6.25
Number of Entries	1.58 ± 0.19	2.00 ± 0.28	1.70 ± 0.23	1.46 ± 0.21	2.12 ± 0.20	1.66 ± 0.21	1.78 ± 0.23
Time in Contact	1.24 ± 2.20	13.2 ± 2.25	23.2 ± 2.49	16.9 ± 2.34	14.7 ± 1.74	16.9 ± 1.79	13.8 ± 2.63
Stretch-Attends	2.99 ± 1.58	2.30 ± 0.69	2.04 ± 0.59	2.13 ± 1.15	1.78 ± 0.51	1.08 ± 0.59	1.67 ± 0.51

Table 7. Effect of IL-2 on Spontaneous Alternation
Performance in a Free-Running Y-Maze

Treatment	% Alternation	Total Arm Entries
Saline	63.76 $\pm$ 1.94	39.47 $\pm$ 3.31
0.05 μ g	64.29 $\pm$ 2.22	44.89 $\pm$ 2.57
0.1 μ g	63.38 $\pm$ 2.62	42.00 $\pm$ 2.96
0.2 μ g	61.63 $\pm$ 2.71	44.06 $\pm$ 2.94
0.4 μ g	64.24 $\pm$ 2.09	40.06 $\pm$ 1.93
0.8 μ g	66.76 $\pm$ 1.62	40.24 $\pm$ 3.50
1.6 μ g	67.86 $\pm$ 2.21	42.92 $\pm$ 2.23

of open-arm or closed-arm entries emitted and the latency to initially enter an open arm (see Table 8). In contrast to IL-2, using the procedures of the present investigation, we observed that the bacterial endotoxin, LPS, as well as IL-1 β , induced a reduction of open-arm entries (Anisman & Merali, 1998; Lacosta et al., 1996,1998). As such, the absence of an effect of IL-2 is not likely attributable to insensitivity of the procedures employed.

Plasma ACTH, corticosterone and biogenic amines

In mice that received acute IL-2 treatment and were then placed in the open-field and Y-maze tests, plasma ACTH and corticosterone levels did not differ from those of saline controls (see Table 9). The possibility existed that the mild stress of exposure to the test situations obfuscated the potential effects of IL-2. Moreover, blood was taken at only one time point following cytokine administration and the possibility existed that hormonal variations would have been evident at other times. Accordingly, an additional study in naive mice evaluated corticosterone concentrations either 1 or 2 h after administration of either 0.4 μ g of IL-2 or saline (0.25 ml) (n = 10/group). Commensurate with the findings in apparatus-exposed mice, plasma corticosterone concentrations measured at either 1 or 2 h after IL-2 treatment ($\bar{x}$ = 5.51 ± 1.20 and 3.45 ± 0.53 , respectively) were comparable to those of saline-treated animals at the same times following injection ($\bar{x}$ = 4.24 ± 0.51 and 3.08 ± 0.60 , respectively).

Acute treatment with IL-2 had limited effects on hypothalamic and extrahypothalamic monoamine levels and turnover. With the exception of the locus coeruleus, neither NE nor MHPG accumulation was affected by the IL-2 treatment within

Table 8. Effect of Acute IL-2 on Behaviour in an Elevated Plus-Maze
Test of Anxiety

Treatment	Saline	IL-2 (0.5 µg)
Time in Open Arms	55.62 ± 8.92	51.31 ± 8.20
Number of Open-Arm Entries	5.62 ± 0.84	6.08 ± 1.02
Number of Closed-Arm Entries	11.62 ± 0.93	11.77 ± 1.12
Latency to Make Initial Entry	33.39 ± 6.16	54.00 ± 20.83

Table 9. Effect of Acute IL-2 on Plasma ACTH
and Corticosterone Concentrations

	ACTH (pg/ml)	Corticosterone (µg/dl)
<u>IL-2 dose</u>		
Saline	174.60 ± 32.92	11.54 ± 1.79
0.05 µg	161.28 ± 20.37	13.43 ± 1.13
0.1 µg	180.98 ± 23.52	16.47 ± 2.21
0.2 µg	191.81 ± 28.77	11.89 ± 2.63
0.4 µg	167.58 ± 26.85	11.99 ± 2.76
0.8 µg	178.87 ± 56.53	10.53 ± 3.95
1.6 µg	187.32 ± 50.27	10.74 ± 1.00

any of the brain regions examined. Within the locus coeruleus, however, the accumulation of MHPG was found to vary with the Cytokine treatment, $F(6, 59) = 3.73$, $p < 0.01$. Newman-Keuls multiple comparisons confirmed that relative to vehicle-treated mice, the 0.1 μg dose significantly increased MHPG accumulation within this brain region (see Figure 7). Likewise, a modest increase of MHPG was evident in the mPFC of IL-2-treated mice, but this effect did not reach statistical significance.

The utilization of DA within the nucleus accumbens was influenced by the Cytokine treatment, $F(6, 64) = 2.30$, $p < 0.05$. Multiple comparisons indicated that within this region, the 0.05, 0.1, 0.4, 0.8 and 1.6 μg doses of IL-2 significantly decreased DOPAC levels, although DA concentrations were unaltered (see Figure 7). In contrast to these effects, the levels of DA and DOPAC within the hypothalamus, mPFC, caudate, substantia nigra and hippocampus did not vary significantly as a function of the IL-2 treatment.

Administration of IL-2 failed to alter 5-HT or 5-HIAA levels in most of the brain regions examined, whereas the concentration of hippocampal 5-HIAA was altered, $F(6, 60) = 2.29$, $p < 0.05$ (see Figure 7). Newman-Keuls multiple comparisons confirmed that the 0.1, 0.8 and 1.6 μg doses of IL-2 significantly increased 5-HIAA levels relative to saline-treated animals, while the effects of the remaining doses did not reach statistical significance. Levels of 5-HT within the hippocampus were also affected by the Cytokine treatment $F(6, 60) = 3.17$, $p < 0.05$, and the multiple comparisons indicated that only the lowest dose of IL-2 (0.05 μg), significantly increased 5-HT levels in this region. However, the latter effect was associated with appreciable variability and may have reflected a spurious outcome.

Figure 7. Mean ($\pm$ S.E.M.) NE and MHPG concentrations in the locus coeruleus (LC), DA and DOPAC concentrations in the nucleus accumbens (NAC), as well as 5-HT and 5-HIAA concentrations in the hippocampus (HC) 50 min following treatment with IL-2 (* $p < 0.05$ relative to saline-treated animals).

Interleukin -2 Dose (ug)

Acute IL-2 effects on biogenic amines within the PVN, median eminence/arcuate nucleus and central amygdala of naive mice

Administration of IL-2 altered the accumulation of MHPG within the PVN, $F(2, 45) = 3.37$, $p < 0.05$, whereas NE levels were unaffected. Newman-Keuls multiple comparisons confirmed that relative to vehicle-treated animals ($\bar{x} \pm \text{SEM} = 4.41 \pm 0.45$ ng/mg protein), MHPG concentrations were significantly elevated in mice that received 0.5 μg of IL-2 ($\bar{x} \pm \text{SEM} = 8.76 \pm 1.66$ ng/mg protein), whereas the effect of the 1.6 μg dose did not quite reach statistical significance ($\bar{x} \pm \text{SEM} = 7.44 \pm 1.20$ ng/mg protein). In contrast to the PVN, IL-2 did not influence NE or MHPG concentrations within the median eminence plus arcuate nucleus, or the central nucleus of the amygdala. Moreover, the variations of locus coeruleus NE, accumbal DA and hippocampal 5-HT utilization seen in mice that had been exposed to a novel environment following IL-2 administration, were not evident among naive mice treated with the cytokine.

Effects of Chronic IL-2 on Behaviour

Open-field exploration and approach to a novel stimulus

In contrast to the effects of acute IL-2 administration, chronic cytokine treatment influenced the time in which mice engaged in locomotor activity, $F(1, 22) = 8.167$, $p < 0.01$ and performance varied as a function of the Sampling period, $F(4, 88) = 24.491$, $p < 0.001$. The multiple comparisons indicated that locomotor activity declined over the course of the test session in both saline and IL-2-treated mice. Moreover, compared to saline controls, mice that received chronic IL-2 spent significantly less time engaged in locomotor activity

(see Figure 8). In contrast, the frequency of rearing responses was not altered by the chronic cytokine treatment. Inactivity was infrequent regardless of whether mice received saline or IL-2 administration, a profile distinct from that ordinarily associated with the profound sickness induced by IL-1 β . Thus, it is unlikely that the altered behaviour observed in the open-field test following chronic IL-2 treatment was secondary to illness elicited by the cytokine.

Contact with the novel stimulus

Although the time to make initial contact with the novel stimulus object was unaffected by the chronic Cytokine treatment ($\bar{x} \pm \text{SEM} = 9.75 \pm 1.89$ and 11.42 ± 2.69 sec, for saline and IL-2-treated animals, respectively), time spent in contact with the novel object was altered by the Cytokine, $F(1, 22) = 5.19$, $p < 0.05$. Over the duration of the testing session, animals in both treatment groups spent progressively less time in contact with the object, $F(4, 88) = 3.90$, $p < 0.01$, and the reduced contact in the IL-2-treated mice persisted throughout the session (see Figure 8).

Spontaneous alternation

Despite the effects of chronic IL-2 administration on open-field behaviour and exploration of a novel stimulus object, spontaneous alternation performance was maintained at a high level following chronic IL-2 treatment. In fact, relative to vehicle-treated animals ($\bar{x} \pm \text{SEM} = 63.00 \pm 3.31$), a small non-significant rise of spontaneous alternation was evident in mice that received the 1.0 or 2.0 μg doses of the cytokine, ($\bar{x} \pm \text{SEM} = 66.96 \pm$

Figure 8. Mean ($\pm$ S.E.M.) time (sec) engaged in locomotor activity and time in contact with a novel stimulus object over time following chronic IL-2 treatment. Each point denotes the behavioural change that occurred within a 2 min epoch over successive 6 min periods.

4.37 and 69.68 ± 2.68 , respectively). Furthermore, the number of arm visits did not differ between treatment groups ($\bar{x} \pm \text{SEM} = 22.67 \pm 1.38$, 29.00 ± 3.97 , 22.00 ± 1.91 , for the saline, 1.0 and 2.0 μg doses, respectively).

Elevated plus-maze

As in the case of acute systemic IL-2 administration, repeated treatment with the cytokine failed to alter behaviour in the elevated plus-maze test of anxiety. Indeed, the time mice spent in the open arms of the maze, the total number of open and closed-arm entries, the latency to first enter an open arm of the maze, as well as the stretch-attend postures emitted, were not affected by the chronic IL-2 treatment (see Table 10). Thus, it does not appear as if chronic IL-2 administration elicits an anxiogenic response any more than acute treatment leads to such an outcome.

Neurochemical determinations

Commensurate with the effects of acute IL-2, chronic treatment with the cytokine failed to influence HPA functioning. Relative to saline-treated animals, plasma ACTH ($\bar{x} \pm \text{SEM} = 130.54 \pm 52.78$ pg/ml) and corticosterone concentrations ($\bar{x} \pm \text{SEM} = 4.65 \pm 0.58$ $\mu\text{g}/\text{dl}$) were comparable to those of animals repeatedly treated with IL-2, ($\bar{x} \pm \text{SEM} = 141.08 \pm 36.44$ pg/ml and 5.88 ± 0.78 $\mu\text{g}/\text{dl}$), respectively.

In mice chronically treated with IL-2, the levels of NE within the locus coeruleus declined, while that of the median eminence plus arcuate nucleus and the central nucleus of the amygdala were increased, F 's (1, 21; 1, 20; 1, 20) = 4.75, 43.05, 12.41, p 's < 0.05 (see

Table 10. Effect of Chronic IL-2 on Behaviour in an Elevated Plus-Maze Test of Anxiety

Treatment	Saline	IL-2 (1.0 µg)
Time in Open Arms	55.82 ± 10.14	67.00 ± 9.01
Number of Open-Arm Entries	6.27 ± 0.83	6.50 ± 0.82
Number of Closed-Arm Entries	13.36 ± 0.74	12.25 ± 0.95
Latency to Make Initial Entry	48.18 ± 13.16	35.00 ± 10.13
Stretch-Attend Postures	21.73 ± 1.78	16.25 ± 1.64

Figure 9). As well, within the median eminence plus arcuate nucleus, the altered NE levels were accompanied by elevated MHPG levels, $F(1, 21) = 8.00$, $p < 0.01$. While hippocampal NE was unaffected by the chronic IL-2 treatment, the accumulation of MHPG was enhanced, $F(1, 20) = 8.12$, $p < 0.01$. Likewise, there was some indication of increased MHPG accumulation within the medial prefrontal cortex and central nucleus of the amygdala; however, these effects did not reach statistical significance. Finally, although the PVN is among the brain regions most readily affected by other cytokines (e.g., IL-1 β), the chronic regimen neither affected NE nor MHPG within this region.

The concentration of DA within the caudate and substantia nigra were significantly reduced following chronic IL-2 administration, $F(1, 20) = 4.66$ and 28.29 , p 's < 0.05 and 0.001 , respectively (see Table 11). In addition, reductions of DOPAC levels were evident within these regions, $F(1, 20; 1, 19) = 4.31$ and 3.82 , p 's < 0.05 , respectively. In contrast, however, IL-2 did not alter either DA or DOPAC concentrations within the PVN, median eminence plus arcuate nucleus, medial prefrontal cortex or nucleus accumbens.

Finally chronic administration of IL-2 failed to alter 5-HT or 5-HIAA levels in the PVN, median eminence plus arcuate nucleus or central nucleus of the amygdala. In contrast, however, the concentration of 5-HT was significantly reduced within the mPFC, $F(1, 20) = 6.07$, $p < 0.05$, while levels within the hippocampus were elevated, $F(1, 20) = 4.50$, $p < 0.05$. In neither region, however, was the level of the 5-HT metabolite, 5-HIAA, affected by the chronic IL-2 treatment (see Table 12).

Figure 9. Mean ($\pm$ S.E.M.) NE (left hand panel) and MHPG (right hand panel) concentrations in the locus coeruleus (LC), paraventricular nucleus (PVN), median eminence plus arcuate nucleus (ME/ARC), hippocampus (HC), central nucleus of the amygdala (CeA) and the medial prefrontal cortex (mPFC), 50 min following chronic administration of IL-2 (* $p < 0.05$ relative to saline-treated animals).

Table 11. Effect of IL-2 on Nigrostriatal Dopamine and DOPAC Concentrations

Treatment	Caudate		Substantia Nigra	
	DA	DOPAC	DA	DOPAC
Saline	211.01 ± 14.96	22.42 ± 1.77	2.43 ± 0.11	2.90 ± 0.41
IL-2 (1.0 µg)	168.02 ± 13.22*	18.16 ± 1.16*	1.55 ± 0.12*	1.93 ± 0.29*

* p < 0.05 relative to saline-treated mice

Table 12. Effect of IL-2 on 5-HT and 5-HIAA Concentrations in the Hippocampus and medial Prefrontal Cortex

Treatment	Hippocampus		medial Prefrontal Cortex	
	5-HT	5-HIAA	5-HT	5-HIAA
Saline	4.50 ± 0.41	5.68 ± 0.65	6.86 ± 0.75	5.01 ± 0.95
IL-2 (1.0 µg)	6.70 ± 0.95*	7.70 ± 1.07	4.57 ± 0.55*	5.02 ± 0.90

* p < 0.05 relative to saline-treated mice

Effects of Acute and Chronic IL-2 Treatment on Morris Water-Maze Performance

Acute IL-2 effects in the fixed platform position paradigm

Predictably, performance in the fixed platform position paradigm was readily acquired, and performance improved significantly over Days, $F(4, 88) = 27.75$, $p < 0.01$ and over Trials within Days, $F(4, 88) = 6.43$, $p < 0.01$. However, the mean latencies to reach the platform on each of 5 successive days did not differ as a function of the acute IL-2 treatment (see Table 13).

Chronic IL-2 effects in the fixed platform position paradigm

Among animals chronically-treated with IL-2, performance was likewise found to vary over Days $F(4, 116) = 25.68$, $p < 0.01$ and over Trials within Days $F(4, 116) = 2.76$, $p < 0.05$. However, neither the main effect of the cytokine treatment nor any of the interactions involving this variable approached statistical significance (see Table 13).

Acute IL-2 effects in the variable platform position paradigm

Acquisition of the correct response in a variable platform position paradigm progressed considerably more slowly than in the fixed platform position paradigm. The analysis of variance indicated that performance in this paradigm improved over Days, $F(7, 189) = 72.41$, $p < 0.01$, as well as over Trials within Days, $F(7, 189) = 15.17$, $p < 0.01$. However, neither the main effect of the cytokine treatment nor the interactions involving this treatment reached statistical significance (see Table 14).

Table 13. Mean ($\pm$ S.E.M.) Latency (sec) to Reach the Platform in a Fixed Platform Position

Paradigm Following Acute and Chronic IL-2 Treatment

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5
<u>Acute</u>					
Saline	25.70 $\pm$ 2.58	20.83 $\pm$ 2.39	16.07 $\pm$ 1.98	10.07 $\pm$ 1.17	12.2 $\pm$ 1.74
1.0 μ g	29.60 $\pm$ 2.85	20.13 $\pm$ 2.33	12.31 $\pm$ 1.79	12.82 $\pm$ 1.73	11.77 $\pm$ 1.62
<u>Chronic</u>					
Saline	32.69 $\pm$ 2.88	22.66 $\pm$ 2.49	17.89 $\pm$ 2.43	14.26 $\pm$ 2.13	14.71 $\pm$ 2.07
1.0 μ g	32.00 $\pm$ 3.08	26.27 $\pm$ 2.96	25.00 $\pm$ 2.86	22.15 $\pm$ 2.93	15.15 $\pm$ 2.02
2.0 μ g	38.86 $\pm$ 2.80	25.12 $\pm$ 2.92	18.06 $\pm$ 2.36	19.44 $\pm$ 3.14	17.06 $\pm$ 2.08

Table 14. Mean ($\pm$ S.E.M.) Latency (sec) to Reach the Platform in a Variable Platform Position Paradigm Following Acute IL-2 Treatment

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Saline	54.31 $\pm$ 1.48	43.85 $\pm$ 2.42	29.43 $\pm$ 2.26	24.26 $\pm$ 2.44	26.03 $\pm$ 2.27	25.86 $\pm$ 2.04	15.14 $\pm$ 1.32	17.06 $\pm$ 1.80
1.0 μ g	55.11 $\pm$ 1.50	46.68 $\pm$ 2.22	38.25 $\pm$ 2.38	32.61 $\pm$ 2.25	33.11 $\pm$ 2.42	26.28 $\pm$ 2.17	19.26 $\pm$ 1.43	24.89 $\pm$ 2.16
2.0 μ g	55.59 $\pm$ 1.51	51.75 $\pm$ 1.92	31.40 $\pm$ 2.38	22.06 $\pm$ 1.93	26.64 $\pm$ 2.24	27.91 $\pm$ 2.31	21.61 $\pm$ 1.76	20.34 $\pm$ 2.01

Chronic IL-2 effects in the variable platform position paradigm

Performance of mice tested in the variable platform position paradigm (where the platform was located in one of the quadrants of the pool) improved over Days in each of the groups, $F(7, 105) = 27.90$, $p < 0.01$, and varied as a function of the Cytokine treatment x Trials interaction, $F(14, 105) = 2.68$, $p < 0.01$. The performance of IL-2 and saline-treated mice is shown in Figure 10 (right-hand panel) over 8 trials (collapsed over days). Multiple comparisons of the simple effects indicated that on the first trial of each day, the 1.0 μg dose of IL-2 was absolutely without effect on performance, whereas the 2.0 μg dose provoked a small but nonsignificant increase of the response latencies. In the saline-treated animals, response latencies decreased between the first and second trials, whereas a comparable decline of response latencies was not evident in mice that received either the 1.0 or 2.0 μg doses of IL-2. As a result, the trial 2 latencies of the cytokine-treated animals were significantly longer than those of saline-treated mice. The between-group differences were also evident on the third and fourth trials, but the between-trial improvements among the cytokine-treated animals culminated in the between-group differences being absent from the fifth trial onward.

Although chronically administered IL-2 influenced motor activity in an open-field exploratory situation, swimming ability was not impaired in the present study. This was reflected by the fact that Trial 1 performance on each of the days was comparable among the groups. Moreover, mice maintained active swimming throughout testing and did not adopt passive floating postures. Indeed, correlational analyses revealed that the distance swum and latency to reach the platform were highly related (data not shown).

Figure 10. Mean ($\pm$ S.E.M.) latency to reach the platform in a variable platform position Morris water-maze paradigm (the position of the platform varied over days, but was always located in the center of a quadrant). Latencies are presented over successive days (left-hand panel) and over trials collapsed over days (right-hand panel) among mice that received either chronic IL-2 or saline treatment.

Figure 11 depicts the effects of chronic IL-2 over successive days (left-hand panel) and trials (right-hand panel) among mice tested in the variable platform position paradigm where the location of the platform was either in the center of the pool, along the perimeter, or in one of the two quadrants. The analysis of variance revealed a significant effect of the Cytokine treatment, $F(1, 10) = 11.15$, $p < 0.01$, indicating that IL-2 hindered performance. The analysis also revealed that performance improved over Days, $F(7, 70) = 13.65$, $p < 0.01$ and over Trials within a Day, $F(7, 70) = 4.16$, $p < 0.01$. Although the Drug x Trials and Drug x Days interactions did not reach statistical significance, multiple comparisons of the simple effects comprising these interactions were conducted based on *a priori* predictions that had been made concerning the effects of IL-2. These comparisons revealed that IL-2 disrupted the latency to reach the escape platform and that this effect was evident throughout the 8 testing days. However, IL-2 treatment did not impair performance on the first trial of each day. The cytokine induced a small disruption on the second trial, markedly interfered with performance from trials 3-5, and elicited a moderate disturbance of performance on trials 6-8 (see Figure 11). Thus, it appears that while IL-2 does not limit the animal's ability to initiate and sustain a response (as reflected by the proficient trial 1 performance), chronic administration of this cytokine disrupted working memory (i.e., the ability of mice to remember the position of the platform following the first training trial on any given day).

Evaluation of the response styles exhibited by cytokine-treated mice did not yield any obvious patterns or biases of responding. The IL-2-treated animals seemed to have difficulty in acquiring the response, irrespective of whether the platform was located along the perimeter, at the center of the pool, or in one of the quadrants. Furthermore, inspection of the

Figure 11. Mean ($\pm$ S.E.M.) latency to reach the platform in a variable platform position Morris water-maze paradigm (the location of the platform was either in the center of the pool, along the perimeter, or in one of two quadrants). Latencies are presented over successive days (left-hand panel) and over trials collapsed over days (right-hand panel) among mice that received either chronic IL-2 or saline treatment.

videotapes indicated that none of the animals adopted perseverative response styles (e.g., tight circling, or swimming in a restricted area along the perimeter of the pool) that could account for the inferior performance.

Chronic IL-1 β Effects in a Variable Platform Position Paradigm

As in earlier studies, mice treated with IL-1 β displayed obvious signs of illness (ptosis, piloerection, curled body posture, diarrhea, immobility) while in the holding cage. However, upon being placed in the Morris water-maze, there was absolutely no indication of illness, and the performance of mice was unhampered by the chronic cytokine administration, (F 's < 1.0 for the Cytokine main effect and the interactions involving this variable). The mean latency to reach the platform in saline and IL-1 β -treated animals, collapsed over Days and Trials, was 21.24 ± 0.92 and 22.09 ± 0.97 sec, respectively. Moreover, on the last testing day, mice of both groups displayed proficient performance ($\bar{x} \pm \text{SEM} = 9.42 \pm 1.35$ and 9.77 ± 1.09 sec, respectively). Thus, these data indicate that illness was not sufficient to induce Morris water-maze impairments.

Discussion

It appears that in several respects, the effects of IL-2 were distinguishable from those of IL-1 β . Unlike the behavioural consequences of IL-1 β treatment, acute systemic IL-2 administration did not influence locomotor behaviour or exploration in an open-field, and failed to provoke an anxiogenic-like effect in an elevated plus-maze test. Furthermore, in contrast to the marked neuroendocrine alterations elicited by IL-1 β , treatment with IL-2 did

not affect HPA functioning, as reflected by plasma ACTH and corticosterone levels. However, acute IL-2 administration provoked a change of NE utilization within the locus coeruleus, increased hippocampal 5-HIAA accumulation, as well as that of DA turnover in the nucleus accumbens. It should be emphasized that in this particular experiment tissue samples were collected from IL-2-treated mice who had received behavioural testing. When a similar experiment was performed in naive mice, amine changes in the locus coeruleus, nucleus accumbens and hippocampus were not evident. Thus, it is possible that the amine variations reflected the conjoint actions of IL-2 and mild stressor exposure (Song et al., 1998).

The effects of acute and chronic IL-2 were likewise distinguishable from one another. Whereas, acute administration of the cytokine did not influence either locomotor activity or exploration in an open-field situation (i.e., including investigation of a novel object), chronic IL-2 treatment diminished both the time mice engaged in locomotor activity, as well as the time animals spent in contact with a novel stimulus object. However, neither acute nor repeated administration of IL-2 provoked anxiogenic-like effects in an elevated plus-maze test or influenced habituation/attention in a spontaneous alternation test. As well, while acute IL-2 treatment did not affect Morris water-maze performance, chronic treatment with the cytokine impaired working memory in a variable position paradigm. Finally, while the central effects of acute IL-2 administration were relatively restricted, chronic IL-2 had more widespread effects on biogenic amine activity and/or levels. Specifically, repeated injection with IL-2 influenced NE, DA and 5-HT processes within the locus coeruleus, median eminence plus arcuate nucleus, hippocampus, central nucleus of the amygdala, mPFC,

caudate nucleus, and substantia nigra.

Experiment 4. Effects of Systemic LPS Administration on Several Tests of Anxiety in Mice

In addition to simply influencing IL-1 β activity, activation of macrophages by bacterial endotoxin provokes the release of several proinflammatory cytokines (e.g., IL-6 and TNF- α) (Zuckerman et al., 1989). Accordingly, the behavioural, neuroendocrine and neurochemical alterations resulting from immune challenge may reflect the conjoint actions of two or more of these cytokines rather than IL-1 β alone. Since LPS, like psychogenic stressors, stimulates HPA activity, (e.g., including activation of PVN CRH), it is possible that like CRH, LPS administration might promote an anxiogenic response. In order to test this possibility the effects of systemically administered LPS were examined in several behavioural paradigms associated with anxiety (e.g., light-dark box, open-field exploration, elevated plus-maze).

4.1.- 4.2. Effects of Systemic LPS Administration on Light-Dark Box and Elevated Plus-Maze Tests of Anxiety

Materials and Methods

The subject characteristics in Experiments 4.1 and 4.2 were the same as in the initial experiment. In Experiment 4.1, mice ($n = 58$) received either an i.p. injection of LPS (Sigma) (0.25, 0.5, 1.0, 5.0, 10.0, 25.0 μ g) or saline and immediately returned to their home cage for a 1 hour period. After this time, animals were individually placed in holding cages (located

in a dimly lit testing room), for 1 hour and then placed in the dark side of a light-dark box and permitted to explore the apparatus for a 10 min period (as described in Experiment 2.2). The number of transitions between the light and dark compartments, the latency to first enter the light compartment, the time spent in each compartment, as well as the number of stretch-attend postures emitted (as defined previously), were recorded using a ceiling-mounted video camera for the ensuing 10 min period and scored as described in Experiment 2.2.

In Experiment 4.2, mice ($n = 40$) received either an i.p. injection of LPS (Sigma) (0.5, 1.0, 5.0 μg) or saline and immediately returned to their home cage for a 2 hour period. Animals were then individually placed in a clear polypropylene open-field situated in a dimly-lit environment (as described in Experiment 3.4.1) for 5 min and immediately thereafter tested on an elevated plus-maze for 5 minutes (as described in Experiment 2.1). Immediately following the behavioural testing, mice were decapitated, trunk blood collected and brains removed and sectioned as described previously. Tissue samples and plasma obtained from the trunk blood were stored at -80°C for subsequent central amine, ACTH and corticosterone determinations.

Data analysis

The effects of LPS on exploratory behaviour in the light-dark box, open-field and in the elevated plus-maze, as well as the neuroendocrine and neurochemical data, were analyzed by a one-way ANOVA. Post-hoc tests were conducted using Newman-Keuls multiple comparisons ($\alpha = 0.05$).

4.3. Effect of Systemic LPS Administration on Neuroendocrine Activity and Biogenic Amine Levels in the Absence of Stressor Exposure

In the previous two experiments, the neuroendocrine and neurochemical effects induced by systemic LPS administration were assessed following exposure to a stressful environment (e.g., light-dark box, open-field, elevated plus-maze). In order to assess the neurochemical/neuroendocrine effects of LPS, uncontaminated by the behavioural tests, an additional study assessed these endogenous substrates following LPS treatment without additional behavioural testing.

Materials and Methods

The characteristics of the mice were the same as that described in Experiment 1.1, except that animals were housed singly in standard polypropylene cages for a minimum of 14 days prior to the start of the experiment. Mice ($n = 40$) received either an i.p. injection of LPS (Sigma) (0.5, 5.0, 10.0 μg) or saline and immediately returned to their home cages for a 2 hour period. During this time, the behaviour of the mice was recorded every 30 min with respect to locomotor activity, rearing and the presence of sickness behaviours (e.g., soporific style, piloerection, ptosis and immobility). Thereafter mice were immediately decapitated, trunk blood taken and brains removed as described previously. Brain regions were sectioned as described in experiments 1.1 and 1.4. Tissue samples and plasma obtained from the trunk blood were stored at -80°C for subsequent central amine, ACTH and corticosterone determinations respectively, as described in the initial experiment.

Data analysis

The neuroendocrine and neurochemical data were analyzed by a one-way ANOVA and post-hoc tests were conducted using Newman-Keuls multiple comparisons ($\alpha = 0.05$).

Results

Light-dark box test

Treatment with LPS appeared to effectively induce an anxiogenic-like effect, which was evident even with low endotoxin doses, and across several of the behavioural paradigms. Although LPS treatment did not influence the latency to initially enter the illuminated compartment of a light-dark box, the time spent in this compartment varied with the Endotoxin treatment, $F(6, 46) = 5.545$, $p < 0.01$. The multiple comparisons confirmed that mice that received 1.0, 5.0 10.0, or 25.0 μg of LPS spent significantly less time in the illuminated part of the apparatus relative to saline-treated animals (see Figure 12).

The total number of transitions between the compartments varied significantly with the Endotoxin treatment, $F(6, 46) = 5.609$, $p < 0.01$, as did the frequency of stretch-attend postures, $F(5, 41) = 9.292$, $p < 0.001$. Paralleling the time spent in the illuminated chamber, multiple comparisons indicated that relative to saline-treated mice, animals injected with 1.0 - 25.0 μg of LPS tended to make significantly fewer crossings and stretch-attend postures than did saline-treated controls. It is important to note that while the LPS-treated animals were less active than controls, they generally did not exhibit a pattern in which they simply sat within the darkened area without moving. To the contrary, when these animals crossed into the light, they left this compartment relatively quickly, and the average time spent in the

Figure 12. Mean ($\pm$ S.E.M.) time mice spent in the light compartment of a light-dark box during a 10 min test following treatment with several doses of LPS (* $p < 0.01$ relative to saline-treated animals).

light (the total time divided by the number of crossings) did not differ significantly between groups. In addition, in a further study it was observed that when LPS-treated animals, like their saline-injected counterparts, were placed into the illuminated compartment at the start of a trial, they immediately left this area (< 2 sec), precluding detection of differences attributable to the treatment. In effect, it appears that the anxiogenic-like actions of LPS could not readily be attributed to the motoric consequences engendered by the endotoxin.

Open-field behaviour and approach to a novel stimulus

The behaviour of mice in the open-field was influenced by treatment with LPS. Both the time animals spent locomoting, as well as the time spent rearing, varied with the Endotoxin treatment, F 's (3, 36) = 20.661 and 15.033, p 's < 0.001 . The multiple comparisons confirmed that mice that received each of the doses of LPS spent significantly less time engaged in these active behaviours relative to saline-treated animals (see Table 15). In addition, LPS administration influenced the time animals spent in contact with the novel stimulus, $F(3, 36) = 5.375$, $p < 0.01$, although the time to make initial contact with the novel object was not affected by the endotoxin. Newman-Keuls multiple comparisons indicated that at each dose of LPS, mice spent significantly less time in contact with the novel object than did saline-treated animals. Finally, LPS influenced the time mice spent sitting immobile, $F(3, 35) = 6.47$, $p < 0.01$. Multiple comparisons indicated that all doses of LPS markedly increased the time animals spent sitting in an immobile posture (see Table 15).

Table 15. Time ($\pm$ SEM) engaged in Open-Field Behaviours Following LPS Treatment

Treatment	Saline	0.5 μ g	1.0 μ g	5.0 μ g
Locomotion	79.4 $\pm$ 4.82	45.8 $\pm$ 4.31**	45.8 $\pm$ 3.32**	36.5 $\pm$ 4.01**
Rearing	43.1 $\pm$ 4.52	19.8 $\pm$ 2.83**	16.7 $\pm$ 1.59**	18.9 $\pm$ 3.15**
Sitting	3.80 $\pm$ 1.49	21.6 $\pm$ 4.08*	25.9 $\pm$ 3.74*	48.7 $\pm$ 13.23*
Contact	43.3 $\pm$ 5.95	25.3 $\pm$ 2.31*	23.3 $\pm$ 4.13*	21.1 $\pm$ 4.39*
Latency	17.2 $\pm$ 4.18	15.0 $\pm$ 1.96	27.3 $\pm$ 5.63	15.8 $\pm$ 3.43

* p < 0.01 relative to saline-treated mice

**p < 0.001 relative to saline-treated mice

Elevated plus-maze test

As in the light-dark box, LPS administration induced an anxiogenic-like effect in the elevated plus-maze. Although LPS did not influence the latency to make an initial entry into an open arm of the maze, the total time mice spent in the open arms of the maze, as well as the number of open-arm entries varied with the Endotoxin treatment, $F's(3, 36) = 3.105$ and 3.146 , $p's < 0.05$, respectively. The multiple comparisons indicated that mice that received the $5.0 \mu\text{g}$ dose of LPS spent significantly less time in the open arms of the maze and made fewer entries relative to saline-treated animals. In contrast, treatment with LPS did not alter the number of closed-arm entries emitted by mice, suggesting that the effects of the endotoxin were not related to motor impairments. Finally, the number of stretch-attend postures were not significantly altered by the LPS treatment, although a modest decrease was evident at the highest dose of LPS ($5 \mu\text{g}$) (see Figure 13).

Home cage behaviour

When mice were treated with LPS and behaviour assessed within the home cage, a marked reduction of active behaviours was evident over the course of the 2 hr post-injection period. Among saline-treated animals, high levels of active behaviour (e.g., locomotor activity and rearing) were evident over each of the 4 sampling periods that took place at 30 min intervals (only 1 animal exhibited inactivity during the last sampling period). In contrast, LPS-treated mice exhibited progressively greater inactivity and more pronounced sickness behaviours (e.g., soporific style, piloerection, ptosis, and immobility) over the 2 hr post-injection interval. At 0.5 hr following a $0.5 \mu\text{g}$ dose of LPS, 60% of animals displayed

Figure 13. Mean ($\pm$ S.E.M.) time spent on the open arms of an elevated plus-maze, the number of open-arm entries emitted, and the number of closed-arm entries emitted during a 5 min test following treatment with several doses of LPS (* $p < 0.05$ relative to saline-treated animals).

some immobility and 40% displayed signs of illness. At 2 hr following this dose of LPS, 40% of animals exhibited piloerection or ptosis, while 70% of animals slept. At the higher doses the inactive profile was more pronounced at the 0.5 hr period (80% of animals at the two higher doses displayed immobility and piloerection) and by 2 hr following treatment, all animals displayed this profile. It ought to be underscored at this juncture that while the inactivity and sickness profile was common in LPS-treated mice, as soon as animals were placed in a novel (and thus potentially threatening) environment, the immobile profile was essentially masked, and animals displayed high levels of activity.

Plasma ACTH and corticosterone

Plasma ACTH levels varied significantly with the LPS treatment, $F(3, 35) = 14.65$, $p < 0.001$. Newman-Keuls multiple comparisons showed that relative to vehicle animals, ACTH levels were elevated in mice that received the 0.5, 5.0 or 10.0 μg doses of LPS. Plasma corticosterone levels likewise varied significantly with the LPS treatment, $F(3, 36) = 40.994$, $p < 0.001$. The multiple comparisons confirmed that each of the doses of LPS significantly increased plasma corticosterone levels relative to saline-treated animals (see Table 16).

Biogenic amines

Treatment with LPS influenced the utilization of NE at both hypothalamic and several extrahypothalamic sites. In particular, while NE concentrations within the PVN, median eminence plus arcuate nucleus, locus coeruleus and the hippocampus did not vary

Table 16. Effect of LPS on Plasma ACTH and Corticosterone Concentrations ($\pm$ SEM)

Treatment	ACTH (ng/ml)	Corticosterone (μ g/dl)
Saline	96.44 $\pm$ 32.82	3.75 $\pm$ 0.32
0.5 μ g	416.91 $\pm$ 45.18*	43.35 $\pm$ 3.09*
5.0 μ g	503.28 $\pm$ 46.50*	45.16 $\pm$ 2.40*
10.0 μ g	450.33 $\pm$ 62.74*	42.15 $\pm$ 4.83*

* p < 0.001 relative to saline-treated mice

significantly as a function of the LPS treatment, MHPG accumulation in these regions was affected by the endotoxin, $F(3, 35) = 3.136, 7.436, 5.40$ and 3.52 , p 's < 0.05 , respectively. The multiple comparisons confirmed that the 5.0 and $10.0 \mu\text{g}$ doses of LPS increased MHPG accumulation within the PVN and in the median eminence plus arcuate nucleus. In the locus coeruleus, the 0.5 and $10.0 \mu\text{g}$ doses of LPS increased MHPG accumulation (see Figure 14). In the case of the hippocampus, MHPG accumulation was increased by the 0.5 and $5.0 \mu\text{g}$ doses, but curiously was not significantly elevated at the highest dose. Neither NE nor MHPG accumulation was affected by the LPS treatment within the amygdala. A modest increase of MHPG was evident in the prefrontal cortex of LPS-treated mice, but this effect did not reach statistical significance. It ought to be noted, that in a separate experiment ($n = 10/\text{group}$) in which animals were mildly stressed (by exposure to an elevated plus-maze test) after injection of LPS, the elevation of MHPG within the prefrontal cortex was statistically significant. However, as the effects of the plus-maze exposure alone were not assessed, it is unclear whether the observed outcome reflected additive or synergistic effects of the LPS and the stressor, or whether the stressor acted in a permissive fashion to permit the actions of LPS to become evident.

The level of DA within the PVN was influenced by the LPS treatment, $F(3, 34) = 5.761$, $p < 0.01$, and Newman-Keuls multiple comparisons indicated that relative to saline-treated mice, the 5.0 and $10.0 \mu\text{g}$ doses of LPS significantly increased DA levels (see Figure 15). In contrast, DA concentrations within the median eminence plus arcuate nucleus and in the nucleus accumbens did not vary significantly as a function of the endotoxin treatment. In addition, it was found that the accumulation of DOPAC was affected by the

Figure 14. Mean ($\pm$ S.E.M.) NE and MHPG concentrations in the paraventricular nucleus (PVN), the median eminence plus arcuate nucleus (ME/ARC), locus coeruleus (LC), and the hippocampus (HC) 2 hrs following administration of LPS (* $p < 0.05$ relative to saline-treated animals).

Figure 15. Mean ($\pm$ S.E.M.) DA and DOPAC concentrations in the paraventricular nucleus (PVN), the median eminence plus arcuate nucleus (ME/ARC), and the nucleus accumbens (NAC) 2 hrs following treatment with several doses of LPS (* $p < 0.05$ relative to saline-treated animals).

endotoxin in each of these regions, F 's (3, 35) = 4.91, 4.22 and 4.92, p 's < 0.05, respectively (see Figure 15). Multiple comparisons indicated that within the PVN, the 5.0 and 10.0 μ g doses of LPS increased the accumulation of the metabolite relative to saline-treated mice, whereas in the median eminence plus arcuate nucleus, the 10.0 μ g dose significantly elevated DOPAC levels. In the nucleus accumbens, the 5.0 μ g dose had such an effect, but the increased metabolite level was absent at the 10.0 μ g dosage. In contrast to the changes within the nucleus accumbens, DA and DOPAC levels within the prefrontal cortex (despite being a stress-sensitive region like the accumbens), were unaltered by the LPS treatment. Likewise, LPS did not alter either DA or DOPAC concentrations within the caudate or the substantia nigra, regions that are typically not sensitive to stressors.

The concentrations of 5-HT within the median eminence plus arcuate nucleus, and the hippocampus were altered by LPS, F (3, 35 and 3, 33) = 2.73 and 2.86, p 's < 0.05 (see Figure 16). The multiple comparisons confirmed that the highest dose of LPS (10.0 μ g) significantly increased 5-HT levels in the median eminence plus arcuate nucleus. While 5-HT levels in the hippocampus were somewhat elevated by each dose of the endotoxin, the increase was only significant at the 0.5 μ g dosage. Furthermore, relative to saline-treated animals, treatment with LPS enhanced 5-HT levels in the PVN at each dose tested, although this effect failed to reach significance.

The concentrations of 5-HIAA within the PVN and hippocampus were influenced by treatment with LPS, F (3, 35 and 3, 36) = 3.924 and 4.275, p 's < 0.05 (see Figure 16). Multiple comparisons indicated that relative to saline-treated mice, the 5.0 and 10.0 μ g doses significantly increased 5-HIAA levels in the PVN, whereas metabolite levels in the

Figure 16. Mean ($\pm$ S.E.M.) 5-HT and 5-HIAA concentrations in the paraventricular nucleus (PVN), the median eminence plus arcuate nucleus (ME/ARC) and the hippocampus (HC) 2 hrs following treatment with several doses of LPS (* $p < 0.05$ relative to saline-treated animals).

hippocampus were enhanced at the 0.5 and 5.0 μg doses. Administration of LPS failed to alter 5-HIAA levels in the median eminence plus arcuate nucleus, although a modest increase of the metabolite was evident at the 10.0 μg dose.

Discussion

Commensurate with previous reports (Dantzer et al., 1996), systemic LPS administration was found to provoke classic signs of illness. Interestingly, however, the sickness profile appears to be dependent upon the environmental context in which the animals were tested. Indeed, LPS-treated mice which were permitted to remain in their home cage, showed signs of illness characterized by reduced exploratory behaviours (e.g., locomotor activity and rearing), increased sleep, piloerection and ptosis. Although endotoxin-treated mice tested in an open-field situation showed a decrease in locomotor activity, rearing, and contact with a novel stimulus object, the sickness profile was much less pronounced than that observed in the home cage situation. Moreover, LPS-induced sickness behaviours were not readily apparent in animals tested in the light-dark box or elevated plus-maze (e.g., environments assumed to be anxiety provoking).

In addition to promoting sickness, LPS provoked an anxiogenic-like response in an open-field exploratory situation, as well as in both a light-dark box and elevated plus-maze test of anxiety. Indeed, LPS decreased the time animals spent in the illuminated portion of a light-dark box and reduced both the number of crossings animals made between the light and dark compartments and the number of stretch-attend postures emitted. Although LPS influences locomotor activity, the behavioural changes do not appear to be secondary to these

motor effects. Indeed, as mentioned previously, LPS-treated animals which were placed directly into the illuminated portion of the light-dark box, quickly left this area (within less than 2 sec) and entered the darkened (less fear provoking) portion of the chamber. Likewise, LPS decreased the number of contacts animals made with a novel stimulus object in an open-field situation, and reduced both the number of open-arm entries and the time animals spent in the open arms of an elevated plus-maze. In contrast, treatment with the endotoxin did not alter the number of closed-arm entries emitted by mice, suggesting that the effects of LPS were not related to motor impairments.

The behavioural changes induced by LPS were accompanied by an increase in plasma ACTH and corticosterone levels, supporting the contention that LPS appreciably influences HPA functioning (Kakucska et al., 1993; Rivier, 1993). As well, LPS increased the turnover of NE, DA and 5-HT in the PVN and median eminence plus arcuate nucleus, NE and 5-HT turnover within the hippocampus, NE turnover within the locus coeruleus and DA turnover within the nucleus accumbens. Although these neurochemical variations were reminiscent of those elicited by stressors, LPS was not particularly effective in modifying DA activity in the prefrontal cortex or NE within the amygdala, variations readily induced by stressors (Anisman et al., 1993; Deutch & Roth 1990; Herman & Cullinan 1997). As will be discussed shortly, the possibility exists that LPS may be interpreted as a stressor and that the apparent anxiogenic-like effects of the endotoxin may be related to some of the central amine changes observed and may reflect stress associated with LPS-induced illness.

Experiment 5. Effects of Immune Challenge on *In Vivo* Neurotransmitter Alterations in Rats

In the preceding studies, neurochemical alterations were demonstrated in post-mortem tissue following cytokine and endotoxin challenge. It is well established, however, that post-mortem analyses may not give an adequate view of monoamine variations induced by various treatments. Among other things, such analyses do not provide an indication of neurotransmitter release, and only an indirect estimate is obtained based on metabolite concentrations. In contrast, *in vivo* microdialysis permits the direct measurement of neurotransmitter release over time by assessing extracellular amine concentrations (Westerink, 1995). Thus, it was of interest to employ *in vivo* microdialysis to investigate the effects of cytokine and immune challenge on neurotransmitter alterations in rats.

5.1. - 5.3. Effects of IL-1 β and Air Puff Stress on Neurotransmission at the Nucleus Accumbens, medial Prefrontal Cortex and Hippocampus

As indicated earlier, most of the data concerning the effects of IL-1 β on central neurotransmitters have been restricted to the hypothalamus, and little information is available concerning the effects of the cytokine on extrahypothalamic monoamine activity. In this respect, there have been only sporadic reports showing post-mortem monoamine changes in some extrahypothalamic sites. In addition, there have been no reports concerning the effects of systemically administered IL-1 β on *in vivo* monoamine variations from mesolimbic sites. It is curious that although IL-1 β is believed to induce stress-like effects (Dunn 1988,1990;

Shintani et al., 1993; Zalcman et al., 1994), limited attention has been devoted to the determination of biogenic amine variations in brain regions where stressors typically have such an effect (e.g., DA in the nucleus accumbens and PFC, as well as NE and 5-HT at the hippocampus). Thus, studies were conducted using *in vivo* microdialysis, in freely moving rats, to assess the effects of systemic IL-1 β administration on interstitial levels of NE, DA, 5-HT, and their respective metabolites at the nucleus accumbens, medial prefrontal cortex and hippocampus. In addition, in this experiment we also evaluated the effects of IL-1 β administration on the neurochemical responses to an environmental stressor (i.e., exposure to a series of mild air puffs).

Materials and Methods

Male Sprague-Dawley rats (n = 8-9/group) weighing approximately 330-380 g were obtained from Charles River Canada (St. Constant, Quebec). Upon arrival from the breeder, they were acclimatized to the laboratory for 1-2 weeks before serving as experimental subjects. Rats were individually housed, maintained on a 12 h light-dark cycle (light phase: 06.30 - 18.30 h) and permitted free access to food and water.

Apparatus and surgical procedure

Rats were anesthetized (60 mg/kg pentobarbital; i.p.) and stereotaxically implanted with a 20 gauge guide cannula containing a removable 24 gauge obturator, aimed at the nucleus accumbens, medial prefrontal cortex or hippocampus. The placement coordinates (obtained from Paxinos and Watson 1986) were nAcb: A/P = +1.8 mm, D/V = -7.5 mm, L

= $\pm$ 1.5 mm, mPFC: A/P = +3.2 mm, D/V = -6.0 mm, L = $\pm$ 0.3 mm, Hippocampus at 14° angle, A/P = -4.8 mm, D/V = -4.0 mm and L = +3.5 mm. The guide cannula, which protruded from a concentric custom made Derelin pedestal, was secured to the skull with four screws and dental cement. After surgical recovery (a minimum of 5 days), animals were transferred to individual testing cages and allowed to acclimatize for 2 days prior to testing.

Microdialysis

On the day prior to testing, rats were briefly anesthetized with halothane, the obturator removed from the guide and replaced with a microdialysis probe. The concentric microdialysis probes had a 2.5 mm (nAcb), 3 mm (mPFC), or 5 mm (hippocampus), membrane (250 μ m O.D.) composed of regenerated cellulose (6000 MW cut-off; spectrum Medical Industries, CA), that protruded into the appropriate nucleus. Probes were secured with retaining screws and connected via PE tubing (Intramedic, Clay Adams, NJ) to a liquid swivel and a 2.5 ml infusion syringe (Hamilton) attached to a pump (Harvard, model 22). Microdialysis probes were perfused overnight on a stand-by rate of 0.5 μ l/min with filtered Krebs Ringer Phosphate (KRB) solution consisting of (in mM) 2.7 K⁺, 145 Na⁺, 1.0 Mg²⁺, 1.35 Ca²⁺, 150 Cl⁻, 0.05 ascorbate, pH 7.4. Collection of dialysate samples began 24 h later (on Day 2). Immediately after collection, each sample (40 μ l) was injected (via Rheodyne (CA) 7161 injector) into a high performance liquid chromatography (HPLC) system equipped with a three-cell electrochemical detector (ESA model 5100A, Bedford, MA) consisting of a guard cell (Model 5021; applied potential, +0.33 V), and a dual-electrode analytical cell (Model 5011, applied potentials, -0.30 V and +0.33 V, respectively). DA,

5-HT, NE and its metabolite, MHPG, were quantified at the high sensitivity second analytical cell, whereas DOPAC, 5-HIAA and HVA were quantified at the first analytical cell which was set at the lower sensitivity due to the presence of larger amounts of these metabolites. Except for the hippocampus, 5-HT was below detection limit.

Separation of the analytes was achieved by their passage through a Nova-Pak C₁₈ reverse-phase analytical column (Waters). The mobile phase consisted of 9.4 mM heptasulfonic acid (HSA; Fisher), 0.27 mM disodium ethylenediaminetetracetate (EDTA; Fisher), 75 mM triethylamine (TEA; Fisher), 6% acetonitrile (Fisher), pH 7.3, and was delivered at a flow rate of 2.0 ml/min using a Spectra-Physics pump (model P1000). Quantification of the various analytes was accomplished by comparing their area under the curve to those of known external standards using the computerized EZChrom[®] chromatography data acquisition system (Scientific Software, CA).

Testing began the next morning and was conducted between 09.00 and 14.00 h. On the test day, the overnight flow rate of the KRB solution was increased to 1.5 µl/min, and 4 consecutive 30 min baseline dialysate samples were collected. Following this, in the first experiment of this series (e.g., where the microdialysis probes were aimed at the nucleus accumbens), rats were randomly assigned to three groups such that they received either an i.p. injection of saline (0.5 ml) plus air puff, IL-1β (1.0 µg in 0.5 ml of saline) plus air puff or IL-1β (1.0 µg in 0.5 ml of saline) only (no air puff). In the subsequent experiments (e.g., where the microdialysis probes were aimed either at the mPFC or hippocampus), two treatment groups were employed, such that rats received either IL-1β or saline, and all animals were subjected to the air puff procedure. Following injection, five 30 minute

dialysate samples were collected. Following the fifth sampling period, rats from two of the treatment groups were exposed to a series of mild air puffs (one 5 second puff/minute for 5 minutes) delivered from an aerosol can, and five additional dialysate samples were then collected at 30 minute intervals. The IL-1 β only group was not subjected to the subsequent air puff stress. The IL-1 β was generously provided by Dr. C. Reynolds (Biological Research Branch, National Cancer Institute, Frederick, Maryland, USA), had a specific activity of 1.9×10^6 Units/50 μ l, protein concentration of 2.1 mg/ml and contained endotoxin of 2.5 EU/mg protein. The dosage of IL-1 β used in this study (1.0 μ g) was based on earlier studies which revealed that this sub-pyrogenic dose was capable of activating the HPA axis.

Histology

Histological verification of the probe placements were conducted at the termination of the experiments. The brains were frozen on dry ice, sliced (30 μ m sections) in a cryostat (Hacker), mounted on gel coated slides and stained with thionine. The position of the probes were verified under a dissecting microscope. Data from only those animals with correct microdialysis probe placements were included in the data analysis.

Data analysis

The average of the first four dialysate samples was considered to make up the baseline and was defined as 100 %. The subsequent data points were expressed as percentage of the individual rat's baseline and each of the amines and metabolites were analyzed by a one-way ANOVA with repeated measures (dialysate samples before and after air puff

exposure). Post-hoc tests were conducted using Newman-Keuls multiple comparisons ($\alpha = 0.05$).

Results

Figure 17 shows the representational view of the coronal sections and probe placements in each region. For the nucleus accumbens placements, 20 of the 24 probe tips were located within the nucleus accumbens, clustered between sections 1.4 and 1.8 mm from bregma and ventral tip position ranging from 8.6 to 8.2 mm below skull surface. In the case of the medial prefrontal cortex placements, 14 of the 16 probe tips were clustered within sections 2.7 and 3.7 mm from bregma and ventral tip position ranging from 5.5 to 7.2 mm below skull surface. In the case of the hippocampal placements, 15 of the 18 probe tips were located within the hippocampus, ventral probe tips being clustered between sections -4.3 and -4.8 mm from bregma and 8.2 to 7.1 mm below the skull surface.

Nucleus Accumbens

The variations of DA, DOPAC, HVA and 5-HIAA in the nucleus accumbens at baseline, following IL-1 β administration and after exposure to air puff stimulation are shown in Figure 18. Analysis of variance of the accumbal 5-HIAA concentrations revealed a significant Treatment x Sampling period interaction, $F(26, 312) = 6.97$, $p < 0.01$. The multiple comparisons ($\alpha = 0.05$) indicated that simply injecting the rats provoked a small (statistically non-significant), increase of 5-HIAA. Treatment with IL-1 β resulted in an increase of 5-HIAA levels, such that they exceeded those of baseline and also exceeded those

Figure 17. Schematic representations of coronal sections of the rat brain showing probe placements in the medial prefrontal cortex, nucleus accumbens and hippocampus. The shaded portion of the probes represents the active region of the microdialysis probe (see description in text for range of placements).

Prefrontal cortex A/P = 3.2 mm

Nucleus accumbens A/P = 1.8 mm

Hippocampus A/P = -4.3 mm

Figure 18. Mean ($\pm$ S.E.M.) concentrations (pg/40 μ l) of extracellular accumbal (a) DA, (b) HVA, (c) DOPAC and (d) 5-HIAA over 30 min samples. The initial four samples served as a baseline period after which rats were injected with either saline or IL-1 β , and five samples collected at 30 min intervals. The mean basal levels of DA, HVA, DOPAC and 5-HIAA were 8.2, 1646.4, 2546.6 and 1150.1 pg/40 μ l dialysate, respectively. The air puff (or no air puff treatment) was then administered and five samples collected at 30 min intervals. Circles denote rats that received IL-1 β and were exposed to the air puff; triangles represent IL-1 β -treated rats not exposed to the air puff; squares denote animals injected with saline and exposed to the air puff. Filled-in symbols denote significant differences from the average of the baseline period; asterisks denote significant differences from saline-treated rats ($p < 0.05$).

of saline-treated rats. In saline-treated animals, application of the air puff provoked a non-significant increase of 5-HIAA levels, which declined over the subsequent sampling periods. The extent of the 5-HIAA increase was considerably more pronounced in IL-1 β -treated rats, in that they significantly exceeded those of control animals exposed to the air puffs or animals injected with IL-1 β but not exposed to the air puffs.

Although neither accumbal DA nor DOPAC varied as a function of the IL-1 β or air puff treatments, analysis of the HVA levels in the nucleus accumbens revealed that the metabolite levels varied as a function of the Treatment x Sampling period interaction, $F(26, 312) = 3.64, p < 0.01$. The multiple comparisons revealed that as in the case of 5-HIAA, in saline-treated animals, the injection and the air puff procedures both provoked transient, non-significant increases of HVA. In IL-1 β -treated rats, the increase of HVA was more sustained, and was further augmented upon introduction of the air puffs. Indeed, the metabolite concentration exceeded both baseline levels and that of saline-treated rats exposed to the air puff, as well as IL-1 β -treated rats that had not received the air puff stimulation.

Prefrontal Cortex

Concentrations of DA in the medial prefrontal cortex were not affected by either the IL-1 β treatment or administration of the air puffs (see Figure 19). However, both DOPAC and HVA concentrations varied as a function of the Treatment x Sampling period interaction, $F(13, 208) = 18.8$ and $14.58, p < 0.01$, respectively. Post-hoc comparisons indicated that administration of IL-1 β alone did not significantly influence either DOPAC or HVA concentrations. Although air puff exposure was without effect in control (saline-treated) rats,

Figure 19. Mean ($\pm$ S.E.M.) concentrations (pg/40 μ l) of extracellular (a) DA, (b) HVA, (c) DOPAC and (d) MHPG from the medial prefrontal cortex over 30 min samples. The mean basal levels of DA, HVA, DOPAC, and MHPG were 6.3, 509.4, 102.1, and 129.9 pg/40 μ l dialysate, respectively. The initial four samples served as a baseline period after which rats were injected with either saline or IL-1 β , and five samples collected at 30 min intervals. The air puff (or no air puff) treatment was then administered and five samples collected at 30 min intervals. Squares denote rats that received saline and were exposed to the air puff; circles denote animals treated with IL-1 β and exposed to the air puff. Filled-in symbols denote significant differences from the average of the baseline period; asterisks denote significant differences from saline-treated rats ($p < 0.05$).

the same stressor in IL-1 β -treated animals elicited a significant rise of the metabolites. The effects on MHPG were also found to vary with the Treatment x Sampling period interaction, $F(13, 208) = 16.33$, $p < 0.01$. Relative to both the baseline levels and that of saline-treated rats, the levels of MHPG were elevated during the fourth and fifth sampling periods following IL-1 β treatment. While the air puff did not appreciably influence MHPG levels in saline treated animals, the MHPG in rats that had received IL-1 β increased significantly from the period preceding the air puff being applied. This effect was maximal 1.0 -1.5 h after air puff exposure, and then declined to the level seen prior to the stimulus being applied. Finally, IL-1 β was also found to influence the concentrations of 5-HIAA and this effect varied over the test session, $F(13, 208) = 2.07$, $p < 0.05$ (data not shown). While the IL-1 β treatment provoked a modest increase of 5-HIAA, upon exposure to the air puff the level of 5-HIAA increased by about 20%, while in saline- treated animals, the rise of 5-HIAA was negligible. As a result, the 5-HIAA accumulation in IL-1 β -treated animals significantly exceeded that of rats that had received saline.

Hippocampus

Figure 20 depicts the hippocampal 5-HT, 5-HIAA, DA and DOPAC concentrations as a function of the IL-1 β and air puff treatments. The analysis of variance revealed that 5-HT concentrations were elevated in rats that had received the IL-1 β treatment, $F(1, 19) = 4.76$, $p < 0.05$, and also varied over Sampling periods, $F(18, 234) = 5.12$, $p < 0.01$. Although the Treatment x Sampling period interaction did not reach statistical significance, a priori comparisons had been made concerning this interaction, and hence between-treatment

Figure 20. Mean ($\pm$ S.E.M.) concentrations (pg/40 μ l) of extracellular hippocampal (a) DA, (b) DOPAC, (c) 5-HT and (d) 5-HIAA over 30 min samples. The mean basal levels of DA, DOPAC, 5-HT and 5-HIAA were 2.05, 24.0, 0.91, and 641.9 pg/40 μ l dialysate, respectively. The initial four samples served as a baseline period after which rats were injected with either saline or IL-1 β , and five samples collected at 30 min intervals. The air puff (or no air puff) treatment was then administered and five samples collected at 30 min intervals. Squares denote rats that received saline and were exposed to the air puff; circles denote animals treated with IL-1 β and exposed to the air puff. Filled-in symbols denote significant differences from the average of the baseline period; asterisks denote significant differences from saline-treated rats ($p < 0.05$).

comparisons were made at the various sampling periods. The treatment groups were not found to differ from one another during the baseline period. In IL-1 β -treated animals, 5-HT levels were greater than those seen in saline injected rats during the third and fourth sampling periods following injection. While the air puffs did not affect 5-HT levels in saline-treated rats, a marked, albeit transient, increase of 5-HT was seen in rats that had received IL-1 β administration. The levels of 5-HT were elevated during the first sampling period following air puff application, and then quickly returned to baseline levels. Analysis of 5-HIAA concentrations revealed a significant interaction between the Cytokine treatment and Sampling period, $F(13, 260) = 3.10$, $p < 0.01$. The individual comparisons revealed that in contrast to the variations of 5-HT, among saline-treated animals the injection procedure and the air puffs both provoked a modest, but significant rise of 5-HIAA levels relative to baseline. While the effects of the injection persisted for only a single sampling period, the effects of IL-1 β were somewhat more extended, being significantly elevated throughout the last four sampling periods following injection. The air puff itself was found to increase 5-HIAA levels in saline-treated rats, as well as in animals that had received IL-1 β , and only during the last two sampling periods did the levels of 5-HIAA begin to decline towards control values. Unlike the variations of 5-HT and 5-HIAA induced by the cytokine, neither hippocampal DA nor DOPAC varied as a function of the IL-1 β or air puff treatments. Likewise, NE and MHPG were not found to be affected by these treatments.

Discussion

In contrast to the post-mortem studies suggesting that IL-1 β enhances DA utilization

in mesocorticolimbic sites, in the present investigation, systemic IL-1 β administration did not significantly influence DA or NE release in the prefrontal cortex (data not shown). Within the nucleus accumbens extracellular HVA was elevated, without any changes of DA. Extracellular 5-HIAA accumulation, however, was enhanced by the cytokine treatment in both the nucleus accumbens and hippocampus. It appears that alterations of DA turnover in mesolimbic sites, which ordinarily are readily influenced by a variety of environmental manipulations (e.g., both aversive and rewarding stimuli) (Fibiger, 1995), is not markedly influenced by systemic IL-1 β administration. Interestingly, the most pronounced effects of the IL-1 β treatment were evident following mild stimulation induced by the air puffs. In particular, while the air puff exposure provoked only small effects on 5-HIAA and HVA in the accumbens of rats treated with saline, these effects were marked and relatively sustained in IL-1 β -treated rats. Likewise, in the medial prefrontal cortex of rats that had received IL-1 β , exposure to the air puffs resulted in greater increases of DOPAC and MHPG than in similarly treated saline injected animals. It seems that although IL-1 β may have only modest effects on central monoamines, this cytokine may influence neuronal reactivity, hence leading to augmented amine changes upon presentation of a stressor. While some of the effects of IL-1 β and mild neurogenic stressors appeared to be of an additive nature, synergistic effects of these treatments were seen in some brain regions (viz. the increased accumbal 5-HIAA and HVA, and the MHPG variations in the prefrontal cortex).

Experiment 6. Time-Dependent *In Vivo* Amine Variations Following Antigenic Challenge (Sheep Red Blood Cells)

In the preceding experiments, IL-1 β was found to promote region specific neurotransmitter alterations. Likewise, systemic administration of the bacterial endotoxin, LPS, was found to provoke central monoamine variations. In each of these cases, the treatment resulted in illness, and hence it is unclear whether the neurochemical changes were actually secondary to the malaise associated with the treatment. Experiment 6 was undertaken to assess, using *in vivo* microdialysis, the effects of immune challenge with sheep red blood cells (SRBC) on the levels of DA and its metabolites in the nucleus accumbens of freely moving rats. Sheep red blood cells promote a T-cell dependent immunological effect, without engendering an illness response. Thus, any variations of neurotransmitter or neuroendocrine functioning cannot be attributed to the malaise associated with the treatment. Furthermore, if the amine changes engendered by stressors stem from the immunological alterations, then it would be expected that the amine variations induced by antigenic challenge would temporally correspond with the immune response being mounted. Accordingly, using *in vivo* procedures, it is possible to examine the amine changes over the course of a developing immune response (e.g., where the time of the peak immune response occurs 4 days following inoculation with SRBC).

It will be recalled that several investigators demonstrated that hypothalamic variations of NE and 5-HT (in post-mortem tissues) varied over time following antigenic challenge (Besedovsky et al., 1983; Carlson et al., 1987; Dunn et al., 1989; Zalcman et al.,

1991). Given the view that immune challenge produces central effects like those elicited by stressors (Dunn, 1988,1990; Shintani et al., 1993; Zalcman et al., 1994), it was of particular interest to establish whether SRBC inoculation would elicit DA changes in the nucleus accumbens, an effect readily elicited by psychological and physical stressors (Anisman et al., 1993; Deutch & Roth, 1990).

It ought to be mentioned at this time that since the mouse brain is too small for use in microdialysis studies, the *in vivo* determinations were conducted using rats. Although one would like to believe that any effects of SRBC would be evident across a variety of species, this is not necessarily the case. Indeed, differences in the time course for an immune response may vary across strains within a species, and various manipulations have been shown to provoke marked differences in inter-strain immune, neuroendocrine and neurotransmitter functioning. Given these inter-strain differences, it would not be remarkable to find pronounced inter-species differences in this respect. Nevertheless, it would be expected that qualitatively similar effects would be elicited by SRBC across rodent species.

Materials and Methods

A total of 20 male Sprague-Dawley rats with characteristics described in the previous experiment, were employed as subjects. Likewise, the surgical procedures for the implantation of cannulae into the nucleus accumbens were the same as in Experiment 5.1.

Antigenic challenge and microdialysis

Testing was conducted between 09.00 and 14.00 h daily for 7 consecutive days. On

Day 0, rats ($n = 10/\text{group}$) were injected with either SRBC (5×10^6 cells, i.p., in a volume of approximately 0.5 ml) or saline (0.5 ml, i.p.). On Day 1, microdialysis probes were inserted into the guide cannula under short halothane anaesthesia. The microdialysis procedure and apparatus were the same as described in Experiment 5. Collection of dialysate samples began 24 h later (on Day 2). Seven 30-min samples were collected daily (0.900-14.00 h) for the ensuing 5 days. Following the collection of samples on each day, the flow rate was reduced to a standby rate of $0.7 \mu\text{l}/\text{min}$. Following collection, dialysates (40 μl) were immediately injected (via Rheodyne (CA) 7161 injector) into the HPLC system as described in Experiment 5, for determination of interstitial biogenic amine levels.

Histology

Histological verification of the probe placements were conducted on the day following the last dialysis session. At the same time blood was collected for determinations of antibody titers. For histological verification of probe placement, rats under deep pentobarbital anesthesia (70 mg/kg; i.p.) were perfused intracardially with 0.9% saline followed by a 10% formalin solution. Frozen brains were sliced (30 μm sections) in a cryostat (Hacker) and mounted on gel-coated slides. Sections were then stained with thionine and viewed under a dissecting microscope.

Immediately prior to perfusion, blood was taken intracardially for determination of antibody titers. Blood was permitted to clot, and samples were centrifuged at $400 \times g$ for 10 min and the supernatant was collected and spun at $600 \times g$ for 10 min. Serum complement was inactivated at 56°C for 30 min. Two-fold serial dilutions of inactivated serum, saline and

a 1% SRBC solution were made in glass microwells. The highest dilution at which aggregation of SRBC was most evident was considered to be the antibody titer and was expressed in $\log_2$ units. Data from only those animals with correct microdialysis probe placements in the nucleus accumbens, and SRBC-inoculated rats showing an immune response, were included in the data analysis. The dialysis results were presented in absolute values (i.e., as pg DA, DOPAC, HVA or 5-HIAA/40 μ l of dialysate).

Data analysis

To analyze the microdialysis results, the average of the first four dialysate samples was considered to make up the baseline and was defined as 100 %. The subsequent data points were expressed as percentage of the individual rat's baseline and each of the amines and metabolites were subjected to a 2 (Drug) x 6 (Day) repeated measures ANOVA, followed by Newman-Keuls multiple comparisons ($\alpha = 0.05$).

Results

Histological verification revealed that the probes of 8 control animals and 9 SRBC-treated rats were located within the nucleus accumbens (see Figure 21). Data from only those animals with correct probe placements were included in the data analysis. The $\bar{x}$ ($\pm$ SEM) antibody titers for rats treated with 5×10^6 SRBC was 3.43 ± 0.36 . One SRBC-treated rat failed to show an immune response and was thus excluded from the analysis.

The analysis of the DA values revealed a significant Treatment x Days interaction, $F(5, 107) = 2.62$, $p < 0.05$. The multiple comparisons confirmed that among saline-treated

Figure 21. Schematic representations of coronal sections of the rat brain. Open and solid squares depict the ventral tip of the probes in control and SRBC-treated rats, respectively.

animals, DA levels remained stable across days. In contrast, in SRBC-treated animals, interstitial DA levels in the nucleus accumbens began to increase on Day 3 following inoculation and reached a significant peak on Day 4 ($p < 0.01$) (see Figure 22). At this time the DA levels in SRBC-treated rats exceeded that of the saline-treated animals. Levels of DA had declined by Day 5, such that they did not differ from baseline. None of the main effects or interactions for DOPAC, 5-HIAA, or HVA levels reached statistical significance. However, in the case of DOPAC and HVA there was a significant effect over days (see Figure 22). Both these metabolites decreased over days under control as well as SRBC conditions.

Discussion

It will be recalled that immunologic challenge, such as viral infection, influences HPA activity, as well as hypothalamic NE and 5-HT function (Dunn et al., 1989). Likewise, SRBC inoculation gives rise to variations of NE within the hypothalamus (Besedovsky et al., 1983; Carlson et al., 1987; Felten et al., 1991; Roszman & Brooks, 1992; Zalcman et al., 1991) possibly reflecting communication between the immune and central nervous system. The results of Experiment 6 make it clear that central transmitter changes engendered by immunologic challenge are not restricted to the hypothalamus but can be observed in the nucleus accumbens. In particular, the present study showed that SRBC administration increased extracellular DA within the nucleus accumbens. Moreover, the maximal increase of DA which reflected neuronal release of the amine, coincided with the peak immune response. Over days following SRBC inoculation, extracellular DA increased progressively,

Figure 22. Mean ($\pm$ S.E.M.) concentrations (pg/40 μ l) of extracellular accumbal (a) DA, (b) HVA, (c) DOPAC and (d) 5-HIAA over days following SRBC immunization or saline treatment.

reached a peak 4 days after SRBC treatment, and then declined to control levels. It is of interest to note that in one animal where SRBC inoculation failed to provoke an immune reaction (the antibody titer equalled 0), the SRBC-induced elevation of accumbal DA release was not evident. In contrast, in each of the remaining mice, where antibody titers ranged from 3-5, changes of extracellular DA were apparent over days. Inasmuch as the DA variations coincided with the peak immune response, it is possible that the amine alterations were secondary to immune activation. Moreover, as animals were not handled during the course of the testing, the observed effect was not attributable to factors such as handling or change in environment, which could potentially influence the DA concentrations observed in post-mortem studies (i.e., where animals are handled and decapitated at various times following inoculation).

7. General Discussion

In addition to the well documented immunological and neuroendocrine consequences observed following cytokine or endotoxin challenge, marked central neurochemical alterations may be elicited by such treatments. As well, cytokine challenge may engender behavioural changes beyond the sickness profile ordinarily observed following cytokine or endotoxin administration. In this respect, however, the effects of IL-1 β and IL-2 are distinctly different from one another, and the outcomes observed may be dependent upon situational factors, as well as the specific cytokine regimen that animals received. In the sections that follow an overview is provided concerning the impact of the cytokine treatments, with particular emphasis being placed on the behavioural effects of the treatments, followed by the neurochemical changes observed.

Effects of IL-1 β on Exploration

Systemic administration of IL-1 β promoted behavioural changes in an open-field exploratory test. In particular, IL-1 β -treated mice spent less time engaged in active behaviours (i.e., locomotor activity, digging and rearing), less time exploring a novel stimulus object, and more time engaged in passive responses (i.e., sitting immobile) either within or outside the container. However, while the cytokine suppressed locomotor activities, these behaviours were not particularly sensitive to dosage differences, at least within the range employed in the present investigation.

In contrast to general activity, exploration of a novel container following IL-1 β treatment varied as a biphasic function of dosage and time following treatment. At the lowest

doses, animals initially made repeated entries into the novel container, after which such responses declined as animals remained inside the container. At intermediate doses the repetitive entries were not emitted during the early period, but instead animals tended to enter the container where they remained. At the highest dosage, animals displayed pronounced illness, made few entries into the container, and unlike animals treated with lower doses, they tended to remain outside the container frequently in a curled body posture. In effect, it appears that while locomotor activity was reduced in cytokine-treated animals, this measure did not distinguish well between doses. In contrast, when locomotor activity is considered together with entries into and time spent within the container, these behaviours were more sensitive to differences in the IL-1 β doses that mice received. These quantifiable, dose-dependent, biphasic behavioural variations may be useful as an index of illness engendered by the cytokine treatment, and may be more sensitive to the effects of the cytokine than relying simply on locomotor changes.

The effects of the cytokine on locomotor activity and exploration in an open-field are entwined, and it has been assumed that reduced locomotor activity and exploration are both indicative of illness. Yet, it was shown that both systemic (Dunn et al., 1991) and intraventricular (Spadaro & Dunn, 1990) administration of IL-1 β reduced exploration (the time mice spent in contact with a novel stimulus), without influencing measures of locomotor activity. Indeed, the variations of exploratory behaviour were sensitive to even very low doses (e.g., in the range of 0.1-10 μ g) of peripherally injected IL-1 β (Dunn et al., 1991). In light of these considerations, the possibility exists that the exploratory changes within the open-field were unrelated to locomotor effects provoked by the cytokine.

To further evaluate the relationship between the effects of IL-1 β on exploration and locomotor activity, performance was assessed in a free-running spontaneous alternation test. As indicated earlier, the number of arm entries (activity) in this test are independent of the pattern of exploration (Kokkinidis & Anisman, 1980). Despite the fact that this paradigm is sensitive to changes in habituation, prior experience in the Y-maze, as well as pharmacological manipulations (e.g., anticholinergics and catecholamine stimulants) (Kokkinidis & Anisman, 1980), in the present investigation alternation performance was unaffected by the cytokine. Mice that received IL-1 β exhibited normal exploratory patterns, and such an outcome was evident in naive mice, those that had received the mild stress of being placed in a novel environment prior to Y-maze testing, and after treatment with IL-1 β on 5 consecutive days. Thus, these data suggest that IL-1 β did not disturb the animal's ability to explore the environment in a systematic fashion. As such, it seems that IL-1 β does not affect the mechanisms (e.g., attention or short-term memory) that might be necessary for adequate alternation performance (Kokkinidis & Anisman, 1980). Of course, this supposition needs to be considered as being highly provisional, pending discrete analysis of attentional and short-term memory processes. Nevertheless, the fact that mice treated with high doses of IL-1 β exhibited control levels of alternation performance represents *prima facie* evidence that variations of open-field performance may well have been secondary to the soporific or motor consequences of the cytokine, rather than effecting habituation processes.

Effect of IL-1 β on Anxiety

As indicated earlier, the majority of studies assessing the behavioural effects of IL-1 β

have focused on the sickness behaviour induced by the cytokine (e.g., soporific effects, anorexia), as well as the processes subserving this behavioural profile. Nevertheless, there have been several studies that assessed behaviours not directly related to illness, including exploration (Bluthe et al., 1996; Dunn et al., 1991; Spadaro & Dunn, 1990) and operant responding for food reinforcement (Bluthe et al., 1989; Bret-Dibat et al., 1995; Crestani et al., 1991). Of course, these behaviours are not independent of IL-1 β -induced illness and it is certainly conceivable that variations of these behaviours are secondary to the malaise engendered by the cytokine.

Further to this same issue, as mentioned previously, it is difficult to dissociate the motivational or anxiogenic effects of IL-1 β from the sickness elicited by the cytokine. However, several recent findings support the view that some of the behavioural outcomes provoked by IL-1 β may be independent of illness and may reflect stress-like actions induced by the cytokine. For example, IL-1ra has been reported to attenuate central neurochemical changes induced by stressors (Shintani et al., 1995a,b) and antagonize disturbances of shuttle escape performance provoked by prior exposure to inescapable shock (Maier & Watkins, 1995).

Inasmuch as IL-1 β is a potent stimulator of CRH, which has been shown to induce anxiogenic effects (Gray, 1991; Heilig et al., 1994), the possibility was entertained that IL-1 β might similarly elicit anxiety, particularly in a novel situation. Moreover, mice treated with the cytokine might display neophobia, as reflected by a reluctance to approach a novel stimulus object. However, as discussed earlier, in the present investigation, the behavioural profile of animals that received IL-1 β did not support the presence of neophobia. Of course,

the absence of neophobia does not necessarily imply that the cytokine was without effect on anxiety. In fact, systemic administration of IL-1 β induced a modest anxiogenic-like effect in an elevated plus-maze test. In this respect, treatment with IL-1 β decreased the total time mice spent in the open arms of the maze and reduced the number of open-arm entries emitted, while increasing both the latency to make an initial entry into an open arm of the maze and the number of stretch-attend postures emitted. Moreover, the effects of the cytokine did not appear to be related to motor impairments, as IL-1 β did not alter the number of closed-arm entries emitted. In a further study conducted in rats, central administration of IL-1 β was also shown to attenuate the number of entries onto the open arms of a plus-maze (Connor et al., 1998), suggesting that IL-1 β induces anxiogenic-like effects via central mechanisms. Interestingly, as the low dose of centrally administered IL-1 β did not elicit a sickness response, it would appear that the anxiogenic effect stemmed from the direct central action of the cytokine rather than being secondary to illness.

While the aforementioned data are consistent with an anxiogenic action of IL-1 β , a caveat should be introduced at this time. In our laboratory, as in others (Hogg, 1996), the elevated plus-maze test has generally been found to be associated with a high degree of between-animal variability. This test appears to be extremely sensitive to lighting conditions, extraneous noise, as well as a variety of organismic variables, such as the strain and age of the animals. Nevertheless, the test appears to be relatively useful in detecting anxiolytic actions of various treatments (Hogg, 1996); however, it may be more difficult to use this paradigm to detect potential anxiogenic effects associated with experimental treatments. In fact, in our laboratory, IL-1 β was not reliably found to promote anxiety. For instance, in a

recently conducted experiment, systemic injection of IL-1 β (doses ranging from 0.1 to 0.8 μ g) did not promote a statistically significant decline in the number of open-arm entries, but provoked an increase in the number of stretch-attend postures emitted.

In addition to the variability ordinarily associated with the elevated plus-maze test (Hogg, 1996), it is important to underscore that the variable consequences of IL-1 β , with respect to the potential anxiogenic actions, may be related to the multiplicity of effects associated with the cytokine treatment. Moreover, it needs to be considered that the time course for the many actions of IL-1 β may differ from one another and thus certain consequences of the treatment may be evident at short intervals, whereas others may only be apparent at long post-treatment periods. It is known, for instance, that although the half-life of IL-1 β is fairly short (in the range of about 30 min), central neurochemical changes may be fairly persistent (Song et al., 1998; see also Experiment 5). Thus, it is uncertain at what time frame following IL-1 β treatment the anxiogenic actions would be most prominent. This issue is further complicated by the fact that illness occurs fairly soon after cytokine administration (certainly within 30 - 60 min following treatment), and may obfuscate more subtle consequences of the compound, including anxiogenic actions. In effect, in addition to assessing the dose-dependent effects of the treatment, it may be essential to evaluate the temporal course of behavioural changes following systemic and central administration of IL-1 β .

In contrast to the anxiogenic-like effects observed in the elevated plus-maze, IL-1 β did not influence either the latency to enter the illuminated portion of a light-dark box or the time animals spent in this compartment. Although cytokine-treated mice made fewer

transitions between the light and dark compartments, it is uncertain whether this had any bearing on anxiety. Once again, however, the scope of the light-dark box experiment was limited with respect to the dosage of the cytokine, the time following cytokine treatment and the phase of the light-dark cycle at which animals were tested. Thus, the fact that the light-dark box test yielded negative results does not necessarily imply the absence of an anxiogenic-like effect.

Finally, it has been suggested that different behavioural tests tap into different aspects of anxiety. For example, one can distinguish between a generalized anxiety response vs that associated with specific environmental stimuli. Likewise, it is possible to dissociate generalized anxiety from a fear-elicited anxiety and both may involve different mechanisms (Lee & Davis, 1997). Thus, the light-dark box test may be an insensitive index of anxiety, at least with respect to the anxiogenic actions of IL-1 β . The inconsistencies observed in the present investigation suggest that the anxiogenic-like effects elicited by this cytokine may be modest and that more sensitive tests of anxiety need to be employed in order to better quantify the role of IL-1 β on anxiogenic processes. For instance, there has been increasing interest in the use of startle and fear-potentiated startle paradigms in the assessment of generalized anxiety vs. cue-related (fear potentiated) anxiety. In this respect, it has been suggested that while the central nucleus of the amygdala may be fundamental in subserving fear-related anxiety, the bed nucleus of the stria terminalis may govern or contribute to generalized anxiety responses (Lee & Davis, 1997). It remains to be established, however, whether cytokines such as IL-1 β , influence either or both of these anxiety-like responses.

It will be recalled that several investigators suggested that, in several respects, IL-1 β

induces effects similar to those elicited by stressors (Anisman & Merali, 1998; Dunn, 1990; Maier & Watkins, 1998). In addition to the potential anxiogenic effects induced by the cytokine, the possibility has been explored that IL-1 β may engender anhedonia. In fact, it has been considered that the anorexia elicited by IL-1 β may, in part, reflect the anhedonic actions of this cytokine. Accordingly, in evaluating the effects of IL-1 β , we also assessed whether this cytokine would attenuate consumption of a highly palatable diet (chocolate milk) in otherwise sated animals (thereby minimizing the contribution of hunger on food consumption). Specifically, following the establishment of stable chocolate milk consumption (i.e., less than 10% variability between days), mice were injected with IL-1 β (0.5 μ g; i.p.) or saline and their consumption was measured over a 1 hr period. Compared with saline controls, consumption was significantly reduced in IL-1 β -treated mice, $F(1, 14) = 9.093$, $p < 0.01$ ($\bar{x} \pm \text{SEM} = 1.40 \pm 0.30$ ml vs 2.70 ± 0.32 ml, respectively). However, the latency to commence drinking was unaffected by the cytokine treatment ($\bar{x} \pm \text{SEM} = 2.14 \pm 0.34$ ml vs 1.57 ± 0.37 ml), suggesting that the decline in chocolate milk consumed in IL-1 β -treated mice was not related to motoric factors. Moreover, upon retesting 24 hr later, in the absence of further treatment, consumption levels returned to baseline values. Parenthetically, in a similar study, we assessed whether IL-2 (0.5 μ g; i.p.) would influence consumption of a highly palatable diet. Unlike IL-1 β , chocolate milk consumption was unaffected by IL-2 administration ($\bar{x} \pm \text{SEM} = 2.00 \pm 0.20$ ml vs 1.91 ± 0.18 ml, for saline and IL-2-treated mice, respectively).

As observed in recent, more detailed studies conducted in our laboratory (Brebner et al., 1998), systemic IL-1 β administration dose-dependently reduced consumption of this

highly palatable food source. Moreover, Brebner et al. (1998) also observed that TNF- α induced a comparable effect, and that IL-1 β and TNF- α acted synergistically to affect consumption of the palatable substance.

Although it is tempting to suggest that the decrease in chocolate milk consumption was related to motivational factors (anhedonia), such an effect may have been more closely aligned with the illness induced by the cytokine, or may have been associated with a change in the perceived palatability of the food. Indeed, several recent studies suggest that IL-1 β has, at best, only limited effects on reward processes. Specifically, treatment with IL-1 β (1.0 μ g) did not alter responding for rewarding brain stimulation from the medial forebrain bundle either 1 hr (e.g., a time corresponding to peak ACTH and corticosterone changes) or 15 min following cytokine injection (Anisman et al., 1996; 1998). However, animals tested with a higher dose of IL-1 β (2.0 μ g), which elicited clear signs of illness, showed a modest reduction of responding. Moreover, although the overall response rate was not significantly different from baseline, responding during the ascending portion of the titration schedule was significantly impaired. This profile was similar to that observed following systemic administration of LPS (Borowski et al., 1998), but clearly different from that provoked by IL-2 (Anisman et al., 1996) or stressors (Anisman et al., 1993). In effect, it appears that the altered responding for rewarding brain stimulation induced by IL-1 β is more likely attributable to the illness induced by the cytokine rather than a direct effect on reward processes (Anisman et al., 1998).

Neuroendocrine Correlates of IL-1 β

Commensurate with reports that IL-1 β stimulates HPA functioning (Blalock 1994; Dunn 1990; Imura et al. 1991; Koenig 1991; Rivier 1995), systemic treatment with the cytokine markedly influenced plasma ACTH and corticosterone concentrations. While elevated corticosterone concentrations were seen across a range of doses, an increase in ACTH was restricted to the highest cytokine dosage. It will be recalled that while ACTH variations induced by various types of treatments are relatively transient, plasma corticosterone alterations tend to be more sustained (Merali et al., 1998). Moreover, in the present investigation, plasma hormone levels were only assessed at a single time point (1 hr post-treatment), and it is certainly possible that changes in ACTH levels might have been detected at other times.

It has been proposed that cytokines and stressors may have synergistic effects with respect to neuroendocrine and central neurotransmitter processes (Anisman & Merali, 1998). In the present investigation, it was observed that among animals that had received the cytokine and then exposed to the novel environment, the endocrine changes were no greater than among mice that had not been exposed to the novel situation (although these comparisons come from two independent studies). It seems that while exposure to novelty may itself have been stressful, thus increasing plasma corticosterone, the effects of novelty and IL-1 β were neither additive nor synergistic. In fact, in an additional study conducted in our laboratory, where IL-1 β and mild stressor application were both manipulated within a single experiment, neither additive nor synergistic effects were evident. This was the case in blood samples taken both 1 and 2 hrs following cytokine treatment (unpublished data).

The fact that IL-1 β enhances HPA functioning, and induces stress-like behavioural effects (e.g., decreased exploration and locomotor activity), has led to the suggestion that immune activation, and particularly release of peripheral cytokines, may be interpreted as a stressor. To be sure, it has been suggested that processive stressors (i.e., those that involve higher-order sensory processing, such as exposure to a novel environment, fear conditioning, exposure to predators) and systemic stressors (i.e., those that influence respiratory, cardiovascular and immune processes) may both influence HPA activity, but only the former would involve amygdaloid processes (Herman & Cullinan, 1997). The finding of the present investigation that cytokine treatment yielded effects like those ordinarily engendered by psychogenic stressors, is consistent with the view of Herman & Cullinan (1997). However, as will be seen shortly, analysis of the central effects of IL-1 β needs to be considered in a broader context than that offered by Herman & Cullinan (1997).

Neurotransmitter Correlates of IL-1 β Administration

Physical and psychological stressors induce a reliable profile of monoamine variations, including NE alterations within the locus coeruleus, hypothalamus, amygdala and prefrontal cortex, as well as DA variations in the nucleus accumbens and in mesocorticolimbic, but not nigrostriatal regions. Thus, it was of interest to ascertain whether the profile of amine changes induced by IL-1 β treatment would be similar to that ordinarily induced by stressors. Indeed, in the present investigation, the transmitter alterations elicited by this cytokine were, in some respects, reminiscent of the amine changes observed in response to processive stressors. However, in several other respects the outcomes could

readily be distinguished from such aversive stimuli. In particular, IL-1 β treatment influenced NE activity within the PVN, locus coeruleus and prefrontal cortex, as well as DA activity within the median eminence plus arcuate nucleus, just as stressors induce such effects (Anisman et al., 1993). In contrast, there was no indication of the cytokine affecting DA within the nucleus accumbens or prefrontal cortex (irrespective of prior exposure to a novel environment), even though the latter region is exquisitely sensitive to the impact of aversive stimuli (Anisman et al., 1993; Deutch & Roth, 1990). In effect, it seems that IL-1 β treatment induces some effects like those engendered by stressors, but the impact of the cytokine, particularly with respect to mesocorticolimbic biogenic amine changes, are distinguishable from those associated with processive stressors.

Effect of IL-1 β on Interstitial Biogenic Amine Levels

While the aforementioned experiments, as well as earlier studies (Dunn 1988; Zalcman et al., 1994) indicated that IL-1 β influences central biogenic amine levels and/or activity, it must be underscored that these data reflect variations observed in post-mortem tissues. As indicated earlier, changes of post-mortem transmitter and/or metabolite levels do not necessarily reflect active utilization at the synaptic level (Westerink, 1995). Accordingly, using *in vivo* microdialysis in freely moving rats, we assessed the effects of IL-1 β on interstitial biogenic amine levels at various brain sites. Furthermore, we used this paradigm to assess whether IL-1 β might potentiate the neurochemical response(s) to environmental stimuli. In fact, it appeared that IL-1 β elicited region-specific changes of the interstitial levels of transmitters and/or their metabolites, and that the response to air puff stimulation was also

enhanced in rats that had been treated with the cytokine.

In contrast to the post-mortem studies suggesting that IL-1 β enhances utilization of DA in mesocorticolimbic sites (Zalcman et al., 1994), in the present investigation systemic administration of IL-1 β did not significantly influence DA or NE release in the prefrontal cortex. Within the nucleus accumbens, extracellular HVA was elevated, but without any changes of DA. In addition, extracellular 5-HIAA accumulation was enhanced by the cytokine treatment in both the hippocampus and nucleus accumbens. Whether the differential effects seen between laboratories with respect to the amine variations in the prefrontal cortex are related to species or dosage differences, or whether they reflect effects associated with the use of *in vivo* vs. *ex vivo* procedures remains to be determined. It is important to underscore that the effects of the IL-1 β treatment were distinctively different from those ordinarily induced by psychogenic factors. In particular, although alterations of DA turnover in mesolimbic sites are readily promoted by both aversive and rewarding stimuli (Fibiger, 1995), systemic IL-1 β administration did not provoke such an outcome. In fact, the most pronounced effects of the cytokine treatment were detectable only after mild air puff stimulation. Specifically, while the air puffs provoked modest 5-HIAA and HVA variations in the accumbens of saline-treated rats, these effects were more pronounced and relatively sustained in IL-1 β -treated animals. Similarly, among rats that had received IL-1 β , the air puffs resulted in greater increases of DOPAC and MHPG in the prefrontal cortex than among saline-treated animals. Evidently, despite the modest monoamine changes elicited by IL-1 β , this cytokine may influence neuronal reactivity, culminating in enhanced monoamine changes upon presentation of a stressor. In fact, while the two treatments had additive effects

at some brain sites, in other regions, IL-1 β and the mild stressor appeared to induce synergistic effects (e.g., elevated 5-HIAA and the HVA in the nucleus accumbens, as well as MHPG alterations in the prefrontal cortex).

Except for the hippocampus, 5-HT was below our limit of detection. However, its metabolite, 5-HIAA was present in relatively large amounts at all regions tested. The relative abundance of the metabolites in relation to the parent amine is a consistent finding in most microdialysis studies. Because of the specific and highly efficient uptake mechanisms for transmitter substances, one has to be in close proximity to the appropriate synapses where the transmitter is being released, or there has to be a high amine turnover rate, in order to detect changes of transmitter utilization. In contrast, it appears that the metabolites can accumulate in neuronal, as well as non-neuronal elements, and can travel greater distances from the biodegradation sites (Benveniste, 1987; Westerink, 1995; Zetterstrom et al., 1988). The fact that we consistently observed changes in the levels of 5-HIAA but not 5-HT, may indicate either that our probes were at some distance from the immediate vicinity of 5-HT release, and/or the levels of 5-HT released were smaller than our detection limit (Deakin et al., 1993). Conclusions about the transmitter utilization based on metabolite levels should be made cautiously, since transmitter and metabolite levels appear to change independently of one another (Wong et al., 1993). For example, whereas d-amphetamine markedly increased the interstitial levels of DA, the levels of its metabolite, DOPAC, changed little if at all. This is thought to occur because d-amphetamine also inhibits the active reuptake of DA and hence retards its metabolism by intraneuronal monoamine oxidase (Paulson & Robinson, 1995; Zetterstrom et al., 1988).

It will be recalled that IL-1 β has been posited as a possible mediator for the central effects engendered by immunologic challenge. For instance, IL-1 β induces neuroendocrine changes similar to those provoked by LPS, and IL-1 antagonists may interfere with the enhanced ACTH secretion promoted by the endotoxin (Rivier, 1990; Rothwell & Hopkins, 1995). Furthermore, LPS administration reduces IL-1 receptor density in some brain regions. However, it is important to note that the contribution of IL-1 β to the central effects associated with antigenic challenge may be dependent on the specific immune challenge being considered. For instance, it seems that the central actions induced by other antigenic challenges, such as SRBC, are distinct from those seen after IL-1 β treatment. In particular, it was observed that SRBC increased extracellular accumbal DA, and this increase corresponded with the time of the peak immune response (Lacosta et al., 1994). Moreover, SRBC treatment was not associated with variations of 5-HT turnover. These effects were distinct from those elicited by IL-1 β , suggesting that the actions of SRBC on central neuronal functioning involve a mechanism other than (or in addition to) IL-1 β activation. Of course, only a single dose of IL-1 β was used in the dialysis studies, and it is conceivable that with other doses, variations of accumbal DA activity might have been detected. Yet, it was noted that this dose was effective in provoking a marked increase of plasma corticosterone and ACTH, and elicited marked behavioural variations.

Summarizing, in some respects the *in vivo* neurochemical alterations stemming from IL-1 β challenge were similar to those observed in post-mortem tissue. Yet, as indicated by Herman & Cullinan (1997), the effects of processive stressors were distinguishable from the *in vivo* amine variations elicited by systemic insults. For instance, the finding that IL-1 β

influences 5-HIAA accumulation in several brain regions is consistent with this proposition, since stressors have reliably been shown to increase 5-HT utilization. However, in other respects the effects of IL-1 β could readily be distinguished from those elicited by stressors. In particular, it has been repeatedly demonstrated that stressors readily induce marked changes of DA activity in mesocorticolimbic sites, particularly within the prefrontal cortex (Abercrombie et al., 1989; Deutch & Roth, 1990). Yet, systemic IL-1 β administration did not influence extracellular DA and had only modest effects on DOPAC and HVA accumulation. Likewise, while the effects of stressors on accumbal DA function are typically less marked than those seen in prefrontal cortex (Abercrombie et al., 1989; Deutch & Roth, 1990), in the present investigation neither accumbal DA nor DOPAC accumulation was affected by IL-1 β , although a marked increase of HVA was evident. Despite the clear differences between the effects of IL-1 β and those elicited by stressors, it was demonstrated that pharmacological manipulations involving IL-1 β may influence the effects of stressors. In this respect, IL-1 receptor antagonists may prevent the hypothalamic biogenic amine variations, the ACTH alterations induced by stressors (Shintani et al., 1995b), as well as the escape disturbances and conditioned fear response introduced by uncontrollable shock (Maier & Watkins, 1995). Such findings have been instrumental in leading to the suggestion that IL-1 β may, in fact, contribute to stress-related pathology.

Effects of IL-2 on Exploratory and Active Behaviours

It will be recalled that in humans undergoing IL-2 immunotherapy, profound neuropsychiatric and cognitive disturbances may be engendered (Capuron et al., 1998;

Caraceni et al., 1992; Denicoff et al., 1987), so much so that discontinuation of therapy may be necessary (Meyers & Valentine, 1995). In the present investigation, acute IL-2 administration did not affect a range of behaviours, whereas chronic cytokine treatment hindered both exploration and Morris water-maze performance. These findings are consistent with reports indicating that immunotherapy in humans induces cognitive deficits, particularly with respect to spatial abilities (Capuron et al., 1998; Caraceni et al., 1992).

When administered acutely, systemic IL-2 did not affect exploratory behaviours, and unlike IL-1 β , did not elicit anxiogenic actions (Petitto et al., 1997). As well, treatment with IL-2 did not influence spontaneous alternation performance, suggesting that habituation processes were unaffected by the cytokine. Paranthetically, other treatments that disrupt ACh activity, such as hippocampal disturbances or administration of anticholinergic agents, have been reported to impair spontaneous alternation performance (Kokkinidis & Anisman, 1980).

In contrast to the effects of acute treatment, repeated IL-2 administration reduced locomotor activity and contact with a novel stimulus object. The latter outcome likely did not reflect neophobia or anxiety, as neither initial contact with the novel object nor plus-maze performance were affected by this treatment. Despite the marked variations of open-field behaviour, animals repeatedly treated with IL-2 still displayed proficient spontaneous alternation performance. It is important to underscore that the effects of IL-2 were distinguishable from those ordinarily associated with amphetamine administration. As discussed earlier, IL-2 immunotherapy was reported to elicit schizophrenic-like symptoms (Caraceni et al., 1992), and elevated IL-2 was reported in the serum and CSF of schizophrenic patients (Licinio et al., 1993; McAllister et al., 1995). In order to model

schizophrenic symptoms in animals, the behavioural and neurochemical effects of amphetamine have frequently been monitored. For instance, in a spontaneous alternation test, acute amphetamine promotes stimulus perseveration (exceedingly low levels of alternation), whereas a haphazard pattern of responding is seen following chronic treatment. The latter effect was taken to stem from an impairment of attentional processes elicited by chronic exposure to the catecholamine stimulant (Kokkinidis & Anisman, 1980). The finding that neither acute nor chronic IL-2 influenced spontaneous alternation, attests to the animal's ability to effectively employ environmental stimuli, unlike the random pattern of exploration observed following chronic amphetamine administration.

Morris Water-Maze Performance

Acute IL-2 treatment did not appreciably affect Morris water-maze performance, whereas chronic systemic IL-2, like repeated central cytokine administration (Hanisch & Quirion, 1996), markedly disrupted performance. However, this effect was only evident in the variable platform position paradigm. It is of particular significance that performance on the first trial of each day was unimpaired (i.e., prior to animals discovering the platform position), suggesting that the cytokine did not influence swimming ability. On ensuing trials performance was disrupted, indicating that chronic cytokine treatment affected working memory. Such an impairment was evident regardless of the platform location within the pool, and the deficits could not be ascribed to either perseverative response tendencies or to the adoption of particular response styles (e.g., thigmotaxis, avoidance of the central aspects of the pool, swimming in small circles). The lack of effect in the fixed platform condition

may be due to performance being readily acquired in this paradigm, and hence less vulnerable to disruption. Alternatively, chronic IL-2 may have selectively acted on working memory without impacting on reference memory processes.

Neurochemical Correlates of IL-2 Administration

In contrast to other cytokines, such as IL-1 β and TNF- α , systemic IL-2 administration did not influence HPA activity (see Anisman & Merali, 1998; Zalcman et al., 1994), just as acute central administration was without effect (Connor et al., 1998). It was reported, however, that repeated central IL-2 treatment increased HPA hormone levels (Hanisch et al., 1994). In the present investigation, neither acute nor chronic systemic IL-2 affected circulating plasma ACTH or corticosterone, regardless of the cytokine dose or time following treatment (1 and 2 hrs).

In vivo studies indicated that IL-2 inhibited accumbal DA release, an effect augmented by a mild stressor (Anisman et al., 1996; Song et al., 1998). It was likewise observed in the present investigation that the effects of IL-2 on central amine functioning differed in naive and mildly stressed mice (i.e., placement in a novel environment following IL-2 treatment). Among naive mice, PVN NE utilization was enhanced by IL-2, but this effect was absent in mildly stressed animals. Interestingly, in the latter condition NE utilization was increased in the locus coeruleus (and to a lesser extent in the mPFC), as was hippocampal 5-HT utilization, whereas accumbal DA utilization was reduced.

Paralleling the behavioural results, the amine variations observed following repeated IL-2 treatment were more pronounced than after acute cytokine administration. In particular,

chronic IL-2 induced widespread variations of neurotransmitter functioning, including increased utilization of NE in the median eminence plus arcuate nucleus and the hippocampus, and to a lesser extent in the mPFC and the central amygdala. In the median eminence plus arcuate nucleus and in the central amygdala, the increased utilization was accompanied by elevations of NE levels, possibly reflecting increased NE synthesis. In contrast, 5-HT utilization was unaffected by chronic IL-2, although 5-HT levels were increased in the hippocampus, and reduced in the mPFC. Finally, as observed in a slice preparation, application of exogenous IL-2 provoked a significant *in vitro* increase of spontaneous and K⁺-evoked striatal DA release (Lapchak, 1992). As indicated earlier, *in vitro* analysis revealed biphasic dose-response effects of IL-2 on DA functioning, with lower doses exciting and higher doses inhibiting amine release (Alonso et al., 1993; Petitto et al., 1997). In the present investigation, acute IL-2 inhibited accumbal DA turnover, as observed *in vivo* (Anisman et al., 1998; Song et al., 1998), but a biphasic dose-response function was not evident. However, the NE variations within the mPFC and locus coeruleus, as well as the hippocampal 5-HIAA alterations, varied biphasically. Whether a similar function would be observed with respect to chronic IL-2 treatment remains to be determined. Reiterating, analyses of neurotransmitter levels and metabolites in post-mortem tissue are difficult to interpret since they do not necessarily reflect the dynamic variations associated with neuronal functioning. Coupled with our earlier findings showing marked *in vivo* amine alterations even after acute IL-2 treatment (Anisman & Merali, 1998), the relatively modest post-mortem amine changes of the present investigation should be interpreted cautiously.

Inasmuch as IL-2 affects ACh release in hippocampal slices (Araujo et al., 1989), and

the hippocampus is involved in spatial memory (Squire, 1992), the IL-2 effects on Morris water-maze performance may stem from cytokine-induced ACh variations. While not denying the importance of ACh, the behavioural effects of chronic IL-2 may involve other neurotransmitter alterations, particularly given the widespread monoamine changes observed. Indeed, serotonergic alterations alone promote memory performance impairments, although these could be distinguished from effects associated with basal forebrain cholinergic variations (Wenk et al., 1987).

Finally, acute IL-2 administration has been shown to promote anhedonic-like effects, as reflected by disturbances of responding for rewarding brain stimulation (Anisman et al., 1996, 1998). The mechanisms supporting these effects have yet to be fully explored, but it is significant that disturbances of DA functioning were observed within the nucleus accumbens following acute IL-2 treatment. Given that DA may subserve reward processes (Fibiger & Phillips, 1988; Wise, 1985), a role for this transmitter (possibly in conjunction with 5-HT reductions within the hippocampus, and NE alterations within both the locus coeruleus and mPFC) ought to be considered in mediating the depressive symptomatology associated with the cytokine. It should be noted, however, that in studies examining self-stimulation performance following acute IL-2 treatment, the performance deficits became increasingly more pronounced with repeated test sessions. These data were reminiscent of the time-dependent sensitization observed with other cytokines, such as IL-1 β (Bartanusz et al., 1993; Tilders et al., 1993a). In particular, following chronic IL-1 β treatment or with the passage of time following an acute treatment, phenotypic variations of CRH and AVP co-expression were induced in the external zone of the median eminence (Tilders et al., 1993b).

Likewise, it was reported that following administration of TNF- α , re-exposure to the cytokine led to progressively greater time-dependent changes of monoamine turnover in some brain regions, whereas at other sites, a tolerance-like effect developed (Hayley et al., 1998). It remains to be established whether the chronic effects of IL-2 observed in the present investigation are similarly a reflection of a time-dependent sensitization effect, and whether normalization of behaviour and central neurochemical status would accompany discontinuation of the cytokine treatment.

In summary, it is clear from the behavioural and neurochemical perspective, that acute and chronic IL-2 treatment elicit profoundly different outcomes. Acute IL-2 treatment did not influence any of the behaviours examined and elicited limited central neurochemical perturbations. In contrast, chronic IL-2 exposure profoundly influenced a wide array of central monoamines and disrupted exploratory behaviour, as well as working memory in a spatial test. Given the increasingly wider utilization of cytokines in immunotherapy, including not only IL-2 but also IL-1 β and TNF- α , greater attention needs to be focused on the behavioural and neurochemical sequelae of chronic, systemically administered cytokine treatment.

Effects of LPS on Exploration

As described earlier, systemic administration of a bacterial endotoxin gives rise to macrophage activation and the subsequent release of IL-1 β , IL-6 and TNF- α . Thus, it should not be surprising to find that LPS has effects beyond those elicited by the individual cytokines. To be sure, there have been reports showing that in some respects LPS elicits

outcomes comparable to those of IL-1 β (Dantzer et al., 1996; Dunn, 1995), but it is also the case that LPS engenders behavioural and neurochemical outcomes beyond those associated with this cytokine (Anisman & Merali, 1998; Borowski et al., 1997; Dunn et al., 1992).

In the present investigation, animals treated systemically with LPS and then returned to their home cage, exhibited sickness behaviour characterized by reduced active responses (e.g., locomotor activity, and rearing), increased sleep, piloerection and ptosis. Moreover, in a recent study conducted in our laboratory, systemic treatment with LPS reduced consumption of a palatable food (chocolate milk) in satiated animals (Brebner et al., 1998). As well, when placed in a novel open-field, LPS-treated mice displayed reduced locomotor activity and exploration (e.g., rearing, contacts with a novel object). However, the sickness profile evident in a novel environment was far less remarkable than that seen in the home cage situation. In a still more threatening environment (i.e., in the elevated plus-maze and light-dark box tests) variations of motor activity were still more limited, and the sickness-related behaviours were not apparent. In effect, as observed by other investigators (Dantzer et al., 1996), LPS induced signs of sickness, but the expression of this profile was influenced by the context in which animals were tested. In the presence of comfortable home-cage cues, animals have the luxury of adopting the sickness profile. These behaviours may be of adaptive significance, in that they minimize energy expenditure and act to conserve body heat, thus enhancing immune functioning and limiting the effects of pathogens (Dantzer et al., 1996; Maier & Watkins, 1998). However, in novel, somewhat threatening environment, it may be maladaptive to adopt these behavioural styles, and instead animals engage in exploration.

In addition to promoting illness, the bacterial endotoxin elicited anxiogenic-like effects in both the light-dark box and the plus-maze tests. Indeed, treatment with LPS, at doses as low as 1.0 μg , reduced the time mice remained in the brightly illuminated portion of the light-dark box (e.g., a pattern believed to reflect anxiety), decreased the total number of transitions made between the dark and light compartments of the apparatus, and diminished the number of stretch-attend postures emitted. These effects were not likely a result of reduced motor activity, as animals placed in the illuminated portion of the test apparatus quickly left this area and remained in the darkened portion of the test chamber. In agreement with the effects observed in the light-dark box, LPS (5.0 μg) reduced both the time mice spent on the open arms of an elevated plus-maze and the total number of open-arm entries emitted. In contrast, closed-arm entries were not affected by the endotoxin, again suggesting that the effects of LPS were not attributable to motoric factors. Finally, while LPS-treated animals did not exhibit a neophobia in terms of the latency to approach and make contact with a novel stimulus object, these animals exhibited reduced contact time with this object. It is certainly possible that this reflected, in part, the reduced motor activity displayed by the LPS-treated animals. Yet, the congruence of effects of LPS in three different test situations, all of which are associated with anxiety, supports the notion that the endotoxin induces an anxiogenic-like response.

Although LPS and IL-1 β elicit common neuroendocrine effects, the actions of these compounds were reported to be behaviourally distinguishable from one another, in that they differentially influenced responding for rewarding brain stimulation (see Anisman & Merali, 1998; Borowski et al., 1997). Yet, the present findings suggest that LPS induces an

anxiogenic effect, just as IL-1 β was reported to provoke such an outcome (Anisman & Merali, 1998; Lacosta et al., 1996). However, the reliability of the IL-1 β effect were less pronounced than that associated with LPS administration. Inasmuch as LPS promotes the secretion of IL-1 β and TNF- α , both of which induce modest anxiogenic actions (Connor et al., 1998), the more pronounced effects of LPS are not unexpected. It remains to be determined whether IL-1 β and TNF- α secretion provoked by LPS act in an additive fashion or in a synergistic manner (Brebner et al., 1998; Plata-Salaman, 1995).

Neuroendocrine Correlates of LPS Administration

The proposition that LPS elicits anxiety is consistent with the finding that the endotoxin induces marked CRH secretion (Kakucska et al., 1993; Rivier, 1993), which is known to promote anxiety (Gray, 1991; Heilig et al., 1994). Of course, as in the case of the IL-1 β studies, it is unclear from the present findings whether the anxiety elicited by LPS was a direct result of changes in CRH or was secondary to the illness attributable to the endotoxin treatment. It might be expected, after all, that animals made ill by LPS would exhibit increased anxiety, particularly when placed in a novel, somewhat threatening situation. Although the anxiogenic-like effects of LPS were evident at low doses tested, signs of illness were also apparent. At this juncture it is necessary to determine whether a sub-illness dosage likewise provokes anxiety, and whether such an effect would be attenuated by either IL-1ra (implicating a role for IL-1) or a CRH antagonist. Likewise, it is essential to ascertain whether a metabolic stressor that does not influence immune functioning would engender anxiety.

As previously observed (Rivier, 1993), LPS increased HPA functioning, as reflected by altered plasma ACTH and corticosterone concentrations. This effect was evident even at the lowest dose of LPS (0.5 μ g). Inasmuch as the anxiogenic effects of LPS in the light-dark box and elevated plus-maze were not evident until higher doses of the endotoxin were used (1.0 and 5.0 μ g, respectively), it is tempting to suggest that the HPA changes and the anxiogenic actions observed were independent of one another. Yet, ACTH and corticosterone levels were only determined 2 hr following LPS treatment, and it is conceivable that the dose response function varied in a time-dependent fashion. Furthermore, the hormonal variations do not necessarily speak to the dosage- and time-dependent effects of LPS on CRH release, which may be more important in the elucidation of an anxiogenic response.

Neurotransmitter Correlates of LPS Administration

Several investigators reported that LPS influenced the activity of biogenic amines in both post-mortem brain (Dunn, 1992; Dunn & Welch, 1991; Masana et al., 1990; Mefford et al., 1991), as well as *in vivo* samples (Lavicky & Dunn, 1995; Linthorst et al., 1995). The present results are commensurate with these findings, in that systemic LPS administration increased NE turnover in the PVN, median eminence plus arcuate nucleus, hippocampus, and locus coeruleus. It is significant that stressful events likewise promote NE variations within these brain regions, and NE changes within the locus coeruleus have been implicated in the provocation of anxiety-like responses (Butler et al., 1990; Weiss, 1991). While not dismissing a role for CRH in the elicitation of the anxiogenic actions of LPS, these findings raise the possibility that alterations of NE within the locus coeruleus might also play a role

in this respect. As well, since NE variations may contribute to the PVN CRH changes elicited by LPS (Dunn, 1995; Pacak et al., 1995), increased activity of hypothalamic NE neurons have also been implicated in anxiogenesis.

It has been reported that both immune activation and treatment with IL-1 β influence 5-HT turnover at the medial hypothalamus and medial prefrontal cortex (Lavicky & Dunn, 1995). The results of the present investigation similarly indicate that systemic LPS enhanced 5-HT levels and turnover within specific hypothalamic nuclei, namely the PVN and the median eminence plus arcuate nucleus, as well as within the hippocampus. At this juncture it is premature to suggest that the 5-HT alterations associated with the LPS treatment contributed to the observed anxiogenic actions. However, it has been shown that 5-HT alterations are readily induced by different types of stressors in several brain regions (Adell et al., 1997; Singewald et al., 1997; Vahabzadeh & Fillenz, 1994), and might thus contribute to behavioural changes exerted by environmental insults.

The effects of LPS were not limited to NE and 5-HT variations. Specifically, LPS stimulated increased DA turnover in the PVN and median eminence plus arcuate nucleus. As well, increased DOPAC accumulation was evident in the nucleus accumbens at the 5.0 μ g dosage, although a comparable elevation was not evident at a higher dose. While these DA changes are reminiscent of those provoked by stressors, systemic LPS administration was without effect on DA activity within the medial prefrontal cortex, a region in which psychogenic and neurogenic stressors reliably induce such an effect (Anisman et al., 1993). Thus, as in the case of the previously discussed IL-1 β findings, the LPS data suggest a differentiation between the effects of systemic and psychogenic/neurogenic stressors. Yet,

the absence of monoamine changes in post-mortem tissues may not be an adequate index of transmitter activity, and the variations of DA changes elicited by LPS or interleukins may be detected more readily through *in vivo* microdialysis (Anisman & Merali, 1998; Borowski et al., 1997; Merali et al., 1997). Accordingly, it may be premature to conclude that LPS was entirely without effect on mesocorticolimbic DA activity. Furthermore, in the present investigation, monoamine variations were assessed following a limited number of LPS doses, and it is conceivable that this endotoxin has dose-dependent (and possibly time-dependent) biphasic effects on DA activity, as do cytokines such as IL-2 (Alonso et al., 1993; Hanisch et al., 1993; Petitto et al., 1997).

Time-Dependent Effects of SRBC on *In Vivo* Mesolimbic DA Levels

As mentioned earlier, it has been suggested that, among other things, the immune system acts as a sensory organ to inform the brain of immunologic challenge (Blalock, 1984), and may interpret such signals as if they were stressors (Dunn et al., 1987, 1991; Dunn & Welch, 1991). It is particularly significant that inoculation with SRBC gives rise to variations of NE within the hypothalamus and that such effects coincided with an immune response being mounted (Besedovsky et al., 1983; Carlson et al., 1987; Zalcman et al., 1991). As the hypothalamus is part of a regulatory loop comprising immune, endocrine and sympathetic processes (Dantzer & Kelley, 1989; Felten et al., 1991; Roszman & Brooks, 1992), the finding that immune alterations affect hypothalamic neurotransmitters is not entirely unexpected. However, as already seen, the central transmitter changes engendered by immunologic challenge, such as bacterial endotoxins, are not restricted to the

hypothalamus. Moreover, there is evidence as discussed earlier, indicating that SRBC inoculation influences hippocampal NE and 5-HT, as well as DA variations in several brain regions. Interestingly, like the effects of stressors on DA activity, regional specificity has been reported concerning the DA alterations associated with immunologic challenge. In particular, both SRBC and stressor exposure, have been shown to profoundly influence mesocortical DA activity, have less pronounced effects on DA activity within the nucleus accumbens, and have little effects on nigrostriatal DA activity (Anisman et al., 1991; Zalcman et al., 1991).

The aforementioned studies involved amine variations in post-mortem tissue and as already indicated, such measures may not provide an accurate index of neuronal activity. Thus, we assessed the effects of systemic SRBC administration (a treatment which is devoid of illness-inducing effects) on extracellular DA levels within the nucleus accumbens. Consistent with the proposition that SRBC would influence central transmitter functioning, and particularly DA within the accumbens (a stress responsive region), antigenic challenge effectively increased accumbal DA efflux. It is of particular significance that the maximal increase of DA, which reflected neuronal release of the amine, coincided with the peak immune response. Over days following SRBC administration, levels of extracellular DA increased with peak effects occurring 4 days after antigenic challenge. Accordingly, it is tempting to argue that the amine alterations were secondary to immune activation. Parenthetically, given that animals were not handled or otherwise disturbed during the post-inoculation period, the DA alterations could not be attributed to stressor-related extraneous factors.

The changes of extracellular DA appeared to be unrelated to the potential effects of the chronic presence of the probe within the brain, as the DA levels in saline-treated animals were stable over the 7-day period. This is concordant with the findings of Georgieva et al. (1993). In contrast, no significant alterations were seen in the extracellular accumbal DA levels of control animals over the 6 days of sampling. However, DOPAC and HVA levels in both SRBC and saline-treated rats were found to decline over days. These results are generally in agreement with previous studies where repeated microdialysis was used (Georgieva et al., 1993; Robinson & Camp, 1991; Westerink & Tuinte, 1986). The decrease in DOPAC concentrations was considered to reflect either a loss of DA fibers or a build up of barrier(s) around the probe (Robinson & Camp, 1991; Westerink & Tuinte, 1986). It was demonstrated, however, that changes of tyrosine hydroxylase fiber density in the vicinity of the probe were minimal, indicating that the decreased DOPAC was not related to neuronal damage (Georgieva et al., 1993). In any case, these factors should not affect the conclusions of the present study, since we used a within subject design with a parallel control group, thus ensuring that the factors associated with chronic sampling are concomitantly monitored in the control condition.

There have been reports that SRBC inoculation gives rise to serotonergic alterations within the hippocampus (Carlson et al., 1987), while IL-1 β provoked alterations of 5-HT within the hypothalamus (Dunn, 1988; Mefford & Heyes, 1990). In the present investigation, there was no indication of SRBC-induced changes of extracellular 5-HT, or its metabolite, 5-HIAA. To be sure, the nucleus accumbens is not rich in 5-HT neurons, and it might not be surprising to find that 5-HT variations would be less notable than in regions such as the

hippocampus or hypothalamus. It is significant, however, that unlike the effects of SRBC, systemic administration of IL-1 β hardly influenced DA activity within the nucleus accumbens, and 5-HT activity was reliably altered by the cytokine treatment. Clearly, SRBC and IL-1 β lead to very different outcomes, making it likely that IL-1 β is probably not involved in mediating the effects of the antigenic challenge.

Conclusions

Just as central neurochemical and neuroendocrine alterations may affect immune functioning, the present investigation revealed that immune activation or products of an activated immune system (e.g., IL-1 β , IL-2), may promote alterations of central nervous system processes. In this respect, we have argued that the immune system may act like a sensory organ, informing the brain of environmental challenge. Moreover, in some respects, the neurochemical effects of stressors and immune/cytokine challenge are similar, supporting the view that the brain may interpret cytokine signals as if they were stressors.

In view of the neurochemical alterations engendered by immune/cytokine challenge, it was of interest to examine the behavioural effects of these treatments. This endeavor was also prompted by the numerous recent reports indicating that depressive illness and schizophrenia were both associated with increases of IL-1 β , IL-2, sIL-2R, IL-6, and sIL6R (Maes, 1998; Sluzewska et al., 1998), and that IL-2 immunotherapy in humans was frequently associated with psychiatric disturbances. Essentially, the possibility was considered that cytokines, by virtue of their effects on hypothalamic-pituitary-adrenal functioning, as well as on hypothalamic and limbic transmitter activity, may actually play

a provocative role in depression and/or schizophrenia. In fact, there is considerable evidence to suggest that depressive illness involves some of the same neurochemical processes as those elicited by stressors and cytokine challenge. For instance, major depressive disorder is characterized by HPA alterations, including variations in the circadian rhythm or levels of plasma ACTH and cortisol, non-suppression of cortisol release following dexamethasone challenge, and a blunted ACTH response to CRH challenge (Holsboer, 1995; Nemeroff, 1996). Further to the same issue, IL-1 β (as well as other cytokines) potently stimulates CRH release, which has also been shown to induce an anxiogenic effect. Although it has previously been found in our laboratory that IL-1 β administration did not produce an anhedonic effect, both this cytokine and administration of LPS elicited anxiogenic-like actions. Yet, the animal's ability to attend to environmental stimuli appeared to be unimpaired by this treatment. The behavioural changes exerted by IL-1 β were accompanied by marked neuroendocrine alterations, as well as several central neurochemical variations, most prominently, NE activity within the PVN, median eminence plus arcuate nucleus, locus coeruleus and prefrontal cortex, as well as 5-HT alterations within the hippocampus. It will be recognized that some of these changes have also been implicated in depressive disorders (or in animal models of depression), and thus it ought to be considered that cytokines may play a fundamental role in the provocation of affective illnesses.

A second aim of the present investigation was predicated on human studies which revealed that when administered chronically, IL-2 may induce cognitive disturbances, characterized by spatial learning deficits (Caraceni, et al., 1992; Denicoff, 1987; Meyers & Valentine, 1995). Commensurate with these findings, it was observed that while acute IL-2

was without effect, repeated administration of this cytokine provoked marked disturbances of exploratory behaviours and disrupted performance in a Morris water-maze test. A similar outcome was not elicited by acute administration of this cytokine. While acutely administered IL-2 has been shown to elicit anhedonic effects, unlike IL-1 β , this cytokine did not engender an anxiogenic-like action or influence exploratory profiles. At this juncture, it can be concluded that chronic IL-2 impairs the spatial learning ability of animals, although the underlying mechanisms can not yet be identified given that this treatment elicited widespread amine variations, and has also been shown to influence hippocampal ACh function.

Although cytokines were long considered within the domain of immune system functioning, recent evidence has pointed to the profound immune-brain interactions that exist and have indicated that immune alterations may come to affect neuroendocrine processes. The present results not only support such findings, but make it clear that much more attention needs to be devoted to the behavioural ramifications associated with immunological disturbances and immunotherapy. This is particularly the case given that the effectiveness of immunotherapy is limited by the potential neuropsychiatric and cognitive sequelae of the treatment.

6. References

- Abercrombie, E.D., Keefe, K.A., DiFrischia, D.S. and Zigmond, M.J. (1989). Differential effects of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. **J. Neurochem.** **52**, 1655-1658.
- Adell, A., Casanovas, J.M., Artigas, F. (1997). Comparative study in the rat of the actions of different types of stress on the release of 5-HT in raphe nuclei and forebrain areas. **Neuropharmacol.** **36**, 735-741.
- Alonso, R., Chaudieu, I., Diorio, J., Krishnamurthy, A., Quirion, R., Boksa, P. (1993). Interleukin-2 modulates evoked release of [³H]dopamine in rat cultured mesencephalic cells. **J. Neurochem.** **61**, 1284-1290.
- Andreis, P.G., Neri, G., Belloni, A.S., Mazzocchi, G., Kasprzak, A., Nussdorfer, G.G. (1991). Interleukin-1 β enhances corticosterone secretion by acting directly on the rat adrenal gland. **Endocrinol.** **129**, 53-57.
- Anisman, H., Kokkinidis, L., Borowski, T., Merali, Z. (1997). Differential effects of interleukin (IL)-1, IL-2 and IL-6 on responding for rewarding lateral hypothalamic stimulation. **Brain Res.** **779**, 177-187.
- Anisman, H., Kokkinidis, L., Merali, Z. (1996). Influence of interleukin-2 on responding for electrical brain stimulation from the hypothalamus. **Brain Res.** **731**, 1-11.
- Anisman, H., Merali, Z. (1998). Anhedonic and anxiogenic effects of cytokine exposure. In: R., Dantzer, E.E., Wollmann, R., Yirmiya (Eds.), **Cytokines, Stress and Depression**. New York: Plenum Press. In press.
- Anisman, H., Ravindran, A.V., Griffiths, J., Merali, Z. (1998). Behavioural, endocrine and cytokine correlates of major depression and dysthymia with typical or atypical features. **Molec. Psychiat.** In press.
- Anisman, H., Shanks, N., Zalcman, S., Zacharko, R.M. (1991). Multisystem regulation of performance deficits induced by stressors: an animal model of depression. In: M.T., Martin-Iverson, (Ed.), **Animal Models in Psychiatry. II.** (pp. 1-55). New Jersey: Humana Press.
- Anisman, H., Zalcman, S., Shanks, N., Zacharko, R.M. (1991). Multisystem regulation of performance deficits induced by stressors: An animal model of depression. In: A., Boulton, G., Baker, M., Martin-Iverson (Eds.), **Neuromethods, vol. 19: Animal Models of Psychiatry.** **11.** (pp. 1-59). New Jersey: Humana Press.

- Anisman, H., Zalcman, S., Zacharko, R.M. (1993). The impact of stressors on immune and central neurotransmitter activity: Bidirectional communication. **Rev. Neurosci.** **4**, 147-180.
- Araujo, B.M., Lapchak, P.A., Collier, B., Quirion, R. (1989). Localization of interleukin-2 immunoreactivity and interleukin-2 receptors in the rat brain: interaction with the cholinergic system. **Brain Res.** **498**, 257-266.
- Ban, E., Haour, F., Lenstra, R. (1992). Brain interleukin 1 gene expression induced by peripheral lipopolysaccharide administration. **Cytokine** **4**, 48-54.
- Ban, E., Marquette, C., Sarrieu, A., Fitzpatrick, F., Fillion, G., Milon, G., Rostene, W., Haour, F. (1993). Regulation of interleukin-1 receptor expression in mouse brain and pituitary by lipopolysaccharide and glucocorticoids. **Neuroendocrinol.** **58**, 581-587.
- Banks, W.A., Kastin, A.J. (1992). The interleukins-1 α , -1 β , and -2 do not acutely disrupt the murine blood-brain barrier. **Int. J. Immunopharmacol.** **14**, 629-636.
- Banks, W.A., Kastin, A.J., Durham, D.A. (1989). Bidirectional transport of interleukin-1 α across the blood-brain barrier. **Brain Res. Bull.** **23**, 433-437.
- Bansil, S., Troiano, R., Cook, S.D., Rohowsky-Kochan, C. (1991). Serum soluble interleukin-2 receptor levels in chronic progressive, stable and steroid-treated multiple sclerosis. **Acta. Neurol. Scand.** **84**, 282-285.
- Bartanusz, V., Jezova, D., Bertini, L.T., Tilders, F.J., Aubry, J.M., Kiss, J.Z. (1993). Stress-induced increase in vasopressin and corticotropin-releasing factor expression in hypophysiotropic paraventricular neurons. **Endocrinol.** **132**, 895-902.
- Bartholomew, S.A., Hoffman, S.A. (1993). Effects of peripheral cytokine injections on multiple unit activity in the anterior hypothalamic area of the mouse. **Brain Behav. Immun.** **7**, 301-316.
- Benveniste, H. (1987). Brain microdialysis. **J. Neurochem.** **49**, 729-734.
- Benveniste, E.N. (1992). Inflammatory cytokines within the central nervous system: sources, functions and mechanisms of action. **Am. J. Physiol.** **263**, C1-C16.
- Benveniste, E.N., Merrill, J.E. (1986). Stimulation of oligodendroglial proliferation and maturation by interleukin-2. **Nature** **321**, 610-613.

- Berkenbosch, F., de Rijk, R., del Rey, A., Besedovsky, H. (1990). Neuroendocrinology of Interleukin-1. In: J.C., Porter, D., Jezova. (Eds.), **Circulating Regulatory Factors and Neuroendocrine Function**. (pp. 303-314). New York: Plenum Press.
- Berkenbosch, F., van Oers, J., del Rey, A., Tilders, F., Besedovsky, H. (1987). Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. **Science** **238**, 524526.
- Berkenbosch, F., Wolvers, D.A.W., de Rijk, R. (1991). Neuroendocrine and immunological mechanisms in stress-induced immunomodulation. **J. Steroid Biochem. Molec. Biol.** **40**, 639-647.
- Bertini, R., Bianchi, M., Ghezzi, P. (1988). Adrenalectomy sensitizes mice to the lethal effects of interleukin-1 and tumor necrosis factor. **J. Exp. Med.** **167**, 1708-1712.
- Besedovsky, H., del Rey, A. (1991). Physiological implications of the immune-neuroendocrine network. In: R., Ader, D. L., Felten, N., Cohen. (Eds.), **Psychoneuroimmunol.** (pp. 589-608). San Diego: Academic Press.
- Besedovsky, H., del Rey, A., Sorkin, E., Da Prada, M., Burri, M., Honegger, C. (1983). The immune response evokes changes in brain noradrenergic neurons. **Science** **221**, 564-566.
- Besedovsky, H., del Rey, A., Sorkin, E., Dinarello, C.A. (1986). Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. **Science** **233**, 652-654.
- Besedovsky, H., Sorkin, E., Felix, D., Haas, H. (1977). Hypothalamic changes during the immune response. **Eur. J. Immunol.** **7**, 325-328.
- Besedovsky, H., Sorkin, E., Keller, M., Muller, J. (1975). Changes in blood hormone levels during the immune response. **Proc. Soc. Exp. Biol. Med.** **150**, 446-470.
- Bignami, G., Michalek, H. (1978). Cholinergic mechanism and aversively motivated behaviours. In: Anisman, H., Bignami, G. (Eds.), **Psychopharmacology of Aversively Motivated Behaviour**. (pp 173-256). New York: Plenum.
- Bindoni, M., Perciavalle, V., Beretta, S., Belluardo, N., Diamantstein, T. (1988). Interleukin 2 modifies the bioelectric activity of some neurosecretory nuclei in the rat hypothalamus. **Brain Res.** **462**, 10-14.
- Blalock, J.E., (1984). The immune system as a sensory organ. **J. Immunol.** **132**, 1067-1070.

- Blalock, J.E. (1994). The syntax of immune-endocrine communication. **Immun. Today**, **15**, 504-544.
- Blatteis, C.M. (1992). Role of the OVLT in the febrile response to circulating pyrogens. In: A., Ernisch, R., Landgraf, H.J., Ruhle (Eds.), **Progress in Brain Research**. vol. 91, (pp. 409-415). Amsterdam: Elsevier
- Bluthe, R.M., Dantzer, R., Kelley, K.W. (1989). CRF is not involved in the behavioural effects of peripherally injected interleukin-1 in the rat. **Neurosci. Res. Commun.** **5**, 149-154.
- Bluthe, R.M., Dantzer, R., Kelley, K.W. (1992). Effects of interleukin-1 receptor antagonist on the behavioural effects of lipopolysaccharide in the rat. **Brain Res.** **573**, 318-320.
- Bluthe, R.M., Walter, V., Parnet, P., Laye, S., Lestage, K., Verrier, D., Poole, S., Stenning, B.E., Kelley, K.W., Dantzer, R. (1994). Lipopolysaccharide induces sickness behaviour in rats by a vagal mediated mechanism. **C.R. Acad. Sci. (Paris)** **317**, 499-503.
- Borowski, T., Kokkinidis, L., Merali, Z., Anisman, H. (1997). Behavioural and central monoamine alterations induced by interleukin-1, interleukin-6 and lipopolysaccharide. **Soc. Neurosci. Abstr.** **23**, 1508.
- Boumpas, D.T., Anastassiou, E.D., Older, S.A., Tsokos, G.C., Nelson, D.L., Balow, J.E. (1991). Dexamethasone inhibits human interleukin 2 but not interleukin 2 receptor gene expression in vitro at the level of nuclear transcription. **J. Clin. Invest.** **87**, 1739-1747.
- Brebner, K., Hayley, S., Lacosta, S., Zacharko, R., Merali, Z., Anisman, H. (1998). Synergistic actions of interleukin (IL)-1 β , IL-6 and TNF- α : central neurochemical, neuroendocrine and behavioural alterations. **Soc. Neurosci. Abstr.** **24**, 2075.
- Breder, C.D., Dinarello, C.A., Saper, C.B. (1988). Interleukin-1 immunoreactive innervation of the human hypothalamus. **Science** **240**, 321-324.
- Bret-Dibat, J., Bluthe, R.M., Kent, S., Kelley, K.W., Dantzer, R. (1995). Lipopolysaccharide and interleukin-1 depress food-motivated behaviour in mice by a vagal-mediated mechanism. **Brain, Behav. Immun.** **9**, 242-246.
- Brown, S.L., Smith, L.R., Blalock, J.E. (1987). Interleukin-1 and interleukin-2 enhance proopiomelanocortin gene expression in pituitary cells. **J. Immunol.** **139**, 3181-3183.

- Brunetti, L., Preziosi, P., Ragazzoni, E., Vacca, M. (1993). Involvement of nitric oxide in basal and interleukin-1 β -induced CRH and ACTH release in vitro. **Life Sci.** **53**, 219-222.
- Brunetti, L., Volpe, A.R., Tagazzoni, E., Preziosi, P., Vacca, M. (1996). Interleukin-1 β -induced specifically stimulates nitric oxide production in the hypothalamus. **Life Sci.** **58**, 373-377.
- Butler, D., Weiss, J.M., Stout, J.C., Nemeroff, C.B. (1990). Corticotropin-releasing factor produces fear-enhancing and behavioural activating effects following infusion into the locus coeruleus. **J. Neurosci.** **10**, 176-183.
- Buttini, M., Boddeke, H. (1995). Peripheral lipopolysaccharide stimulation induces interleukin-1 β messenger RNA in rat brain microglial cells. **Neuroscience** **65**, 523-530.
- Cambronero, J.C., Rivas, F.J., Borell, J., Guaza, C. (1992). Interleukin-2 induces corticotropin-releasing hormone release from superfused rat hypothalami: Influence of glucocorticoids. **Endocrinol.** **131**, 677-683.
- Capuron, L., Ravaut, A., Radat, F., Dantzer, R., Goodall, G. (1998). Affects of interleukin-2 and alpha-interferon cytokine immunotherapy on the mood and cognitive performance of cancer patients. **Neuroimmunomod.** **5**, 9.
- Caraceni, A., Martini, C., Belli, F., Mascheroni, L., Rivoltini, L., Arienti, F., Cascinelli, N. (1992). Neuropsychological and neurophysiological assessment of the central effects of interleukin-2 administration. **Euro. J. Cancer**, **29A**, 1266-1269.
- Carlson, S.L., Felten, D.L., Livnat, S., Felten, S.Y. (1987). Alterations of monoamines in specific central autonomic nuclei following immunization in mice. **Brain Behav. Immunol.** **1**, 52-63.
- Carmelia, G., Pietro, G., De Simoni, M.G. (1991). Activation of the hypothalamic serotonergic system by central interleukin-1. **Eur. J. Pharmacol.** **209**, 139-140.
- Chesnokova, V., Busick, J., Chesnokov, V., Jakobson, G., Ivanova, L. (1997). Different effects of IL-2 in vivo treatment and emotional stress on POMC gene expression in the pituitary of mice. **Horm. Metab. Res.** **29**, 88-89.
- Chiueh, C.C., Zukowska-Grojec, Z., Kirk, K.L., Kopin, J.J. (1983). 6-fluorocatecholamine as a false adrenergic neurotransmitter. **J. Pharm. Exp. Therap.** **225**, 529-533.

- Chuluyan, H., Saphier, D., Rohn, W., Dunn, A.J. (1992). Noradrenergic innervation of the hypothalamus participates in the adrenocortical responses to interleukin-1. **Neuroendocrinol.** **56**, 106-111.
- Coceani, F., Lees, J., Dinarello, C.A. (1988). Occurrence of interleukin-1 in cerebrospinal fluid of the conscious cat. **Brain Res.** **446**, 245-250.
- Coehlo, M.M., Souze, G.E., Pela, I.R. (1992). Endotoxin-induced fever is modulated by endogenous glucocorticoids in rats. **Am. J. Physiol.** **263**, R423-R427.
- Connor, T.J., Song, C., Leonard, B.E., Merali, Z., Anisman, H. (1998). Comparative effects of interleukin-1 β , -2, -6, and tumor necrosis factor- α administration on some behavioural, neurochemical, endocrine and immune parameters. **Neuroscience** **84**, 923-933.
- Costa, A., Trainer, P., Besser, M., Grossman, A. (1993). Nitric oxide modulates the release of corticotropin-releasing hormone from the rat hypothalamus in vitro. **Brain Res.** **605**, 187-192.
- Cunningham, E.T., De Souza, E.B. (1993). Interleukin-1 receptors in the brain and endocrine tissues. **Immunol. Today** **14**, 171-176.
- Cunningham, E.T., Sawchenko, P.E. (1988). Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. **J. Comp. Neurol.** **274**, 60-76.
- Cunningham, E.T., Wada, E., Carter, D.B., Tracey, D.E., Battey, J.F., De Souza, E.B. (1992). In situ histochemical localization of type I interleukin-1 receptor messenger RNA in the central nervous system, pituitary, and adrenal gland of the mouse. **J. Neurosci.** **12**, 1101-1114.
- Dantzer, R., Bluthé, R.M., Aubert, A., Goodall, G., Bret-Dibat, J-L., Kent, S., Goujon, E., Laye, S., Parnet, P., Kelley, K.W. (1996). Cytokine actions on behaviour. In: N.J., Rothwell (Ed.), **Cytokines and the Nervous System**. (pp. 117-144). London: Landes.
- Dantzer, R., Kelley, K.W. (1989). Stress and immunity: An integrated view of relationships between the brain and the immune system. **Life Sci.** **44**, 1995-2008.
- Dawson, T.M., Snyder, S.H. (1994). Gases as biological messengers: Nitric oxide and carbon monoxide in the brain. **J. Neurosci.** **14**, 5147-5159.
- Deakin, J.F.W., Graeff, F.G. and Guimaraes, F.S. (1993). Microdialysis studies with 5-HT reuptake inhibitors. Reply. **Trends Pharmacol. Sci.** **14**, 398.

- Denicoff, K.D., Rubinow, D.R., Papa, M.Z., Simpson, L., Seipp, L.A., Lotze, M.T., Change, A.E., Rosenstein, D., Rosenberg, S.A. (1987). The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. **Ann. Int. Med.** **107**, 293-300.
- Denicoff, K.D., Durkin, T.M., Lotze, M.T., Quinlan, P.E., Davis, C.L., Listwak, S.J., Rosenberg, S.A., Rubinow, D.R. (1989). The neuroendocrine effects of interleukin-2 treatment. **J. Clin. Endocr. Metab.** **69**, 402-410.
- De Sarro, G.B., Ascioti, M.G., Audino, V., Rispoli, V., Nistico, G. (1989). Behavioural and ECoG spectrum changes induced by intracerebral microinfusion of interferons and interleukin-2 in rats are antagonized by naloxone. In: J., Hadden, K., Masek, G. Nistico. (Eds.), **Interactions among Central Nervous System, Neuroendocrine and Immune Systems**. (pp. 351-356). Milan: Pythagora Press.
- De Sarro, G.B., Masuda, Y., Ascioti, C., Audino, M.G. Nistico, G. (1990). Behavioural and ECoG spectrum changes induced by intracerebral infusion of interferons and interleukin 2 in rats are antagonized by naloxone. **Neuropharmacol.** **29**, 167-179.
- De Simoni, M.G., Del Bo, R., De Luigi, A., Simard, S., Forloni, G. (1995). Central endotoxin induces different patterns of interleukin (IL)-1 β and IL-6 messenger ribonucleic acid expression and IL-6 secretion in the brain and periphery. **Endocrinol.** **136**, 897-902.
- Derijk, R., Paul, J.L., Strijbos, M., Van Rooijen, N., Rothwell, N., Berkenbosch, F. (1993). Fever and thermogenesis in response to bacterial endotoxin involve macrophage-dependent mechanisms in rats. **Am. J. Physiol.** **265**, R1179-R1183.
- Derijk, R., Van Rooijen, N., Tilders, F.J., Besedovsky, H.O., Del Rey, A., Berkenbosch, F. (1991). Selective depletion of macrophages prevents pituitary-adrenal activation in response to subpyrogenic, but not to pyrogenic, doses of bacterial endotoxin in rats. **Endocrinol.** **129**, 330-338.
- Deutch, A.Y., Roth, R.H. (1990). The determinants of stress-induced activation of the prefrontal cortical dopamine system. In: H., Uylings, C., Van Eden, J., De Bruin, M., Corner, M., Feenstra. (Eds.), **Progress in Brain Res.** vol. 85, (pp. 367-403). New York: Elsevier.
- Deutch, A.Y., Tam, S-Y. and Roth, R.H. (1985). Footshock and conditioned stress increase 3,4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. **Brain Res.** **333**, 143-146.
- Dinareello, C.A. (1984a). Interleukin-1. **Rev. Infect. Dis.** **6**, 51-95.

- Dinareлло, C.A. (1984b). Interleukin-1 and the pathogenesis of the acute-phase response. **N. Engl. J. Med.** **311**, 1413-1418.
- Dinareлло, C.A. (1988). Biology of interleukin 1. **FASEB J.** **2**, 108-115.
- Dinareлло, C.A., Cannon, J.G., Wolff, S.M., Bernheim, H.A., Beutler, B., Cerami, A., Figari, I.S., Palladino, M.A., O'Connor, J.V. (1986). Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces the production of interleukin-1. **J. Exp. Med.** **163**, 1433-1450.
- Dinareлло, C.A., Wolff, S.M. (1993). The role of interleukin-1 in disease. **New Engl. J. Med.** **328**, 106-113.
- Dunn, A.J. (1988). Systemic interleukin-1 administration stimulates hypothalamic norepinephrine metabolism paralleling the increased plasma corticosterone. **Life Sci.** **43**, 429-435.
- Dunn, A.J. (1990). Interleukin-1 as a stimulator of hormone secretion. **Prog. NeuroEndocrinImmunol.** **3**, 26-34.
- Dunn, A.J. (1992). Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: comparison with interleukin-1. **J. Pharmacol. Expt. Therapeut.** **261**, 964-969.
- Dunn, A.J. (1993). Nitric oxide synthase inhibitors prevent the cerebral tryptophan and serotonergic responses to endotoxin and interleukin-1. **Neurosci. Res. Comm.** **13**, 149-156.
- Dunn, A.J. (1995). Interactions between the nervous system and immune system. In: F.E., Bloom, D.J., Kupfer. (Eds.), **Psychopharmacology, 4th Generation of Progress.** (pp.719-731). New York: Raven Press.
- Dunn, A.J., Antoon, M., Chapman, Y. (1991). Reduction of exploratory behaviour by intraperitoneal injection of interleukin-1 involves brain corticotropin-releasing factor. **Brain Res. Bull.** **26**, 539-542.
- Dunn, A.J., Chapman, Y., Antoon, M. (1992). Endotoxin-induced behavioural changes of mice in the multicompartiment chamber are distinct from those of interleukin-1. **Neurosci. Res. Comm.** **10**, 63-69.
- Dunn, A.J., Chuluyan, H.E., (1992). The role of cyclo-oxygenase and lipoxygenase in the interleukin-1-induced activation of the HPA axis: dependence on the route of injection. **Life Sci.** **51**, 219-225.

- Dunn, A.J., Powell, M.L., Meitin, C., Small, P.A. (1989). Virus infection as a stressor: Influenza virus elevates plasma corticosterone and brain concentrations of MHPG and tryptophan. **Physiol. Behav.** **45**, 591-594.
- Dunn, A.J., Powell, M.L., Moreshead, W.V., Gaskin, J.M., Hall, N.R. (1987). Effects of Newcastle disease virus administration to mice on the metabolism of cerebral biogenic amines, plasma corticosterone, and lymphocyte proliferation, **Brain Behav. Immun.** **1**, 216-230.
- Dunn, A.J., Vickers, S.L., (1994). Neurochemical and neuroendocrine responses to Newcastle disease virus administration in mice. **Brain Res.** **645**, 103-112.
- Dunn, A.J., Welch, J. (1991). Stress- and endotoxin-induced increases in brain tryptophan and serotonin metabolism depend on sympathetic nervous system activity. **J. Neurochem.** **57**, 1615-1622.
- Ebisui, O. Fukata, J., Murakami, N., Kobayashi, H., Segawa, H., Muro, S., Hanaoka, I., Naito, Y., Masui, Y., Ohmoto, Y., Imura, H., Nakao, K. (1994). Effect of IL-1 receptor antagonist and antiserum to TNF- α on LPS-induced plasma ACTH and corticosterone rise in rats. **Am. J. Physiol.** **266**, E986-E992.
- Eckman, P.L., King, W.M., Brunson, J.G. (1958). Studies on the blood brain barrier, I. Effects produced by a single injection of the gram-negative endotoxin influencing the permeability of the cerebral vessels. **Am. J. Pathol.** **34**, 631-639.
- Eisenberg, S.P., Evans, R.J., Arend, W.P., Verderber, E., Brewer, M.T., Hannum, C.H., Thompson, R.C. (1990). Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. **Nature** **343**, 341-346.
- Elenkov, I.J., Kovacs, K., Kiss, J., Bertok, L., Vizi, E.S. (1992). Lipopolysaccharide is able to bypass corticotropin-releasing factor in affecting plasma ACTH and corticosterone levels: evidence from rats with lesions of the paraventricular nucleus. **J. Endocrinol.** **133**, 231-236.
- Ellison, M., Krieg, R.J. Povlishock, J.T. (1990). Differential central nervous system responses following single and multiple recombinant IL-2 infusions. **J. Neuroimmunol.** **28**, 249-260.
- Ellison, M., Merchant, R.E. (1991). Appearance of cytokine-associated central nervous system myelin damage coincides temporally with serum tumor necrosis factor induction after recombinant interleukin-2 infusion in rats. **J. Neuroimmunol.** **33**, 245-251.

- Elmquist, J., Ackermann, M.R., Register, K.B., Rimler, R.B., Ross, L.R., Jacobson, C.D. (1993). Induction of Fos-like immunoreactivity in the rat brain following *Pasteurella multocida* endotoxin administration. **Endocrinol.** **133**, 3054-3057.
- Ericsson, A., Kovacs, K.J. Sawchenko, P.E. (1994). A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. **J. Neurosci.** **14**, 897-913.
- Farrar, W.L., Kilian, P.L., Ruff, M.R., Hill, J.M., Pert, C.B. (1987). Visualization and characterization of interleukin-1 receptors in brain. **J. Immunol.** **139**, 459-463.
- Felten, D.L., Cohen, N., Ader, R. & Felten, S.Y., Carlson, S.L., Roszman, T.L. (1991). Central neural circuits involved in neural-immune interactions. In: R., Ader, D.L. Felten, N., Cohen (Eds.), **Psychoneuroimmunology**. (pp. 3-25). San Diego: Academic Press.
- Fibiger, H.C. (1995). Neurobiology of depression: Focus on dopamine. In: G., Gessa, W., Fratta, L., Pani, G., Serra (Eds.), **Depression and Mania: From Neurobiology to Treatment**. (pp. 1-17). New York: Raven Press.
- Fibiger, H.C., Phillips, A.G. (1988) Mesocorticolimbic dopamine systems and reward. In: P.W., Kalivas, C.B., Nemeroff. (Eds.), **The mesocorticolimbic dopamine system**. (pp.206-215). New York: Acad. Sciences.
- File, S.E. (1996). Recent developments in anxiety, stress, and depression. **Pharm. Biochem. Behav.** **54**, 3-12.
- Fleshner, M., Goehler, L.E., Hermann, J., Relton, J.K., Maier, S.F., Watkins, L.R. (1995). Interleukin-1 β induced corticosterone elevation and hypothalamic NE depletion is centrally mediated. **Brain Res. Bull.** **37**, 605-610.
- Franklin, K.B.J., Paxinos, G. (1997). **The mouse brain in stereotaxic coordinates**. Academic Press, San Diego, CA.
- Fukata, J., Imura, H., Nakao, K. (1993). Cytokines as mediators in the regulation of the hypothalamic-pituitary-adrenocortical function. **J. Endocrinol. Invest.** **16**, 141-155.
- Gabellec, M-M., Griffais, R., Fillion, G., Haour, F. (1996). Interleukin-1 receptors type I and type II in the mouse brain: kinetics of mRNA expressions after peripheral administration of bacterial lipopolysaccharide. **J. Neuroimmunol.** **66**, 65-70.
- Gaillard, R.C. (1994). Neuroendocrine-immune system interactions. The immune-hypothalamo-pituitary-adrenal axis. **Trends Endocrinol. Metab.** **5**, 303-309.

- Ganguli, R., Brar, J.S., Chengappa, K.R., Deleo, M., Yang, Z.W., Shurin, G., Rabin, B. (1995). Mitogen-stimulated interleukin-2 production in never-medicated first episode schizophrenics - the influence of age of onset and negative symptoms. **Arch. Gen. Psychiat.** **52**, 668-672.
- Gardier, A.M., Kachaner, S., Shaghghi, E.K., Blot, C., Bohuon, C., Jacquot, C., Pallardy, M.J. (1994). Effects of a primary immune response to T-cell dependent antigen on serotonin metabolism in frontal cortex: in vivo microdialysis study in freely moving Fischer 344 rat. **Brain Res.** **645**, 150-156.
- Gatti, S., Bartfai, T. (1993). Induction of tumor necrosis factor- α mRNA in the brain after peripheral endotoxin treatment: Comparison with interleukin-1 family and interleukin-6. **Brain Res.** **624**, 291-294.
- Gaykema, R., Dijkstra, I., Tilgers, F. (1995). Subdiaphragmatic vagotomy suppresses endotoxin-induced activation of hypothalamic corticotropin-releasing hormone neurons and ACTH secretion. **Endocrinol.** **136**, 4717-4720.
- Gemma, C., Ghezzi, P., De Simoni, M.G. (1991). Activation of the hypothalamic serotonergic system by central interleukin-1. **Eur. J. Pharmacol.** **209**, 139-140.
- Georgieva, J., Luthman, J., Mohring, B., Magnusson, O. (1993). Tissue and microdialysate changes after repeated and permanent probe implantation in the striatum of freely moving rats. **Brain Res. Bull.** **31**, 663-470.
- Gillis, S., Grabtree, G.R., Smith, K., (1979). Glucocorticoid-induced inhibition of T cell growth factor production. I the effect of mitogen-induced lymphocyte proliferation. II. The effect of the in vitro generation of cytolytic T cells. **J. Immunol.** **123**, 1624-1638
- Goujon, E., Parnet, P., Aubert, A., Goodnall, G., Dantzer, R. (1995). Corticosterone regulates behavioural effects of lipopolysaccharide and interleukin-1 β in mice. **Am. J. Physiol.** **269**, R154-R159.
- Gray, T.S. (1991). Amygdala: Role in autonomic and neuroendocrine responses to stress. In: J.A., McCubbin, P.G., Kaufmann, C.B., Nemeroff. (Eds.), **Stress, Neuropeptides and Systemic Disease**. (pp. 37-53). New York: Academic Press.
- Hayley, S., Brebner, K., Staines, W., Merali, Z., Anisman, H. (1998). Sensitization effects of tumor necrosis factor- α : behavioral, neuroendocrine and neurochemical alterations. **Soc. Neurosci. Abstr.** **24**, 1861.

- Haour, F., Marquette, C., Ban, E., Crumeyrolle-Arias, M., Rostene, W., Tsiang, H., Fillion, G. (1995). Receptors for interleukin-1 in the central nervous and endocrine systems. **Ann. d'Endocrinol. (Paris)**, **56**, 173-179.
- Hashimoto, K., Nishioka, T., Tojo, C., Takao, T. (1995). Nitric oxide plays no role in ACTH release induced by interleukin-1 β , corticotropin-releasing hormone, arginine vasopressin and phorbol myristate acetate in rat pituitary cell cultures. **J. Endocrine**, **42**, 435-439.
- Hanisch, U.K., Neuhaus, J., Rowe, W., VanRossum, D., Moller, T., Kettenmann, H., Quirion, R. (1997). Neurotoxic consequences of central long-term administration of interleukin-2 in rats. **Neuroscience** **79**, 799-818.
- Hanisch, U.K., Quirion, R. (1996). Interleukin-2 as a neuroregulatory cytokine. **Brain. Res. Rev.** **21**, 246-284.
- Hanisch, U.K., Rowe, W., Sharma, S., Meaney, M.J., Quirion, R. (1994). Hypothalamic-pituitary-adrenal activity during chronic central administration of interleukin-2. **Endocrinol.** **135**, 2465-2472.
- Hanisch, U.K., Seto, D., Quirion, R. (1993). Modulation of hippocampal acetylcholine release: Potent central action of interleukin-2. **J. Neurosci.** **13**, 3368-3374.
- Hannum, C.H., Wilcox, C.J., Arend, W.P., Joslin, F.G., Dripps, D. J., Heimdahl, P.L., Armes, L.G., Sommer, A., Eisenberg, S.P., Thompson, R.C. (1990). Interleukin-1 receptor antagonist activity of a human IL-1 inhibitor. **Nature** **343**, 336-340.
- Haour, F., Ban, E., Milon, G., Baran, D., Fillion, G. (1990). Brain interleukin-1 receptors: characterization and modulation after lipopolysaccharide injection. **Prog. NeuroEndocrinImmunol.** **3**, 1-9.
- Harbuz, M.S. Stephanou, A., Knight, R.A., Chover-Gonzalez, A.J., Lightman, S.L. (1992). Action of interleukin-2 and interleukin-4 on CRF mRNA in the hypothalamus and POMC mRNA in the anterior pituitary. **Brain Behav. Immun.** **6**, 214-222.
- Hart, B.L. (1988). Biological basis of the behaviour of sick animals. **Neurosci. Biobehav. Rev.** **12**, 123-137.
- Heilig, M., Koob, G.F., Ekman, R., Britton, K.T. (1994). Corticotropin releasing factor and neuropeptide Y: role in emotional integration. **Trends Neurosci.** **17**, 80-84.

- Helerstein, M.K., Meydani, S.N., Meydani, M., Wu, K., Dinarello, C.A. (1989). Interleukin-1-induced anorexia in the rat. Influence of prostaglandins. **J. Clin. Invest.** **84**, 228-235.
- Herman, J.P., Cullinan, W.E. (1997). Neurocircuitry of stress: Central control of hypothalamo-pituitary-adrenocortical axis. **Trends Neurosci.** **20**, 78-84.
- Ho, B.T., Lu, J.G., Huo, Y.Y., Meyers, C.A., Tansey, L.W., Payne, R., Levin, V.A. (1994). Neurochemical basis of interleukin 2-modified discrimination behaviour. **Cytokine** **6**, 365-367.
- Hoffman, F.M., Von Hanwehr, R.I., Dinarello, C.A., Mizel, S.B., Hinton, D., Merrill, J.E. (1986). Immunoregulatory molecules and IL-2 receptors identified in multiple sclerosis brain. **J. Immunol.** **136**, 3239-3245.
- Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. **Pharm. Biochem. Behav.** **54**, 21-30.
- Holsboer, F. (1995). Neuroendocrinology of mood disorders. In: F.E., Bloom, D.J., Kuper (Eds.), **Psychopharmacology: The Fourth Generation of Progress.** (pp. 957-959). New York: Raven Press.
- Hopkins, S.J., Rothwell, N.J. (1995). Cytokines and the nervous system I: expression and recognition. **Trends Neurosci.** **118**, 83-88.
- Hosli, E., Lefkovits, M., Kaser, H., Hosli, L. (1992). Evidence for receptors for arginine vasopressin, atrial natriuretic peptide and bradykinin on cultured astrocytes. **Proc. Ann. Eur. Neurosci. Assoc.** **5**, 32.
- Imura, H., Fukata, J. I., Mori, T. (1991). Cytokines and endocrine function: an interaction between the immune and neuroendocrine systems. **Clin. Endocrinol.** **35**, 107-115.
- Imperato, A., Angelucci, L., Casolini, P., Zocchi, A., Puglisi-Allegra, S. (1992). Repeated stressful experiences differently affect limbic dopamine release during and following stress. **Brain Res.** **577**, 194-199.
- Johnson, R.W., Propes, M.J. Shavit, Y. (1996). Corticosterone modulates behavioural and metabolic effects of lipopolysaccharide. **Am. J. Physiol.** **270**, R192-R198.
- Kabiersch, A., del Rey, A., Honegger, C.G., Besedovsky, H.O. (1988). Interleukin-1 induces changes in norepinephrine metabolism in the rat brain. **Brain Behav. Immun.** **2**, 267-274.

- Kakucska, I., Qi, Y., Clark, B.D., Lechan, R.M. (1993). Endotoxin-induced corticotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus is mediated centrally by interleukin-1. **Endocrinol.** **133**, 815-821.
- Kapcala, L.P., He, J.R., Gao, Y., Pieper, J.O., De Tolla, L. (1996). Subdiaphragmatic vagotomy inhibits intra-abdominal interleukin-1 β stimulation of adrenocorticotropin secretion. **Brain Res.** **728**, 247-254.
- Karant, S., McCann, S.M. (1991). Anterior pituitary hormone control by interleukin 2. **Proc. Natl. Acad. Sci.** **88**, 2961-2965.
- Katsuura, G., Gottschall, P.E., Arimura, A. (1988a). Identification of a high-affinity receptor for interleukin-1 β in rat brain. **Biochem. Biophys. Res. Commun.** **156**, 61-67.
- Katsuura, G., Gottschall, P.E., Dahl, R.R., Arimura, A. (1988b). Adrenocorticotropin release induced by intracerebroventricular injection of recombinant human interleukin-1 in rats: Possible involvement of prostaglandin. **Endocrinol.** **122**, 1773-1779.
- Keller, S.E., Schleifer, S.J., Demetrikopoulos, M.K. (1991). Stress-induced changes in immune function in animals: Hypothalamo-pituitary-adrenal influences. In: R., Ader, D.L., Felten, N., Cohen. (Eds.), **Psychoneuroimmunol.** (pp. 771-787). San Diego: Academic.
- Kent, S., Rodriguez, F., Kelley, K.W., Dantzer, R. (1994). Reduction of food and water intake induced by microinjection of interleukin-1 β in the ventromedial hypothalamus of the rat. **Physiol. Behav.** **56**, 1031-1036.
- Kent, S., Bluthé, R.M., Dantzer, R., Hardwick, A., Kelley, K.W., Rothwell, N.J., Vannice, J.L. (1992a). Different receptor mechanisms mediate the pyrogenic and behavioural effects of interleukin 1. **Proc. Natl. Acad. Sci.** **89**, 9117-9120.
- Kent, S., Bluthé, R.M., Kelley, K.W., Dantzer, R. (1992b). Sickness behaviour as a new target for drug development. **Trends Pharmacol. Sci.** **13**, 24-28.
- Kintzel, P.E., Calis, K.M. (1991). Recombinant interleukin-2: A biological response modifier. **Drug Rev.** **10**, 110-128.
- Klir, J.J., McClellan, J.L., Kozak, W., Szelenyi, Z., Wong, G.H., Kluger, M. (1995). Systemic but not central administration of tumor necrosis factor- α attenuates LPS-induced fever in rats. **Am. J. Physiol.** **268**, R480-R486.

- Klir, J.J., Roth, J., Szelenyi, Z., McClellan, J.L., Kluger, M. (1993). Role of hypothalamic interleukin-6 and tumor necrosis factor- α in LPS fever in rat. **Am. J. Physiol.** **265**, R512-R517.
- Kluger, M.J., (1991). Fever: role of endogenous pyrogens and cryogens. **Physiol. Rev.** **71**, 93-127.
- Koenig, J.I. (1991). Presence of cytokines in the hypothalamic-pituitary axis. **Prog. NeuroEndocrinImmunol.** **4**, 143-153.
- Koenig, J.I., Snow, K., Clark, B.D., Toni, R., Cannon, J.G., Shaw, A.R., Dinarello, C.A., Reichlin, S., Lee, S.L., Lechan, R.M. (1990). Intrinsic pituitary interleukin-1 β is induced by bacterial lipopolysaccharide. **Endocrinol.** **126**, 3053-3058.
- Kokkinidis, L., Anisman, H. (1981). Amphetamine psychosis and schizophrenia: A dual model. **Neurosci. Biobehav. Rev.** **5**, 449-461.
- Kokkinidis, L., Anisman, H. (1980). Amphetamine models of paranoid schizophrenia models: an overview and elaboration of animal experimentations. **Psychol. Bull.** **88**, 551-579.
- Komaki, G., Arimura, A., Koves, K. (1992). Effect of intravenous injection of IL-1 β on PGE₂ levels in several brain areas as determined by microdialysis. **Am. J. Physiol.** **262**, E246-E251.
- Knudsen, P.J., Dinarello, C.A., Strom, T.B. (1987). Glucocorticoids inhibit transcriptional and post-transcriptional expression of interleukin 1 in U937 cells. **J. Immunol.** **139**, 4129-4134.
- Kluger, M.J. (1991). Fever: role of pyrogens and cryogens. **Physiol. Rev.** **71**, 93-127.
- Lacosta, S., Kulczycki, J., Merali, Z., Anisman, H. (1996). Behavioural, endocrine and central neurochemical alterations induced by interleukin-1. **Soc. Neurosci. Abstr.** **22**, 2059.
- Lacosta, S., Merali, Z., Anisman, H. (1995). Cytokine-induced endocrine, neurochemical and behavioural Alterations. **Soc. Neurosci. Abst.** **21**, 1397.
- Lacosta, S., Merali, Z., Anisman, H. (1998). Influence of interleukin-1 β on exploratory behaviours, plasma ACTH, corticosterone, and central biogenic amines in mice. **Psychopharmacol.** **137**, 351-361.

- Lacosta, S., Merali, Z., Zalcman, S., Anisman, H. (1994). Time-dependent in vivo mesolimbic dopamine variations following antigenic challenge. **Brain Res.** **664**, 225-230.
- Langhans, W., Savoldelli, D., Weingarten, S. (1993). Comparison of the feeding responses to bacterial lipopolysaccharide and IL-1 β . **Physiol. Behav.** **53**, 643-649.
- Lapchak, P.A. (1992). A role for IL-2 in the regulation of striatal dopaminergic function. **NeuroReport** **3**, 165-168.
- Lapchak, P.A., Araujo, D.M. (1993). Hippocampal IL-1, IL-2, and IL-3, but not IL-6 levels are elevated in Alzheimer's but not Parkinson's disease. **Soc. Neurosci. Abstr.** **19**, 191.
- Lapchak, P.A., Araujo, D.M., Quirion, R., Beaudet, A. (1991). Immunoautoradiographic localization of interleukin 2-like immunoreactivity and interleukin 2 receptors (Tac antigen-like immunoreactivity) in the rat brain. **Neuroscience** **44**, 173-184.
- Laviano, A., Renvyle, T., Meguid, M.M., Yang, Z.J., Cangiano, C., Fanelli, F.R. (1995). Relationship between interleukin-1 and cancer anorexia. **Nutrition** **11**, 680-683.
- Lavicky, J., Dunn, A.J. (1995). Endotoxin administration stimulates cerebral catecholamine release in freely moving rats as assessed by microdialysis. **J. Neurosci. Res.** **40**, 407-413.
- Laye, S., Bluthé, R.M., Kent, S., Combe, C., Medina, C., Parnet, P., Kelly, K., Dantzer, R. (1995). Subdiaphragmatic vagotomy blocks induction of IL-1 β mRNA in mice brain in response to peripheral LPS. **Am. J. Physiol.** **37**, R1327-R1331.
- Laye, S., Parnet, P., Goujon, E., Dantzer, R. (1994). Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice. **Mol. Brain Res.** **27**, 157-162.
- Lee, Y., Davis, M. (1997). Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. **J. Neurosci.** **17**, 6434-6446.
- Lee, S.W., Tsou, A.P., Chan, H., Thomas, J., Petrie, K., Eugui, E.M., Allison, C.A. (1988). Glucocorticoids selectively inhibit the transcription of the interleukin 1 β gene and decrease the stability of interleukin 1 β -mRNA. **Proc. Natl. Acad. Sci. USA** **85**, 1204-1208.

- LeMay, L.G., Otterness, I.G., Vander, A.J., Kluger, M.J. (1990a). In vivo evidence that the rise in plasma IL-6 following injection of a fever-inducing dose of LPS is mediated by IL-1 β . **Cytokine** **2**, 199-204.
- LeMay, L.G., Vander, A.J., Kluger, M.J. (1990b). The role of interleukin-6 in fever in the rat. **Am. J. Physiol.** **258**, R798-R803.
- Licinio, J., Seibyl, J.P., Altemus, M., Charney, D.S., Krystal, J.H. (1993). Elevated CSF levels of interleukin-2 in neuroleptic-free schizophrenic patients. **Am. J. Psychiat.** **150**, 140-1410.
- Licinio, J., Wong, M., Gold, P.W. (1991). Localization of interleukin-1 receptor antagonist mRNA in rat brain. **Endocrinol.** **129**, 562-564.
- Linthorst, A.C., Flachskamm, C., Muller-Preuss, P., Holsboer, F., Reul, J.M. (1995). Effect of bacterial endotoxin and interleukin- β on hippocampal serotonergic neurotransmission, behavioural activity, and free corticosterone levels: an in vivo microdialysis study. **J. Neurosci.** **15**, 2920-2934.
- Lisanby, S.H., Rokoske, L.D., Ritchie, J.C., Sundar, S.K., Weiss, J.M. (1990). Central Lipopolysaccharide infusion induces interleukin-1 production in the adult rat brain in vivo. **Soc. Neurosci. Abstr.** **16**, 1212.
- Lister, R.G. (1987). The use of a plus-maze to measure anxiety in the mouse. **Psychopharmacol.** **92**, 180-185.
- Long, M.C., Morimoto, A., Nakamori, T., Murakami, M. (1992). Systemic injection of TNF- α attenuates fever due to IL-1 β and LPS in rats. **Am. J. Physiol.** **263**, R987-R991.
- Long, M.C., Otterness, K., Kunkel, S.L., Vander, A.J., Kluger, M.J. (1990). Roles of interleukin-1 β and tumor necrosis factor in lipopolysaccharide fever in rats. **Am. J. Physiol.** **259**, R724-R728.
- Luber-Narod, J., Rogers, J. (1988). Immune system associated antigens expressed by cells of the human central nervous system. **Neurosci. Lett.** **94**, 17-22.
- Maes, M. (1995). Evidence for an immune response in major depression: a review and hypothesis. **Prog. Neuro-Psychopharmacol. Biol. Psychiat.** **19**, 11-38.
- Maes, M. (1998). The inflammatory response system in major depression. In: R., Dantzer (Ed). **Cytokines, Stress and Depression**. New York: Plenum. In press

- Maes, M., Meltzer, H.Y., Bosmans, E., Bergmans, R., Vandoolaeghe, E., Ranjan, R., Desnyder, R. (1995). Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. **J. Affect. Disord.** **34**, 301-309.
- Maes, M., Smith, R., Scharpe, S. (1995). The monocyte T-lymphocyte hypothesis of major depression. **Psychoneuroendocrinol.** **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 Res.** **695**, 279-282.
- Maier, S.F., Watkins, L.R. (1998). Cytokines for psychologists: Implications of bidirectional immune-to-brain communication for understanding behaviour, mood, and cognition. **Psychol. Rev.** **105**, 83-107.
- Maier, S.F., Wiertelak, E.P., Martin, D., Watkins, L.R. (1993). Interleukin-1 mediates the behavioural hyperalgesia produced by lithium chloride and endotoxin. **Brain Res.** **623**, 321-324.
- Marquette, C., Van Dam, A.M., Van Rooijen, N., Berkenbosch, F., Haour, F. (1994). Peripheral macrophage depletion prevents down regulation of central interleukin-1 receptors in mice after endotoxin challenge. **Psychoneuroendocrinol.** **19**, 189-196.
- Masana, M.I., Heyes, M.P., Mefford, I.N. (1990). Indomethacin prevents increased catecholamine turnover in rat brain following systemic endotoxin challenge. **Prog. Neuro-Psychopharmacol. Biol. Psychiat.** **14**, 609-621.
- Matta, S.G., Weatherbee, J., Sharp, B.M. (1992). A central mechanism is involved in the secretion of ACTH in response to IL-6 in rats: Comparison to and interaction with IL-1 β . **Neuroendocrinol.** **56**, 516-525.
- Mazzocchi, G., Rocco, S., Malendowicz, L.K., Rebuffat, P., Nussdorfer, G.G. (1995). Bacterial lipopolysaccharide stimulates glucocorticoid secretion in hypophysectomized rats. **Endocrine Res.** **21**, 525-536.
- McAllister, C.G., van Kammen, D.P., Rehn, T.J., Miller, A.N., Gurklis, J., Kelley, M.E., Yao, J., Peters, J.L. (1995). Increases in CSF levels of interleukin-2 in schizophrenia: effects of recurrence of psychosis and medication status. **Am. J. Psychiat.** **152**, 1291-1297.

- McCarthy, D.O., Kluger, M.J., Vander, A.J. (1985). The role of interleukin-1 in the suppression of food intake during infection: is interleukin-1 involved? **Am. J. Clin. Nutr.** **42**, 1179-1182.
- McCarthy, D.O., Kluger, M.J., Vander, A.J. (1986). Effect of centrally administered interleukin-1 and endotoxin on food intake of fasted rats. **Physiol. Behav.** **36**, 745-749.
- McClellan, C.L., Rogan, G.J., Tou, J., Baie, C.A., Joy, W.D. (1992). Food intake and body temperature responses of rats to recombinant human interleukin-1 β and a tripeptide interleukin-1 β antagonist. **Physiol. Behav.** **52**, 1155-1160.
- McGeer, P.L., Rogers, J. (1992). Anti-inflammatory agents as a therapeutic approach to Alzheimer's disease. **Neurobiol.** **42**, 447-449.
- Meaney, M.J., Aitken, D.H., Sharma, S., Viau, V. (1992). Basal ACTH, corticosterone and corticosterone-binding globulin levels over diurnal cycle and hippocampal type I and type II corticosteroid receptors in young and old handled and nonhandled rats. **Neuroendocrinol.** **55**, 204-213.
- Mefford, I.N., Heyes, M.P. (1990). Increased biogenic amine release in mouse hypothalamus following immunological challenge: antagonism by indomethacin. **J. Neuroimmunol.** **27**, 55-61.
- Mefford, I.N., Masters, C.F., Heyes, M.P., Eskay, R.L. (1991). Cytokine-induced activation of the neuroendocrine stress axis persists in endotoxin-tolerant mice. **Brain Res.** **557**, 327-330.
- Merrill, J.E. (1990). Interleukin-2 effects in the central nervous system. **Ann. NY Acad. Sci.** **594**, 188-199.
- Merali, Z., Lacosta, S., Anisman, H. (1997). Effects of Interleukin-1 β and mild stress on alterations of norepinephrine, dopamine and serotonin neurotransmission: a regional microdialysis study. **Brain Res.** **761**, 225-235.
- Meyers, C.A., Valentine, A.D. (1995). Neurological and psychiatric adverse effects of immunological therapy. **CNS Drugs** **3**, 56-68.
- Minden, S.L., Schiffer, R.B. (1990). Affective disorders in multiple sclerosis: review and recommendations for clinical research. **Arch. Neurol.** **47**, 98-104.

- Moghadden, B., Bunney, B. (1989). Ionic composition of microdialysis perfusion solution alters the pharmacological responsiveness and basal outflow of striatal dopamine. **J. Neurochem.** **53**, 652-654.
- Mohankumar, P.S., Quadri, S.K. (1993). Systemic administration of interleukin-1 stimulates norepinephrine release in the paraventricular nucleus. **Life Sci.** **52**, 1961-1967.
- Mohankumar, P.S., Thyagarajan, S., Quadri, S.K. (1991). Interleukin-1 stimulates the release of dopamine and dihydroxyphenylacetic acid from the hypothalamus in vivo. **Life Sci.** **48**, 925-930.
- Mohankumar, P.S., Thyagarajan, S., Quadri, S.K. (1993). Interleukin-1 β increases 5-hydroxyindoleacetic acid release in the hypothalamus in vivo. **Brain Res. Bull.** **31**, 745-748.
- Morimoto, A., Murakami, N., Nakamori, T., Watanabe, T. (1987). Evidence for separate mechanisms of induction of biphasic fever inside and outside the blood-brain barrier in rabbits. **J. Physiol.** **383**, 629-637.
- Morrow, L.E., McClellan, J.L., Conn, C.A., Kluger, M.J. (1993). Glucocorticoids alter fever and IL-6 responses to psychological stress and to lipopolysaccharide. **Am. J. Physiol.** **264**, R1010-R1016.
- Muller, N., Ackenheil, M. (1998). Psychoneuroimmunology and the cytokine action in the CNS: Implications for psychiatric disorders. **Prog. Neuropsychopharmacol. Biol. Psychiat.** **22**, 1-33.
- Naito, Y., Fukata, J., Tominaga, T., Masui, Y., Hirai, Y., Murakami, N., Tamai, S., Mori, K., Imura, H. (1989). Adrenocorticotrophic hormone-releasing activities of interleukins in a homologous in vivo system. **Biochem. Biophys. Res. Commun.** **164**, 1262-1267.
- Navarra, P., Tsagarakis, S., Faria, M.S., Rees, L.H., Besser, G.M., Grossman, A.B. (1990). Interleukin-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. **Endocrinol.** **128**, 37-44.
- Nemeroff, C.B. (1996). The corticotropin-releasing factor (CRF) hypothesis of depression: New findings and new directions. **Mol. Psychiat.** **1**, 336-342.
- Nemni, R., Iannaccone, S., Quattrini, A., Smirne, S., Sessa, M., Lodi, M., Erminio, C., Canal, N. (1992). Effect of chronic treatment with recombinant interleukin-2 on the central nervous system of adult and old mice. **Brain Res.** **591**, 248-252.

- Neta, R., Perlstein, R., Vogel, S.N., Ledney, G.D., Abrams, J. (1992). Role of interleukin 6 (IL-6) in protection from lethal irradiation and in endocrine responses to IL-1 and tumor necrosis factor. **J. Exp. Med.** **175**, 689-694.
- 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-1 β protein in the rat. **J. Neurosci.** **18**, 2239-2246.
- Nieto-Sampedro, M., Chandy, K.G. (1987). Interleukin-2 like activity in injured brain. **Neurochem. Res.** **12**, 723-727.
- Niimi, M., Sato, M., Wada, Y., Takahara, J., Kawanishi, K. (1996). Effect of central and continuous intravenous injection of interleukin-1 β on brain c-fos expression in the rat: involvement of prostaglandins. **Neuroimmunomod.** **3**, 87-92.
- Nistico, G. (1993). Communication among central nervous system, endocrine and immune systems: Interleukin-2. **Prog. Neurobiol.** **40**, 463-575.
- Nistico, G., De Sarro, G. (1991a). Is interleukin-2 a neuromodulator in the brain? **Trends Neurosci.** **14**, 146-150.
- Nistico, G., De Sarro, G. (1991b). Behavioural and electrocortical spectrum power effects after microinfusion of lymphokines in several areas of the rat brain. In: W., Pierpaoli, N., Fabris. (Eds.), **Physiological Senescence and its Postponement**. vol. 621. (pp. 119- 134). New York: Ann. N.Y. Acad. Sci.
- Ohta, K., Hirata, Y., Imai, T., Marumo, F. (1993). Interleukin-1 β induces nitric oxide production by a mouse pituitary tumor cell line (AtT20/D16). **J. Endocrinol.** **138**, 429-435.
- O'Reilly, B., Vander, A. J., Kluger, M. J. (1988). Effects of chronic infusion of lipopolysaccharide on food intake and body temperature of the rat. **Physiol. Behav.** **42**, 287-291.
- Ottersness, I.G. Seymour, P.A., Golden, H.W., Reynolds, J.A. Daumy, G.O. (1988). The effects of continuous administration of murine interleukin-1 β in the rat. **Physiol Behav.** **43**, 797-804.
- Pacak, K., Armando, I., Fukuhara, K., Kvetnansky, R., Palkovits, M., Kopin, I.J., Goldstein, D.S. (1992). Noradrenergic activation in the paraventricular nucleus during acute and chronic immobilization stress in rats: an in vivo microdialysis study. **Brain Res.** **589**, 91-96.

- Pacak, K., Palkovits, M., Kopin, I.J., Goldstein, D.S. (1995). Stress-induced norepinephrine release in the hypothalamic paraventricular nucleus and pituitary-adrenocortical and sympathoadrenal activity: in vivo microdialysis studies. **Front. Neuroendocrinol.** **16**, 89-150.
- Palazzolo, D.L., Quadri, S.K. (1990). Interleukin-1 stimulates catecholamine release from the hypothalamus. **Life Sci.** **47**, 2105-2109.
- Palkovits, M., Brownstein, M.J. (1988). **Maps and guide to microdissection of the rat brain**. New York: Elsevier Science.
- Paulson, P.E., Robinson, T.E. (1995). Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: A microdialysis study in behaving rats. **Synapse** **19**, 56-65.
- Pardy, K., Murphy, D., Carter, D., Hui, K.M. (1993). The influence of interleukin-2 on vasopressin and oxytocin gene expression in the rodent hypothalamus. **J. Neuroimmunol.** **42**, 131-13.
- Parker, J., Smarr, K., Anderson, S., Hewett, J., Walker, S., Bridges, A., Caldwell, W. (1992). Relationship of changes in helplessness and depression to disease activity in rheumatoid arthritis. **J. Rheumatol.** **19**, 1901-1905.
- Paxinos, G., Watson, C. (1986). **The rat brain in stereotaxic coordinates**. New York, Academic Press.
- Perlstein, R.S., Mougey, E.H., Jackson, W.E., Neta, R. (1991). Interleukin-1 and interleukin-6 act synergistically to stimulate the release of adrenocorticotrophic hormone in vivo. **Lymphokine Cytokine Res.** **10**, 141-146.
- Perlstein, R.S., Whitnall, M.H., Abrams, J.S., Mougey, E.H. (1993). Synergistic roles of interleukin-6, interleukin-1 and tumor necrosis factor in the adrenocorticotropin response to bacterial lipopolysaccharide in vivo. **Endocrinol.** **132**, 946-952.
- Petitto, J.M., Huang, Z. (1994). Molecular cloning of a partial cDNA of the interleukin-2 receptor- β in normal mouse brain: in situ localization in the hippocampus and expression by neuroblastoma cells. **Brain Res.** **650**, 140-145.
- Petitto, J.M., McCarthy, D.B., Rinker, C.M., Huang, Z., Getty, T. (1997). Modulation of behavioural and neurochemical measures of forebrain dopamine function in mice by species-specific interleukin-2. **J. Neuroimmunol.** **73**, 183-190.

- Pezeshki, G., Pohl, T., Schobitz, B. (1996). Corticosterone controls interleukin-1 β and sickness behaviour in the rat. **J. Neuroendocrinol.** **8**, 129-135.
- Pitossi, F., del Rey, A., Kabiersch, A., Besedovsky, H. (1997). Induction of cytokine transcripts in the CNS and pituitary following peripheral administration of endotoxin to mice. **J. Neurosci. Res.** **48**, 287-298.
- Plata-Salaman, C.R. (1989). Immunomodulators and feeding regulation: A humoral link between the immune and nervous systems. **Brain Behav. Immun.** **3**, 193-213.
- Plata-Salaman, C.R., (1994). Meal patterns in response to the intracerebroventricular administration of interleukin-1 β in rats. **Physiol. Behav.** **55**, 727-733.
- Plata-Salaman, C.R., (1995). Cytokines and feeding suppression: An integrative view from neurologic to molecular levels. **Nutrition** **11**, 674-677.
- Plata-Salaman, C.R., Borkoski, J.P. (1993). Centrally administered bacterial lipopolysaccharide depresses feeding in rats. **Pharmacol. Biochem. Behav.** **46**, 787-791.
- Puglisi-Allegra, S.P., Imperato, A., Angelucci, L., Cabib, S. (1991). Acute stress induces time-dependent responses in dopamine mesolimbic system. **Brain Res.** **554**, 217-22.
- Quan, N., Sundar, S.K., Weiss, J.M. (1994). Induction of interleukin-1 in various brain regions after peripheral and central injections of lipopolysaccharide. **J. Neuroimmunol.** **49**, 125-134.
- Raber, J., Bloom, F.E. (1994). IL-2 induced vasopressin release from the hypothalamus and the amygdala: Role of nitric oxide mediated signaling. **J. Neurosci.** **14**, 6187-6195.
- Raber, J., Koob, G.F., Bloom, F.E. (1995). Interleukin-2 (IL-2) induces corticotropin-releasing factor (CRF) release from the amygdala and involves a nitric oxide-mediated signaling: Comparison with the hypothalamic response. **J. Pharmacol. Exp. Ther.** **272**, 815-824.
- Rabin, B.S., Cohen, S., Ganguli, R., Lysle, D.T., Cunnick, J.E. (1989). Bidirectional interaction between the central nervous system and the immune system. **Crit. Rev. Immunol.** **9**, 279-312.
- Rada, P., Mark, G.P., Vitek, M.P., Mangano, R.M., Blume, A.J., Beer, B., Hoebel, B.G. (1991). Interleukin-1 β decreases acetylcholine measured by microdialysis in the hippocampus of freely moving rats. **Brain Res.** **550**, 287-290.

- Reed, J.C., Abidi, A.H., Alpers, J.D., Hoover, R.G., Robb, R.J., Nowell, P. C. (1986). Effect of cyclosporin A and dexamethasone on interleukin 2 receptor gene expression. **J. Immunol.** **137**, 150-153.
- Reinisch, N., Wolkersdorfer, M., Kahler, C., Ye, K., Dinarello, C.A., Wiedermann, C.J. (1994). Interleukin-1 receptor type 1 mRNA in mouse brain as affected by peripheral administration of bacterial lipopolysaccharide. **Neurosci. Lett.** **166**, 165-167.
- Rivest, S., Torres, G., Rivier, C. (1992). Differential effects of central and peripheral injection of Interleukin-1 β on brain c-fos expression and neuroendocrine functions. **Brain Res.** **587**, 13-23.
- Rivest, S., Rivier, C. (1995). The role of corticotropin-releasing factor and interleukin-1 in the regulation of neurons controlling reproductive functions. **Endocr. Rev.** **16**, 177-199.
- Rivier, C. (1990). Role of endotoxin and interleukin-1 in modulating ACTH, LH and sex steroid secretion. In: J.C., Porter, D., Jezova (Eds.), **Circulating Regulatory Factors and Neuroendocrine Function**. (pp. 295-302). New York: Plenum
- Rivier, C. (1993). Effect of peripheral and central cytokines on the hypothalamic-pituitary-adrenal axis of the rat. **Ann. N.Y. Acad. Sci.** **697**, 97-105.
- Rivier, C. (1995). Influence of immune signals on the hypothalamic-pituitary axis of the rodent. **Front. Neuroendocrinol.** **16**, 151-182.
- Rivier, C., Shen, G. (1994). In the rat, endogenous nitric oxide modulates the response of the hypothalamic-pituitary-adrenal axis to interleukin-1 β , vasopressin and oxytocin. **J. Neurosci.** **14**, 1985-1993.
- Rivier, C., Chizzonite, R., Vale, W. (1989). In the mouse, the activation of the hypothalamic-pituitary-adrenal axis by a lipopolysaccharide (endotoxin) is mediated through interleukin-1. **Endocrinol.** **125**, 330-338.
- Robinson, T.E., Camp, D.M. (1991). The feasibility of repeated intracerebral microdialysis. In: H., Rollema, B.H., Westerink, W.J., Drijfhout (Eds.), **Monitoring Molecules in Neuroscience**. (pp. 51-55). Meppel: Krips Repro.
- Rosenberg, S.A. (1990). Adoptive immunotherapy for cancer. **Sci. Am.** **262**, 62-69.
- Roszman, T.L., Brooks, W.H. (1992). Signaling pathways of the neuroendocrine-immune network. In: J.E., Blalock (Ed.), **Neuroimmunoendocrinology**. (pp. 170-190). Basel: Karger.

- Roth, J., Conn, C., Kluger, M.J., Zeisberger, E. (1992). Release of interleukin-6 and tumor necrosis factor during lipopolysaccharide induced fever in guinea pigs. In: P., Lomax, E., Schonbaum. (Eds.), **Thermoregulation: The Pathophysiological Basis of Clinical Disorders**. (pp. 28-32). Basel: Karger.
- Rothwell, N.J. (1990). Mechanisms of the pyrogenic actions of cytokines. **Eur. Cytokine Net.** **1**, 211-213.
- Rothwell, N.J. (1991). Functions and mechanisms of interleukin-1 in the brain. **Trends Pharmacol. Sci.** **12**, 430-436.
- Rothwell, N.J., Hopkins, S.J. (1995). Cytokines and the nervous system II: actions and mechanisms of action. **Trends Neurosci.** **18**, 130-136.
- Sandi, C., Guaza, C. (1995). Evidence for a role of nitric oxide in the corticotropin-releasing factor release induced by interleukin-1 β . **Eur. J. Pharmacol.** **274**, 17-23.
- Saper, C.B., Breder, C.D. (1994). The neurologic basis of fever. **New Engl. J. Med.** **330**, 1880-1886.
- Saperstein, A., Brand, H., Audhya, T., Nabriski, D., Hutchinson, B., Rosenzweig, S., Hollander, C.S. (1992). Interleukin-1 β mediates stress-induced immunosuppression via corticotropin-releasing factor. **Endocrinol.** **130**, 152-158.
- Saphier, D., Abramsky, O., Mor, G., Ovadia, H. (1987). Multiunit electrical activity in conscious rats during an immune response. **Brain Behav. Immun.** **1**, 40-51.
- Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P., Vale, W. (1987). Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. **Science** **238**, 522-524.
- Saris, S., Rosenberg, A., Friedman, R.B., Rubin, J.T., Barba, D., Olffeld, E.H. (1988). Penetration of recombinant interleukin-2 across the blood cerebrospinal fluid barrier. **J. Neurosurg.** **69**, 29-34.
- Sauer, J., Rupprecht, M., Arzt, E., Stalla, G.K., Rupprecht, R. (1993). Glucocorticoids modulate soluble interleukin-2 receptor levels in vivo depending on the state of immune activation and the duration of glucocorticoid exposure. **Immunopharm.** **25**, 269-276.
- Schwartz, J.A., Speed, N.M., Brunberg, J.A., Brewie, T.L., Brown, M., Greden, J.F. (1993). Depression in stroke rehabilitation. **Biol. Psychiatry** **33**, 696-699.

- Schmidt, E.D., Janszen, J.W., Wouterlood, F.G., Tilders, F. (1995). Interleukin-1-induced long-lasting changes in hypothalamic corticotropin-releasing hormone (CRH)-neurons and hyperresponsiveness of the hypothalamus-pituitary-adrenal axis. **J. Neurosci.** **15**, 7417-7426.
- Schobitz B, De Kloet E.R., Holsboer F. (1994). Gene expression and function of interleukin-1, interleukin-6 and tumor necrosis factor in the brain. **Prog. Neurobiol.** **44**, 397-432.
- Schotanus, K., Tilders, F., Berkenbosch, F. (1993). Human recombinant interleukin-1 receptor antagonist prevents ACTH but not interleukin-6 responses to low doses of bacterial endotoxin rats. **Endocrinol.** **133**, 2461-2468.
- Schreiber, W., Pollmacher, T., Fassbender, K., Gudewill, S., Vedder, H., Wiedemann, K., Galanos, C., Holsboer, F. (1993). Endotoxin- and corticotropin-releasing hormone-induced release of ACTH and cortisol. **Neuroendocrinol.** **58**, 123-128.
- Seegal, R.F., Brosch, K.O., Bush, G. (1986). High-performance liquid chromatography of biogenic amines and metabolites in brain, cerebrospinal fluid, urine and plasma. **J. Chromatog.** **377**, 131-144.
- Seto, D., Karr, S., Quirion, R. (1997). Evidence for direct and indirect mechanisms in the potent modulatory action of interleukin-2 on the release of acetylcholine in rat hippocampal slices. **Br. J. Pharmacol.** **120**, 1151-1157.
- Shanks, N., Francis, D., Zalcman, S., Meaney, M.J., Anisman, H. (1994). Alterations in central catecholamines associated with immune responding in adult and aged mice. **Brain Res.** **666**, 77-87.
- Shalaby, M.R., Waage, A., Aarden L., Espevik, T. (1989). Endotoxin, tumor necrosis factor- α and interleukin 1 induce interleukin 6 production in vivo. **Clin. Immunol. Immunopathol.** **53**, 488-498.
- Shek, P.N., Sabiston, B.H. (1983). Neuroendocrine regulation of immune processes: Change in circulating corticosterone levels induced by the primary antibody response in mice. **Int. J. Immunopharm.** **5**, 23-33.
- Shimojo, M., Imai, Y., Nakajima, K., Mizushima, S., Uemura, A., Kohsaka, S. (1993). Interleukin-2 enhances the viability of primary cultured rat neurocortical neurons. **Neurosci. Lett.** **151**, 170-173.

- Shintani, F., Kanba, S., Nakaki, T., Nibuya, M., Kinoshita, N., Suzuki, E., Yagi, G., Kato, R., Asai, M. (1993). Interleukin-1 β augments release of norepinephrine, dopamine, and serotonin in the rat anterior hypothalamus. **J. Neurosci.** **13**, 3574-3581.
- Shintani, F., Nakaki, T., Kanba, S., Kato, R., Asai, M. (1995a). Role of interleukin-1 in stress responses. **Molec. Neurobiol.** **10**, 47-71.
- Shintani, F., Nakaki, T., Kanba, S., Sato, K., Yagi, G., Shiozawa, M., Aiso, S., Kato, R., Asai, M. (1995b). Involvement of interleukin-1 in immobilization stress-induced increase in plasma adrenocorticotrophic hormone and in release of hypothalamic monoamines in the rat. **J. Neurosci.** **15**, 1961-1970
- Singewald, N., Kaehler, S., Hemeida, R., Philippu, A. (1997). Release of serotonin in the rat locus coeruleus: effects of cardiovascular, stressful and noxious stimuli. **Eur. J. Neurosci.** **9**, 556-562.
- Sironi, M., Breviario, F., Proserpio, P., Biondi, A., Vecchi, A., Van Damme, J., Dejana, E., Mantovani, A., (1989). IL-1 stimulates IL-6 production in endothelial cells. **J. Immunol.** **142**, 549-553.
- Sluzewska, A. (1998) Indicators of immune activation in depressed patients. In: R., Danzer (Ed.), **Cytokines, stress and depression**. New York: Plenum. In press.
- Smith, K.A. (1990). Interleukin-2. **Sci. Am. March**, 26-33.
- Smith, B.K., Kluger, M.J. (1992). Human IL-1 receptor antagonist partially suppresses LPS fever but not plasma levels of IL-6 in Fischer rats. **Am. J. Physiol.** **253**, R653-R655.
- Song, C., Merali, Z., Anisman, H. (1998). Systemically administered interleukin-1, interleukin-2 and interleukin-6 and mild stress influence in vivo biogenic amine variations in the nucleus accumbens. **Neuroscience**. In press.
- Song, C., Merali, Z., Lacosta, S., Anisman, H. (1996). Effects of cytokines and stress on neurotransmitter release in the nucleus accumbens: An in vivo microdialysis study. **Psychoneuroimmunol. Res. Soc.**, 1996.
- Spadaro, F., Dunn, A.J. (1990). Intracerebroventricular administration of interleukin-1 to mice alters investigation of stimuli in a novel environment. **Brain. Behav. Immun.** **4**, 308-322.
- Spangelo, B.L., Judd, A.M., Isakson, P.C., MacLeod, R.M. (1991). Interleukin-1 stimulates interleukin-6 release from rat anterior pituitary cells in vitro. **Endocrinol.** **128**, 2685-2692.

- Squire, L.R. (1992). Memory and the hippocampus: a synthesis from findings from rats, monkeys, and humans. **Psychol. Rev.** **99**, 195-231.
- Stanford, S.C. (1995). Central noradrenergic neurons and stress. **Pharmac. Ther.** **68**, 297-342.
- Sundar, S.K., Becker, K.J., Cierpial, M.A., Carpenter, M.D., Rankin, L.A., Fleener, S.L., Ritchie, J.C., Simson, P.E., Weiss, J.M. (1989). Intracerebroventricular infusion of interleukin-1 rapidly decreases peripheral cellular immune responses. **Proc. Natl. Acad. Sci.** **86**, 6398-6402.
- Sundar, S.K., Cierpial, M.A., Long, S., Ritchie, J.C., Weiss, J.M. (1990). Localization of interleukin-1 synthesis in diencephalon region of rats centrally infused with lipopolysaccharide. **Soc. Neurosci. Abstr.** **16**, 1212.
- Takao, T., Culp, S.G., De Souza, E.B. (1993a). Reciprocal modulation of interleukin-1 β (IL-1 β) and IL-1 receptors by lipopolysaccharide (endotoxin) treatment in the mouse brain-endocrine-immune axis. **Endocrinol.** **132**, 1497-1504.
- Takao, T., Tracey, D.E., Mitchell, W.M., De Souza, E.B. (1993b). Interleukin-1 receptors in mouse brain: characterization and neuronal localization. **Endocrinol.** **127**, 3070-3078.
- Takao, T., Nakata, H., Tojo, C., Kurokawa, H., Nishioka, T., Hashimoto, K., De Souza, E.B. (1994). Regulation of interleukin-1 receptors and hypothalamic-pituitary-adrenal axis by lipopolysaccharide treatment in the mouse. **Brain Res.** **649**, 265-270.
- Tanaka, S., Watanabe, N., Nishioka, K. (1990). Serum soluble interleukin-2 receptor levels in juvenile rheumatoid arthritis. **J. Med. Pharmacol. Sci.** **24**, 1221-1226.
- Tancredi, V., Zona, C., Velotti, F., Eusebi, F., Santoni, A. (1990). Interleukin-2 suppresses established long-term potentiation and inhibits its induction in the rat hippocampus. **Brain Res.** **525**, 149-151.
- Tazi, A., Dantzer, R., Crestani, F., Le Moal, M. (1988). Interleukin-1 induces conditioned taste aversion in rats: A possible explanation for its pituitary-adrenal stimulating activity. **Brain Res.** **473**, 369-371.
- Tilders, J., DeRijk, R., Van Dam, A.M., Vincent, V., Schotanus, K., Persoons, J. (1993a). Activation of the hypothalamus-pituitary-adrenal axis by bacterial endotoxins: Routes and intermediate signals. **Psychoneuroendocrinol.** **19**, 209-232.
- Tilders, F.J., Schmidt, E.D., De Goeij, D.C.E. (1993b). Phenotypic plasticity of CRF neurons during stress. **Ann. NY. Acad. Sci.** **697**, 39-52.

- Tominaga, T., Fukata, J., Maito, Y., Usui, T., Murakami, N., Fukushima, M., Makai, Y., Hirai, Y., Imura, H. (1991). Prostaglandin-dependent in vitro stimulation of adrenocortical steroidogenesis by interleukins. **Endocrinol.** **128**, 526-531.
- Vahabzadeh, A., Fillenz, M. (1994). Comparison of stress-induced changes in noradrenergic and serotonergic neurons in the rat hippocampus using microdialysis. **Eur. J. Neurosci.** **6**, 1205-1212.
- Van Dam, A-M., Brouns, M., Louisse, S., Berkenbosch, F. (1992). Appearance of interleukin-1 in macrophages and in ramified microglia in the brain of endotoxin-treated rats: a pathway for the induction of non-specific symptoms of sickness? **Brain Res.** **588**, 291-296.
- Van Dam, A-M., Brouns, M., Man-A-Hing, W., Berkenbosch, F. (1993). Immunocytochemical detection of prostaglandin E₂ in microvasculature and in neurons of rat brain after administration of bacterial endotoxin. **Brain Res.** **613**, 331-336.
- Villemain, F., Girard, J.M., Owens, T. (1990). Immunohistochemical detection of interleukin-2 in normal mouse brain. **Soc. Neurosci. Abstr.** **16**, 1213.
- Waguespack, P.J., Banks, W.A., Kastin, A.J. (1994). Interleukin-2 does not cross the blood-brain barrier by a saturable transport system. **Brain Res. Bull.** **34**, 103-109.
- Waldmann, T.A. (1993). The IL-2/IL-2 receptor system: a target for rational immune intervention. **Immunol. Today** **14**, 264-270.
- Watanabe, T., Morimoto, A., Murakami, N. (1991). ACTH response in rats during biphasic fever induced by interleukin-1. **Am. J. Physiol.** **261**, R1104-R1108.
- Watanabe, H., Sasaki, S., Takebe, K. (1994). Effects of intravenous administration of interleukin-1 β on the release of prostaglandin E₂, corticotropin-releasing factor and arginine vasopressin in several hypothalamic areas of freely moving rats: Estimation by push-pull perfusion. **Neuroendocrinol.** **60** (Suppl. 1), 43-62.
- Watkins, L., Maier, S.F., Goehler, L.E. (1995a). Cytokine-to-brain communication: A review & analysis of alternative mechanisms. **Life Sci.** **57**, 1011-1026.
- Watkins, L., Goehler, L., Relton, J., Tartaglia, N., Silbert, L., Martin, D., Maier, S. (1995b). Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for mediation of immune-brain communication. **Neurosci. Lett.** **183**, 27-31.

- Wehrenberg, W.B., Saudan, P., Corder, R., Voltz, D.M., Giustina, A., Gaillard, R.C. (1994). Acute and chronic effects of systemic administration of interleukin-1 β and endotoxin on the hypothalamic-pituitary-adrenal axis in the rat. **Endocrine J.** **2**, 273-277.
- Weidenfeld, J., Abramsky, O., Ovadia, J. (1989). Evidence for the involvement of central adrenergic system in interleukin-1-induced adrenocortical response. **Neuropharm.** **28**, 1411-1414.
- Weidenfeld, J., Amir, I., Shohami, E. (1993). Role of glucocorticoids in the regulation of brain prostaglandin biosynthesis under basal conditions and in response to endotoxin. **Endocrinol.** **132**, 941-945.
- Weiss, J.M. (1991). Stress-induced depression: critical neurochemical and electrophysiological changes. In: J., Madden (Ed.), **Neurobiology of Learning, Emotion and Affect**. (pp. 123-154). New York: Raven Press.
- Wenk, G., Hughey, D., Boundy, V., Kim, A. (1987). Neurotransmitters and memory: Role of cholinergic, serotonergic and noradrenergic systems. **Behav. Neuropsychol.** **101**, 325-332.
- Westerink, B.H. (1995). Brain microdialysis and its application for the study of animal behaviour. **Behav. Brain Res.** **70**, 103-124.
- Westerink, B.H.C., Tuinte, M.H.J. (1986). Chronic use intracerebral dialysis for the in vivo measurement of 3,4-dihydroxyphenylethylamine and its metabolites 3,4-dihydroxyphenylacetic acid. **J. Neurochem.** **46**, 181-185.
- Whishaw, I.Q. (1985). Formation of a place learning-set by the rat: a new paradigm for neurobehavioural studies. **Physiol. Behav.** **35**, 139-143.
- Wise, R.A. (1985). The anhedonia hypothesis; Mark III. **Behav. Brain Sci.** **8**, 178-186.
- Wispelwey, B., Lesse, A.J., Hansen E.J., Scheld, W.M. (1988). *Haemophilus influenza* lipopolysaccharide-induced blood brain barrier permeability during experimental meningitis in the rat. **J. Clin. Invest.** **82**, 1339-1346.
- Wong, P.T-H., Teo, W.L., Lee, T. (1993). A microdialysis study on striatal dopamine, 5-HT and metabolites in conscious rats after various treatments: Evidence for extravesicular release of dopamine. **Neuroreport** **5**, 53-56.
- Yirmiya, R. (1996). Endotoxin produces a depressive-like episode in rats. **Brain Res.** **711**, 163-174.

- Zacharko, R. M., Bowers, W. J., Kelley, M. S., Anisman, H. (1984). Prevention of stressor-induced disturbances of self-stimulation by desmethylinipramine. **Brain Res.** **321**, 175-179.
- Zacharko, R.M., Bowers, W.J., Kokkinidis, L., Anisman, H. (1983). Region specific reductions of intracranial self-stimulation after uncontrollable stress: Possible effects on reward processes. **Behav. Brain Res.** **9**, 129-141.
- Zalcman, S., Green-Johnson, J., Murray, L., Nance, D.M., Dyck, D., Anisman, H., Greenberg, A. (1994). Cytokine-specific central monoamine alterations induced by interleukin (IL)-1, IL-2 and IL-6. **Brain Res.** **643**, 40-49.
- Zalcman, S., Shanks, N., Anisman, H. (1991). Time-dependent variations of central norepinephrine and dopamine following antigen administration. **Brain Res.** **557**, 69-76.
- Zanetti, G., Heumann, D., Gerain, J., Kohler, J., Abbet, P., Barras, C., Lucas, R., Glauser, M.P., Baumgartner, J.D. (1992). Cytokine production after intravenous or peritoneal gram negative bacterial challenge in mice. **J. Immunol.** **148**, 1890-1897.
- Zetterstrom, T., Sharp, T., Collin, A.K., Ungerstedt, U. (1988). In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs; implications for the origin of extracellular DOPAC. **Eur. J. Pharmacol.** **148**, 327-334.
- Zhou, D., Kusnecov, A.W., Shurin, M.R., DePaoli, M., Babin, B.S. (1993). Exposure to physical and psychological stressors elevates plasma interleukin 6: Relationship to the activation of hypothalamic-pituitary-adrenal axis. **Endocrinol.** **133**, 2523-2530.
- Zuckerman, S.H., Shelhaas, J., Butler, L.D., (1989). Differential regulation of lipopolysaccharide-induced interleukin 1 and tumor necrosis factor synthesis: effects of endogenous and exogenous glucocorticoids and the role of the pituitary-adrenal axis. **Europ. J. Immunol.** **19**, 301-305.

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