INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

**Synergistic Effects of Macrophage Derived Cytokines: Consumption of a Highly Palatable
Substance and Plasma Corticosterone Alterations**

A thesis presented to the Faculty of Graduate Studies and Research, Carleton University, in
partial fulfillment of the requirements of the degree of Master of Science in Psychology

Carleton University
Ottawa, Ontario

National Library
of Canada

Acquisitions and
Bibliographic Services

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale
du Canada

Acquisitions et
services bibliographiques

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-27043-2

The undersigned hereby recommend to the Faculty of Graduate Studies and Research
acceptance of the thesis submitted by

Karen Brebner

in partial fulfillment of the requirements for the degree of

Master of Science

.....
Chair, Department of Psychology

.....
Thesis Supervisor

Carleton University

September, 1997

Abstract

It is becoming apparent that the proinflammatory cytokines, interleukin-1 β (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α), mediate some of the behavioral and hormonal responses to pathophysiological conditions. In addition to the illness behavior associated with immune and cytokine challenge (e.g., reduced eating, exploration, locomotion, the appearance of curled body posture, and soporific effects), such treatments may also provoke anhedonia. Moreover, there is reason to believe that these treatments may act synergistically in promoting at least some of the actions associated with endotoxin challenges. The present investigation assessed the potential synergistic effects of macrophage-derived cytokines on feeding and on plasma corticosterone concentrations. Male CD-1 mice were trained to drink chocolate milk, and dose-dependent alterations in consumption were measured following the separate administration of intraperitoneal (i.p.) doses of lipopolysaccharide (LPS), IL-1, IL-6 and TNF- α . Corresponding alterations in plasma corticosterone levels were established in naive CD-1 mice. It was observed that LPS, IL-1 and TNF- α each reduced chocolate milk consumption and increased corticosterone levels. Based on preliminary findings, sub-optimal doses of the cytokines were chosen in order to assess the synergistic actions of combinations of IL-1 + IL-6, IL-6 + TNF- α , and IL-1 + TNF- α on consumption and hormone alterations. Cytokine combinations were administered in two successive i.p. injections and consumption of chocolate milk was measured one hour later. Plasma corticosterone levels were again measured in a naive group of mice. The combination of IL-1 and TNF- α synergistically reduced consumption of chocolate milk and increased corticosterone levels. In contrast, neither the combinations of IL-1 + IL-6 nor IL-6 + TNF- α induced synergistic effects on behaviour or endocrine functioning. The data are discussed in terms of (a) the anorexic vs the anhedonic actions of cytokine treatment, and (b) the occurrence and function of synergistic effects of various cytokines.

Acknowledgements

I would like to express my sincere thanks to Dr. Robert Zacharko and Dr. Hymie Anisman, without whom none of this would have been possible. I am also extremely grateful to Dr. Jerzey Kulczycki who spent so much time analyzing my blood samples.

To everyone in the lab, I will always be grateful for your support, your good humour and your neverending encouragement in times of stress. In particular I am indebted to Yael Sela who was always there to offer her opinion and her invaluable assistance when it was most needed.

To my family and friends, I appreciate your patience, your understanding, and your encouragement (not to mention your computer!).

This research was supported by a MRC grant.

Table of Contents

Title	i
Recommendation	ii
Abstract.....	iii
Acknowledgements.....	iv
Table of Contents.....	v
List of Figures.....	vi
I. Introduction	1
II. Multisystem Interactions	8
III. Cytokine Involvement in Endocrine/CNS Transmitter Alterations.....	11
IV. Purpose.....	21
Experiments 1-4	21
Experiments 5-7	36
General Discussion	45
References	60
Appendix A.....	73
Appendix B.....	80
Appendix C.....	81
Appendix D.....	82
Appendix E.....	83
Appendix F.....	84
Appendix G.....	85
Appendix H.....	86
Appendix I.....	87

List of Figures

Figure 1. Mean ($\pm$ S.E.M.) consumption (mls) of a highly palatable food among mice that received peripheral lipopolysaccharide (LPS) or saline. Various doses of LPS (0.5 μ g, 0.75 μ g or 1 μ g) were administered intraperitoneally to male CD-1 mice and consumption of chocolate milk was measured over a 1 hr. period commencing 90 minutes following injection. Baseline values were calculated by averaging consumption over the three day period immediately prior to testing. Recovery data were collected 24 hr. following testing.

Figure 2. Mean ($\pm$ S.E.M.) consumption (mls) of a highly palatable food among mice receiving peripheral interleukin-1 β (IL-1), tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6) or saline. Various doses of IL-1 (0.025 μ g, 0.05 μ g, 0.1 μ g, 0.2 μ g, or 0.8 μ g), TNF- α (1 μ g, 2 μ g, or 4 μ g) or IL-6 (0.1 μ g, 0.2 μ g, 0.8 μ g or 1.6 μ g) were administered intraperitoneally to male CD-1 mice and consumption of chocolate milk was measured over a 1 hr. period commencing either 45 minutes (for IL-1 and IL-6) or 30 minutes (for TNF- α) following injection. Baseline values were calculated by averaging consumption over the three day period immediately prior to testing. Recovery data were collected 24 hr. following testing.

Figure 3 Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (μ g/dl) among mice that received peripheral lipopolysaccharide (LPS) or saline. Various doses of LPS (0.5 μ g or 1 μ g) were administered intraperitoneally to male CD-1 mice and corticosterone concentrations were measured 2 hr later.

Figure 4. Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (μ g/dl) in mice receiving peripheral interleukin-1 β (IL-1), tumor necrosis factor-alpha (TNF- α),

interleukin-6 (IL-6), or saline. Various doses of IL-1 (0.025 μ g, 0.05 μ g or 0.1 μ g), IL-6 (0.1 μ g or 0.8 μ g), or TNF- α (1 μ g, 2 μ g or 4 μ g) were administered intraperitoneally to male CD-1 mice and plasma corticosterone concentrations were measured 1 hr later.

Figure 5. Effect of intraperitoneal administration of 0.025 μ g IL-1 + 0.1 μ g IL-6 (upper panel), 0.1 μ g IL-6 + 1 μ g TNF- α (middle panel) or 0.025 μ g IL-1 + 1 μ g TNF- α (lower panel) on mean ($\pm$ S.E.M.) consumption (mls) of a highly palatable food. Male CD-1 mice were injected with the cytokine combinations or 0.025 μ g IL-1 + Veh, 0.1 μ g IL-6 + Veh, or 1 μ g TNF- α + Veh and consumption of chocolate milk was measured over a 1 hr. period commencing 1 hr following injection. Baseline values were calculated by averaging consumption over the three day period immediately prior to testing. Recovery data were collected 24 hr. following testing.

Figure 6. Effect of intraperitoneal administration of 0.025 μ g IL-1 + 0.1 μ g IL-6, 0.1 μ g IL-6 + 1 μ g TNF- α or 0.025 μ g IL-1 + 1 μ g TNF- α on mean ($\pm$ S.E.M.) plasma corticosterone concentrations (μ g/dl). Male CD-1 mice were injected with the cytokine combinations or 0.025 μ g IL-1 + Veh, 0.1 μ g IL-6 + Veh, or 1 μ g TNF- α and plasma corticosterone levels were measured 1 hr following injection.

Figure 7. Mean ($\pm$ S.E.M.) consumption (in mls) of a highly palatable food by capsaicin- and vehicle-treated mice following intraperitoneal administration of lipopolysaccharide (LPS) or saline. Neo-natal CD-1 mice were treated with subcutaneous capsaicin or vehicle at 2 days of age. At 60 days of age both groups of mice were injected with 0.5 μ g LPS or saline, and consumption of chocolate milk was measured over a 1 hr. period commencing 90 minutes following injection. Baseline values were calculated by averaging consumption over the three day period immediately prior to testing. Recovery data were collected 24 hr. following testing.

Figure 8. Mean ($\pm$ S.E.M.) plasma corticosterone concentrations (μ g/dl) in capsaicin- or vehicle-treated mice receiving intraperitoneal lipopolysaccharide (LPS) or saline. Neonatal male CD-1 mice were treated with subcutaneous capsaicin or vehicle at 2 days of age. At 60 days of age both groups of mice were injected with 0.5 μ g LPS or saline and plasma corticosterone concentrations were measured 2 hr later.

Introduction

It has become clear that the immune and central nervous systems do not act independently of one another. Central nervous system (CNS) alterations come to affect immune functioning, and conversely immune activation may promote increased endocrine and central neurotransmitter activity (Besedovsky, del Ray, Sorkin, Da Prada, Burri & Honegger, 1983). Indeed, the view has been offered that the immune system stimulates CNS activity, and like environmental stimuli, may be associated with psychopathology. In particular, it is thought that immune activation acts like a stressor to provoke activation of the hypothalamic-pituitary-adrenal (HPA) endocrine system, and may also elicit variations of central monoamines (Zalcman, Shanks & Anisman, 1993). As a result, behavioral alterations may occur which are reminiscent of those elicited by stressors (Dantzer & Kelley, 1989).

The mechanisms by which the immune system influences CNS processes remain to be deduced. However, several candidates have been proposed in this respect. While these systems are not mutually exclusive, it has been suggested that activation of the vagal system, for example, may play a significant role in CNS-immune communication (Watkins et al., 1994; Laye et al., 1995). In particular, it is thought that immune activation by way of vagal innervation may ultimately come to excite hypothalamic factors (Gaykema, Dijkstra & Tilders, 1995; Fleshner, Goehler, Hermann, Relton, Maier & Watkins, 1995). Other investigators, however, have emphasized the role of cytokines released from activated immune cells, particularly macrophages (Besedovsky, del Rey, Sorkin & Dinarello, 1986). In particular, antigen-stimulated macrophages release proinflammatory cytokines (interleukin-1, interleukin-6 and tumor necrosis factor- α) which are instrumental in signaling other immunological cells, including T helper cells. In addition, however, these cytokines may gain access to the CNS, albeit in limited quantities, and among other things, promote the release of corticotropin releasing hormone (CRH) from the paraventricular

nucleus (PVN) of the hypothalamus, and either directly or indirectly elicit increased activity of forebrain monoamines (Anisman, Zalcman & Zacharko, 1993; Lavicky & Dunn, 1995).

There have been several reports consistent with the suggestion that cytokines play a role in affecting CNS processes (see Schobitz, De Kloet & Holsboer, 1994; Watkins, Maier & Goehler, 1995a for review). Curiously, however, scant attention has been devoted to the behavioral effects of cytokines, other than “sickness behavior” which often accompanies immunological challenge. In addition, although macrophage activation promotes concurrent release of interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), most studies that have considered the hormonal, neurotransmitter or behavioral effects of these cytokines have assessed such effects individually. Yet, the few studies that evaluated the conjoint action of these cytokines have indicated that IL-1 and IL-6 may act synergistically with respect to HPA functioning (Matta, Weatherbee & Sharp, 1992; Klir, Roth, Szelenyi, McClellan & Kluger, 1993; Perlstein, Whitmall, Abrams, Mougey & Neta, 1993; Zhou, Shanks, Riechman, Liang, Kusnecov & Rabin, 1996). The purpose of the present investigation is to detail some of the synergistic effects of these cytokines. In particular, administration of a bacterial endotoxin, such as lipopolysaccharide (LPS), which promotes IL-1 secretion (Rivier, Chizzonite, & Vale, 1989; Mefford, Masters, Heyes, & Eskay, 1991; Zanetti et al., 1992), has been shown to elicit sickness behavior, of which anorexia and anhedonia are primary symptoms (Hart, 1988; Dantzer & Kelley, 1989; Plata-Salaman & Borkoski, 1993; Exton, Bull & King, 1995; Yirmiya, 1996; Anisman, Kokkinaides & Merali, 1996). The present investigation was conducted in order to determine whether IL-1, IL-6 and TNF- α influence anorexia/anhedonia, and whether such behavioral effects are subject to the synergistic activity of these cytokines. Additionally, a preliminary study was conducted to ascertain whether destruction of vagal afferent fibers attenuates the illness behavior provoked by the bacterial endotoxin LPS, known to activate IL-1, IL-6 and TNF- α (Rivier et al., 1989; Zanetti et al., 1992).

Cytokines and Vagal Nerve Influence Immune-Brain Communication

There is sufficient evidence to suggest that communication between the CNS, pituitary–adrenal endocrine system, and the immune system is multidirectional. Activation of immune cells resulting from pathophysiological stimuli produces a CNS response which, in turn, modulates the immune system. It is thought that this communication occurs via alterations in neurotransmitter and hormonal activity (Blalock, 1984; Blalock & Smith, 1985; Berkenbosch, Wolvers & Derijk, 1991; Dunn, 1990). The immune system, recognizing that injury or infection has occurred, initiates metabolic processes aimed at eliminating antigenic or pathogenic factors, while the neuroendocrine system ensures that the metabolic demands of the host are met (Watkins et al., 1995; Blalock, 1994). Typically, endogenous or exogenous threats to homeostatic mechanisms, which include injury, bacterial infection or inflammation, give rise to a cascade of immunological alterations. In this context, the host organism's primary attempt to restore homeostasis is characterized by metabolic and neuroendocrine changes collectively referred to as the acute phase response (see Kent, Bluthé, Kelley & Dantzer, 1992b for review). This response may be mediated by, among other things, activity of the HPA axis. In fact, it appears that IL-1 is exquisitely effective in promoting secretion of CRH from the PVN, and is fundamental in the release of pituitary adrenocorticotropin releasing hormone (ACTH) and adrenal corticosterone (Sapolsky, Rivier, Yamamoto, Plotsky & Vale, 1987; Berkenbosch, Van Oers, Del Rey, Tilders & Besedovsky, 1987; Turnbull & Rivier, 1995). It was proposed, as well, that IL-1 (and possibly other cytokines) may influence hypothalamic norepinephrine (NE), which in turn may come to affect CRH release (Dunn, 1988; Dunn, 1990; Lavicky & Dunn, 1995). Additionally, IL-1 may also influence mesolimbic NE and dopamine (DA) and might thus influence affect (Anisman et al., 1993).

In assessing the involvement of immune activation on endocrine and neurotransmitter processes, numerous investigators have opted to evaluate the effects of the endotoxin, LPS, which is a subcellular component derived from the outer membrane of

gram-negative bacteria. Endotoxins, normally present in a healthy host are typically not found in circulation, but under pathological conditions may gain access to the bloodstream. As a result, LPS challenge may cause neuroendocrine and behavioral changes similar to those seen during the acute phase response to infection (Dantzer & Kelley, 1989; Kent et al., 1992b).

It is generally believed that LPS does not cross the blood-brain barrier, but rather induces its effects through locally mediated signals which are relayed to neuronal targets (Tilders, DeRijk, Van Dam, Vincent, Schotanus & Persoons, 1993). Peripheral administration of LPS releases CRH from the hypothalamus, and hence, increases plasma ACTH and corticosterone levels (Kakucska, Qi, Clark, & Lechan, 1993). Inasmuch as the effects of LPS are attenuated or blocked by an IL-1 receptor antagonist (IL-1ra), it is likely that the effects of LPS are subserved by IL-1 (Schotanus, Tilders & Berkenbosch, 1993; Ebisui et al., 1994). Moreover, there is reason to suspect that IL-1 may be acting conjointly with IL-6 and TNF- α to promote the endocrine effects engendered by LPS (Long, Otterness, Kunkel, Vander & Kluger, 1990; Dunn, 1992a; Zanetti et al., 1992; Perlstein et al., 1993). Such an outcome is, of course, not surprising, given that LPS first promotes the appearance of TNF- α in the circulation, followed by IL-1 and IL-6 (Zanetti et al., 1992). It remains unclear, however, whether CRH release is a direct result of the action of the cytokines on the brain or is secondary to endotoxin-induced cytokine-release in the periphery.

Recently, investigators have reported that subdiaphragmatic vagotomy can suppress CRH and ACTH secretion ordinarily resulting from LPS-induced HPA activation (Gaykema et al., 1995). These data suggest a putative vagal contribution to the immune response following endotoxin administration (Laye et al., 1995). The sectioning of vagal afferents has also been shown to block IL-1 induced ACTH secretion (but not corticosterone secretion) (Kapcala, He, Gao, Peiper & DeTolla, 1996), providing additional support for the view that cytokines may be influencing the brain via primary

sensory afferents. Gaykema et al. (1995) studied c-fos expression in CRH neurons in the PVN following endotoxin administration. Complete subdiaphragmatic vagotomy blocked the ACTH response to low doses, and attenuated the response to high doses of LPS in the rat, while leaving the corticosterone response intact. In addition, Fos expression in CRH neurons in the PVN was suppressed following subdiaphragmatic vagal transection. Taken together, these studies indicate that immune-brain communication may be mediated by vagal afferents, possibly through cytokine activation of peripheral sites.

Illness Behaviour: Signs and Symptoms

Following immune challenge, animals often exhibit non-specific symptoms of illness, including fever, anorexia, decreased water intake, hyperalgesia, increased somnolence, piloerection, lethargy and reduced social exploration (see Hart, 1988 for review). While such behaviors were once considered a secondary response to illness, they are now regarded as an active part of the host organism's attempt to restore homeostasis (Dantzer & Kelley, 1989). One component of the febrile response includes increased body temperature owing to endogenous pyrogen release, which stimulates the immune response. Other sickness behaviors that elevate body temperature (eg. shivering and piloerection among others), and reduce the incidence of metabolically expensive activities (eg. somnolence, reduced libido, reduced social exploration), may well maximize the organism's ability to contend with environmental challenges (Kent et al., 1992b). It has been demonstrated that behaviors associated with illness are not secondary to fever, as the behavioral effects of inflammatory stimuli can be dissociated from the pyrogenic effects of the treatments. (O'Reilly, Vander & Kluger, 1988; Johnson, Propes & Shavit, 1996)

Evidence that pro-inflammatory cytokines, such as IL-1, IL-6 and TNF- α , are involved in the mediation of endotoxin-induced illness has been derived from investigations which demonstrated that systemic administration of IL-1 or TNF- α prior to LPS challenge mitigates the pyrogenic effects of the endotoxin (Long, Morimoto, Nakamori & Murakami, 1992; Klir, McClellan, Kozak, Szelenyi, Wong & Kluger, 1995). In addition, the

peripheral administration of IL-1, IL-6 and TNF- α results in behaviors reminiscent of those seen in response to LPS challenge (Shimomura et al., 1990; McLaughlin, Rogan, Tou, Baile & Joy, 1992; Chance & Fischer, 1992; Goujon, Parnet, Aubert, Goodall & Dantzer, 1995). Predictably, cytokine receptor antagonists, as well as antibodies directed against the cytokines, prevent or limit the effects of LPS on these behaviors (Bluthe, Dantzer & Kelley 1992; Plata-Salman & French-Mullen, 1992). Taken together, these studies support the notion that the behavioral components of the immune response to endotoxin challenge may be mediated by some of the cytokines.

It would be premature to conclude that all sickness behaviors are mediated by an identical mechanism, or conversely, that only one mechanism can mediate individual behavioral components of the illness response. Langhans, Savoldelli and Weingarten (1993), for example, demonstrated that in the rat, peripheral administration of either IL-1 or LPS provoked anorexia. While the anorexia associated with LPS resulted from a reduction in meal frequency, the IL-1 associated appetite suppression was expressed as a reduction in both meal frequency and meal size. Paracetamol, which inhibits brain cyclooxygenase, attenuated the IL-1-, but not the LPS-induced anorexia, suggesting that IL-1 affects feeding through a direct action on the brain (likely involving the pyrogenic effects of IL-1), whereas other mechanisms may mediate the effects of LPS on feeding behavior.

It should be emphasized that the pyrogenic and behavioral effects of the cytokines do not appear to be mediated exclusively through central mechanisms. DeRijk, Van Rooijen, Tilders, Besedovsky, Del Rey and Berkenbosch (1991) reported that in rats, the elimination of peripheral macrophages (which secrete IL-1), attenuated the pyrogenic response to LPS. Klir et al. (1995) found that systemic administration of TNF- α attenuated LPS-induced fever in rats, whereas central administration had no such effect. Bluthe et al., (1992) demonstrated that, in rats, the decreased social exploration and reduced body weight associated with peripheral LPS injection was attenuated by peripheral

IL-1ra, but not by intraventricular IL-1ra administration. Taken together, these studies indicate that peripheral cytokines are intimately involved in the sickness response associated with immune activation, but these data do not preclude the involvement of central mechanisms in this respect.

There are several sources of evidence suggesting that ACTH and/or corticosterone may be integral to some of the behavioral/illness effects associated with immune activation (Goujon et al., 1995; Johnson et al., 1996). For instance, while the febrile response associated with endotoxin challenge is likely subserved, in part, by activation of prostaglandin E (PGE), it appears that endogenous glucocorticoids may also play a role in this respect (Morrow, McClellan, Conn & Kluger, 1993). It was reported that pretreatment of rats with dexamethasone abolished the fever produced by an acute systemic injection of LPS. Conversely, adrenalectomized rats showed an increased febrile-response to LPS administration, which could be inhibited by acute or chronic treatment with dexamethasone (Coelho, Souza & Pela, 1992). Further, pretreatment with the glucocorticoid antagonist, RU-38486 (following oral, or intracerebroventricular administration), resulted in elevated fevers relative to those in evidence among vehicle-treated rats following acute LPS administration (Morrow et al., 1993; McClellan, Klir, Morrow & Kluger, 1994).

In addition to the potential effects on fever, there is evidence suggesting that glucocorticoids may be involved in other symptoms of illness which are associated with LPS treatment. Specifically, it was demonstrated that administration of LPS to adrenalectomized rats provoked sickness behavior (eg. increased body temperature, reduced social exploration and reduced body weight) and that such an effect could be antagonized by corticosterone implants (Johnson et al., 1996). Moreover, Goujon et al. (1995) found that in mice, subcutaneous IL-1 or LPS administration reduced social exploration, an effect which could be enhanced by adrenalectomy. The same effect was evident among intact animals given subcutaneous injections of RU-38486, indicating that a reduction in glucocorticoid concentration was likely related to the enhanced susceptibility to

the behavioral effects of LPS. Indeed, corticosterone implants had a protective effect on the adrenalectomized rats injected with IL-1, and partially protected mice injected with LPS. In a similar fashion, Pezeshki, Pohl and Schobitz (1996) demonstrated that LPS administration in adrenalectomized rats, in doses which ordinarily induce fever and anorexia, promoted a more profound reduction of locomotor activity relative to intact endotoxin-treated animals. In effect, it seemed that the absence of corticosterone permitted greater development of illness behavior. As might be expected, the behavioral effects (with the exception of the reduction in locomotor activity) could be attenuated by pretreatment with corticosterone prior to LPS administration.

An alternative accounting for some of the behavioral effects of immune activation, including sickness behavior, is derived from the work of Blalock and colleagues (Smith, Meyer & Blalock, 1982; Blalock, 1984; Blalock & Smith, 1985). It was proposed that activated lymphocytes secrete an ACTH-like substance which may directly or indirectly stimulate CNS processes. In fact, it was shown that following hypophysectomy, immune challenge still increased plasma ACTH levels, possibly reflecting the release of this hormone from activated lymphocytes (Blalock, 1984). Subsequent studies conducted by Dunn, Powell and Gaskin (1987) suggested that some of the observations derived by Blalock may have been due to incomplete pituitary ablation, which afforded an ACTH source distinct from lymphocyte-ACTH secretion. While incomplete hypophysectomy may have been a factor in the detection of ACTH release, it is likely that pituitary endocrines released during immune activation promote CNS effects.

MULTISYSTEM INTERACTIONS

Sympathetic Nervous System and the HPA Axis

As indicated earlier, the similarities between the effects of stressors and immune challenge on endocrine and CNS processes gave rise to the proposition that immune challenge may be interpreted by the brain as if it were a stressor (Dunn, 1990). Upon detection of a stressor, the sympathetic nervous system is activated, causing rapid

alterations in catecholamine levels in the blood. It has been suggested that the initial stressor-provoked peripheral NE release may be essential for activating immune processes (Felten & Felten, 1991). Indeed, it appears that the sympathetic nervous system innervates a wide variety of organs and tissues, including the primary and secondary lymphoid organs, as well as the spleen. Moreover, NE receptors are present on lymphocytes, and these cells may be activated by factors that provoke sympathetic NE release (e.g., stressors) (Madden & Livnat, 1991). Like NE, it has been demonstrated that glucocorticoid receptors are present in the spleen, thymus and lymph nodes, as well as on the cell surface of lymphocytes. Activation of these glucocorticoid receptors have the effect of reducing immune activity (Keller, Schleifer & Demetrikopoulos, 1991). It has been suggested that one of the functions of circulating corticosterone is that of limiting the extent to which the immune system is activated by stressors, hence reducing the potential autoimmune effects that might otherwise occur (Munck, Guyre, & Holbrook, 1984). In effect, it seems that the immune system is intrinsically linked to both HPA functioning and sympathetic nervous system activity.

Immune-CNS Communication

Consistent with the notion that immune activation has CNS effects, it has been shown that antigenic challenge promotes catecholamine turnover in the brain and the periphery. In particular, in rats and mice, administration of sheep red blood cells (SRBC) results in changes of NE turnover in the locus coeruleus and hypothalamus (Zalcman et al., 1991), and DA turnover in mesocortical sites (Lacosta, Merali & Anisman, 1995). In addition, SRBC administration alters serotonin (5HT) levels in the paraventricular and supraoptic nuclei (Carlson, Felten, Livnat & Felten, 1987). These changes, along with increased electrophysiologic activity in the PVN and in the locus coeruleus (Besedovsky, Sorkin, Felix & Haas, 1977), corresponded with the immune response being mounted (Carlson et al., 1987; Zalcman et al., 1991; Lacosta et al., 1995).

Like the effects of SRBC, it was recently demonstrated that neuroendocrine activation may occur as a result of viral challenge or infectious disease. In particular, pathogens such as Newcastle disease virus (NDV) and influenza virus are associated with HPA activation (Dunn, Powell, Meitin & Small, 1989), and increased hypothalamic NE release (Dunn & Vickers, 1994). Intraperitoneal injection of NDV also increased hypothalamic concentrations of tryptophan, the NE catabolite 3-methoxy 4-hydroxyphenylethyleneglycol (MHPG), and the 5-hydroxytryptamine (5-HT) catabolite 5-hydroxyindoleacetic acid (5-HIAA). In view of the observation that hypophysectomy blocked elevations in plasma corticosterone following NDV and LPS challenge, it seems that the corticosterone changes were probably not a result of viral stimulation of the adrenal, but rather involved HPA activation.

It will be recalled that there are sufficient data to suggest that IL-1 may function as an immunotransmitter to provoke endocrine secretion following immune challenge. Among other things, IL-1 is a potent stimulator of CRH release, and hence ACTH and corticosterone secretion (Besedovsky et al., 1986; Berkenbosch et al., 1987; Sapolsky et al., 1987; Wehrenberg, Saudan, Corder, Voltz, Giustina & Gaillard, 1994; Melink Parsadaniantz, Levin, Lenoir, Roberts & Kerdelhue, 1994). Moreover, central pretreatment with antagonists of IL-1 or CRH limited the ACTH and corticosterone variations induced by either IL-1 or LPS treatment (Dunn, 1992a; Schotanus et al., 1993; Ebisui et al., 1994). Not unexpectedly, the increased paraventricular CRH messenger RNA (mRNA) observed following intraperitoneal administration of LPS in rats, could also be abolished by intraventricular IL-1ra pre-treatment (Kakucska et al., 1993). In addition, dexamethasone was found to inhibit IL-1ra mRNA levels in endotoxin-stimulated human monocytes, and inhibited the secretion of IL-1ra. These effects could be reversed by the glucocorticoid antagonist RU 38486 (Arzt et al., 1994).

In addition to these endocrine variations, there is reason to suppose that the effects of immune challenge on extrahypothalamic NE, DA, and 5-HT may involve cytokines. In

particular, it was demonstrated that systemic administration of IL-1 promotes several distinctive monoamine changes (eg. DA turnover in the prefrontal cortex), which are reminiscent of the effects associated with stressors (Merali, Lacosta, & Anisman, 1997, in press). Yet, IL-1 and SRBC have distinctly different effects with respect to some of the central neurochemical changes observed. In particular, while SRBC provokes in vivo accumbal DA release (Lacosta et al. 1995) IL-1 does not produce such an outcome, and IL-6 reduces DA release (Merali et al., 1997 in press).

CYTOKINES INFLUENCE ENDOCRINE/CNS TRANSMITTERS

Immune activation involves the release of numerous cytokines, including IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF- α , and interferon- γ among others. Most of these cytokines have not been assessed extensively insofar as their endocrine and behavioral effects are concerned (see Berkenbosch, de Rijk, Del Rey & Besedovsky, 1990; Schobitz et al., 1994; Turnbull & Rivier, 1995 for review). It is conceivable that in addition to the pro-inflammatory cytokines, one or more of these other components may act to signal endocrine/CNS processes. Nevertheless, the available data have suggested a prominent role for IL-1, IL-6 and TNF- α in promoting an interface between endocrine and CNS activity. Accordingly, the present review has been limited to these cytokines. In discerning potential individual or synergistic effects of this subset of cytokines, one would be remiss if a more detailed description of their actions were not described.

There are three forms of IL-1, IL-1 α , IL-1 β and IL-1ra. Although IL-1 β is the major secreted form, IL-1 α and IL-1 β nevertheless appear to have similar effects on numerous systems (Rothwell & Luheshi, 1994). Studies in rats suggest, however, that IL-1 β has more prominent effects than IL-1 α with respect to HPA activation. For instance, IL-1 β is more effective than IL-1 α in promoting CRH release from the PVN and hence ACTH release from the pituitary (Harbuz, Stephanou, Sarlis & Lightman, 1992). In addition, the fever and thermogenesis induced by intracerebroventricular (icv)

administration of IL-1 β , but not IL-1 α , can be blocked with a central injection of a CRF receptor antagonist (Long et al., 1990; Rothwell & Luheshi, 1994). Likewise, the receptor antagonist, IL-1ra counteracts the HPA stimulating action of IL-1 β (Dunn, 1995).

TNF exists in two forms, TNF- α and TNF β , which share approximately 50% sequence homology. TNF- α (or cachectin) is secreted by a variety of immune cells, whereas TNF- β is exclusively secreted by activated lymphocytes. IL-6 is a B-cell growth factor that (along with IL-1) augments T-lymphocyte mitogenesis. Under homeostatic conditions, the expression of IL-1, IL-6 and TNF- α is ordinarily low in most tissues, peripheral and central sites, while a marked increase in synthesis and secretion is detected following inflammation or infection. Like IL-1, elevations of IL-6 and TNF- α not only have paracrine and endocrine-like effects, but also autocrine effects which inhibit their own activity, hence limiting immune overshoots (Schobitz et al., 1994).

The mechanisms by which the cytokines signal the brain following infection has yet to be fully elucidated. Receptors for IL-1, IL-6 and TNF- α have been localized in various brain regions (including the hippocampus and hypothalamus), although it is unclear how cytokines gain access to the brain, as they are large, lipophobic proteins that are unlikely to pass through the blood-brain barrier (BBB). It appears, however, that the cytokines may gain access to the brain via carrier-mediated transport, or possibly via regions lacking a blood-brain barrier (eg. the organum vasculosum of the lamina terminalis) (Watkins et al., 1995a). An alternative explanation for the influence of cytokines on behavioral change is derived largely from the observation that illness behaviour can be induced in the absence of detectable levels of LPS or cytokines in the blood. In effect, central activation may not be dependent on blood-borne cytokines. Rather, it has been suggested that cytokines may activate peripheral afferent nerves at or near the site of release. It will be recalled, for example that subdiaphragmatic vagotomy blocks many of the illness responses following IL-1, TNF- α and LPS, suggesting a system of immune-brain communication that is based

on humoral rather than blood-borne factors (Laye et al., 1995, Watkins et al., 1994; Bluthé et al., 1994, Gaykema et al., 1995).

Alterations in IL-1 levels have been implicated in a number of physiological changes resulting from LPS administration. In order to assess the role of IL-1 in LPS-induced pyrogenesis, Derijk, Van Rooijen, Tilders, Besedovsky, Del Rey & Berkenbosch, 1991 and Marquette, Van Dam, Van Rooijen, Berkenbosch and Haour (1994) eliminated peripheral macrophages through the administration of dichloromethylene diphosphonate (Cl₂MDP) liposomes. This treatment attenuated the rise in plasma IL-1 concentrations in response to LPS and also abrogated the thermogenic and fever response to LPS in rats. These data suggest that peripherally produced IL-1 is involved in the pyrogenic effects of LPS. However, peripheral injection of LPS has been shown to induce IL-1 mRNA in rat brain microglial cells (Buttini & Boddeke, 1995) and induce IL-1 activation in ramified microglia, as well as activated macrophages in the meninges and choroid plexus of the rat brain (Van Dam, Brouns, Louisse & Berkenbosch, 1992). Thus, there appears to be a central role of IL-1 in LPS-induced neural activation. Interestingly, depletion of macrophages also prevented the plasma ACTH and corticosterone responses to sub-pyrogenic doses of LPS. However, this effect on ACTH and corticosterone was not evident in response to pyrogenic doses of LPS (Derijk et al., 1991). Evidently, the pyrogenic actions of IL-1 can be disassociated from the ACTH variations following endotoxin treatment.

There are distinct variations in the kinetics of the various cytokines following LPS administration (de Simoni, Del Bo, De Luigi, Simard & Forloni, 1995). It has also been demonstrated that the pattern of mRNA induction in rat brain following peripheral LPS injection is different for each of the cytokines (Gatti & Bartfai, 1993), indicating that they may play temporally linked divergent roles following immune activation. For example, TNF- α levels peak approximately one hour following LPS administration, whereas, IL-1 and IL-6 peak within 2-4 hours (Zanetti et al., 1992; Derijk et al., 1993; Schotanus et al.,

1993; Fantuzzi, Di Santo, Sacco, Benigni & Ghezzi, 1995). In addition, de Simoni et al. (1995) noted that the levels of TNF- α decline more rapidly than those of IL-1 and IL-6, suggesting that TNF- α may initiate HPA activation following LPS administration, while IL-1 and IL-6 sustain the activation once it has been triggered

Evidence in favour of the conjoint action of IL-1, IL-6 and TNF- α in mediating the endocrine effects of LPS is derived from investigations evaluating the effect of cytokine antibodies. For instance, the fact that systemic administration of IL-1 itself induces marked HPA alterations suggests that the role this cytokine is apparently more significant than sustaining a previously excited HPA system. Of course, the possibility cannot be dismissed that the relative contributions of the three pro-inflammatory cytokines are dissimilar with respect to initiating vs. maintaining HPA activation. Moreover, recent investigations involving the synergistic effects of IL-1, IL-6 and TNF- α revealed that these cytokines may interact with respect to the activation of the HPA axis following LPS administration (Zhou et al., 1996).

Perlstein et al. (1993) reported that IL-6 antibody pre-treatment prevented the LPS-induced ACTH response ordinarily observed 2 and 4 hours after endotoxin administration. Pretreatment with either IL-1 α antibody or TNF- α antibody also blocked the 4 hour ACTH response, but did not block the response observed 2 hours after endotoxin treatment. A combination of IL-1 α and TNF- α -antibody limited the 2 hour ACTH response. In addition, it was observed that combined doses of TNF- α and IL-6, as well as TNF- α and IL-1, increased production of circulating ACTH to a greater extent than that produced by these cytokines administered separately. Moreover, pretreatment with an IL-6 antibody completely blocked the conjoint effects of TNF- α and IL-1 on the ACTH response. Since TNF- α and IL-1 both stimulate IL-6 release, the data of this investigation suggest that LPS directly stimulates IL-6 release 2 hours after administration, but that TNF- α and IL-1 synergistically induce the variations of IL-6 observed at the 4 hour interval.

In contrast to the results reported by Perlstein et al. (1993), which emphasized the contribution of IL-6 in mediating the endocrine variations associated with LPS treatment, Schotanus et al. (1993) suggested that this cytokine was not fundamental in the provocation of the ACTH alterations. In particular, it was demonstrated that peripheral IL-1 administration increased plasma ACTH, corticosterone and IL-6 concentrations. At low doses, however, the ACTH and corticosterone response to peripheral IL-1 were not accompanied by changes in plasma IL-6 concentrations, indicating that the latter cytokine was not involved in the ACTH response. In a subsequent study, dose-dependent elevations of plasma ACTH, corticosterone and IL-6 concentrations were seen following peripheral administration of LPS. The dose required to induce the median IL-6 response was 4 times higher than the dose required to induce the ACTH and corticosterone elevations. These results, along with the demonstration that the ACTH, but not the IL-6, response to endotoxin was blocked by peripheral IL-1ra, indicate that IL-6 did not play a prominent role in the initiation of the HPA response following endotoxin administration.

Additional evidence that IL-6 is not an essential mediator in the inflammatory response to endotoxin is derived from investigations involving IL-6 deficient mice. Fattori et al. (1994) found that these mice were able to generate an inflammatory response to LPS administration. Corticosterone, as well as IL-1 levels in the IL-6 deficient mice were similar to the values detected in controls following LPS administration. Similarly, the reduction in body weight and food intake following LPS administration was comparable in both groups of animals. Unexpectedly, levels of TNF- α induced by LPS were three-fold higher in IL-6 deficient mice than in controls, which prompted the suggestion that the increased TNF- α may compensate for the absence of IL-6. In effect, it appears that the corticosterone response following LPS does not require IL-6, and IL-6 is not a necessary component of the inflammatory response to endotoxin administration.

As indicated earlier, the mechanisms that mediate HPA activation following pyrogenic doses of endotoxin may be distinct from those that mediate low doses of

endotoxin. Indeed, it has been demonstrated that LPS induces dose-dependent variations of glucocorticoids, and that the relative contributions of various cytokines vary as a function of the LPS dose employed. Ebisui et al. (1994), for instance, found that when small amounts of LPS (0.03 $\mu\text{g/kg}$) were administered intravenously to rats, the maximum corticosterone response could be almost completely abolished by TNF- α -antiserum, but not by an IL-1 receptor antagonist. However, either TNF- α -antiserum or IL-1ra attenuated the response when larger amounts of LPS (10 $\mu\text{g/kg}$) were administered. These results suggest that, at low doses, TNF- α alone was responsible for mediating the effects of LPS on the HPA axis, while IL-1 participated in stimulating ACTH secretion following high doses. Moreover, following LPS administration, recruitment of IL-1 and TNF- α in the provocation of ACTH release may occur at different times.

Anorexia is Influenced by Synergistic Actions of Cytokine Treatment

Although it is clear that IL-1, IL-6 and TNF- α all participate in the HPA response to endotoxin, it is difficult to determine which cytokine initiates the HPA response. It will be recalled that multiple behavioral and endocrine interactions exist between the cytokines. Not only do the cytokines act in synergistic ways, depending on the combination of cytokines activated, but IL-1, IL-6 and TNF- α can all modulate the synthesis and secretion of one another. In view of the relative paucity of data concerning the interactive effects of cytokines in modulating endocrine functioning, it is not surprising that still fewer studies have focused on the behavioral synergisms that occur in response to cytokine challenge.

As indicated earlier, anorexia is a prominent behavioral response evident following immune challenge, and has been assessed following administration of endotoxins. Dose-dependent reductions in food intake and body weight have been demonstrated following both peripheral (O'Reilly et al., 1987) and central (McCarthy, Kluger, & Vander, 1986) administration of LPS. It has been suggested that the endotoxin-induced reduction in feeding may be mediated, in part, by the proinflammatory cytokines. Indeed, IL-1, IL-6 and TNF- α , all of which are induced by LPS administration, suppress food intake (Plata-

Salaman, Oomura & Kai, 1988; Tracey et al., 1988; Socher, Freeman & Martinez, 1988; Michie, Sherman, Spriggs, Rounds, Christie & Wilmore, 1989; Fantino & Wieteska, 1993; Langhans et al., 1992; Fattori et al., 1994; Plata-Salaman, 1994). Inasmuch as the anorexic effects can be induced by either peripheral or central administration of IL-1 raises the possibility that this effect is, at least in part, centrally mediated (Shimomura et al., 1990, Chance & Fischer, 1991; McLaughlin et al., 1992). Commensurate with this view, peripheral administration of IL-1 antagonists blocked the anorexic effects of systemic IL-1 (McLaughlin et al., 1992), and central administration of IL-1ra attenuated the anorexia induced by icv IL-1 (Plata Salaman & French-Mullen, 1992; Plata-Salaman & Borkoski, 1993). Kent, Bret-Dibat, Kelley & Dantzer (1996) demonstrated that central administration of IL-1 profoundly decreased operant responding for food reinforcement, and this effect could be prevented by central IL-1ra pretreatment. Interestingly, however, central IL-1ra did not completely block the effect of peripheral IL-1, nor did peripheral IL-1ra block the effects of central IL-1. These results suggest that IL-1 influences both peripheral and central mechanisms to reduce food-motivated behavior.

TNF- α and IL-6, it will be recalled, have also been shown to have a direct action in the CNS, and it has been suggested that TNF- α exerts its anorectic effects through centrally mediated mechanisms. Rogers, Mctigue and Hermann (1996) demonstrated that the nausea influence of gastric relaxation, could be evoked through unilateral TNF- α injections into the dorsal vagal complex. In addition, Fantino & Wieteska (1992) demonstrated that, in rats, central administration of TNF- α resulted in a decrease in food and water intake, whereas peripheral administration had no effect. Moreover, electrophoretically applied TNF- α suppressed the activity of glucose-sensitive neurons in the lateral hypothalamus. (Plata-Salaman et al., 1988). Since IL-1 was found to produce similar effects, it was posited that some of the effects of TNF- α may have been secondary to the induction of IL-1.

It has been suggested that IL-6 may play a role in the attenuation of anorexia following IL-1 receptor blockade. Oldenburg et al. (1993) investigated the effects of IL-6 and IL-1r antibodies on anorexia in the mouse following sterile turpentine abscess. The reduction in food intake and resulting weight loss were attenuated by both IL-1r and IL-6 antibody administration. The co-administration of IL-6 and IL-1r antibodies did not, however, result in a significantly greater reduction in the anorexic effects than those in evidence following the administration of the each separate antibody. Based on the additional observation that IL-1r blockade attenuated production of IL-6, the investigators concluded that IL-6 may be the more direct mediator of anorexia and weight loss in this model.

Although there is evidence that the anorexic effects of LPS and IL-1 are similar to one another, there are conflicting reports regarding the effects of endotoxin on meal patterns. For example, O'Reilly et al. (1988) reported that prolonged exposure to LPS did not result in tolerance to the anorexic effects, whereas Langhans et al. (1993) demonstrated that tolerance did develop following prolonged LPS exposure. Interestingly, Langhans et al. also reported that intraperitoneal LPS reduced food intake by decreasing meal frequency, without affecting meal size. In contrast to this finding, however, Plata-Salaman (1994) found that LPS significantly reduced meal size. It is important to note that while Plata-Salaman (1994) administered LPS centrally, Langhans et al. (1993) used peripheral injections to induce anorexia. Thus, it is conceivable that different mechanisms subserve the anorexia, with only the central processes affecting meal size.

Although numerous effects of LPS, as already described, have been attributed to activation of IL-1, there is evidence that LPS and IL-1 induced anorexia are mediated through different mechanisms. Whereas repeated injections of IL-1 lead to sensitization of the anorexic effects, prolonged exposure to LPS lead to tolerance, with no evidence of cross-tolerance or cross-sensitization between the compounds (Langhans et al., 1993). Moreover, the cyclooxygenase inhibitor, paracetamol, significantly attenuated the IL-1-

but not the LPS-induced anorexia, suggesting a role for prostaglandins in the IL-1 mediated effects.

Vagotomy and Feeding

It will be recalled that several investigators argued that communication between the immune system and the CNS may occur through the vagal nerve. This proposition was based on, among other things, the finding that subdiaphragmatic vagotomy attenuated the hormone variations otherwise provoked by immune challenge or bacterial endotoxins (Gaykema et al., 1995). Severing the vagus nerve eliminates sensory afferent fibres which carry information concerning the gastrointestinal tract to the central nervous system. In addition, efferent fibres that are involved in motor activities of the intestine and stomach are also severed, which eliminates integral sources of information concerning intestinal content, as well as CNS control over the secretory and motilic functions of the stomach (Shapiro & Miselis, 1985). Given the important contribution of the vagus to intestinal processes, it should not be surprising that subdiaphragmatic vagotomy, which involves severing of the vagal–afferent and efferent innervation, influences feeding in humans and in infrahuman subjects (Yang, Ratto, Gleason, Bellantone, Crucitti & Meguid, 1992). Such effects are not dietary dependent, and are readily apparent when dry food or more palatable liquid diets are provided (Davis, Smith & Kung, 1994; Bernstein, 1995).

Davis et al. (1994) demonstrated that, in rats, the reduced consumption of a liquid diet following vagotomy could be attributed to a decline in the rate of licking during the meal, which was not related to motor dysfunction. Analysis of the microstructure of the licking response revealed that vagotomized rats had shorter licking bouts at the beginning of the test, and a characteristically defined rapid decline in the licking rate during the test. This behavioral profile was independent of the type of diet provided (milk, or one of 3 concentrations of sucrose solution) suggesting that neither motivational factors, nor nutritional content, were particularly relevant in inducing the pattern of behavioral change in evidence. It was suggested that the decline in the rate of licking during the meal was

attributable to an accelerated clearance of fluid from the stomach, which enhanced negative feedback signals from the gastrointestinal tract ordinarily conveyed to the CNS through extravagal pathways.

Recently, it has been suggested that the reduction in food intake following vagotomy can be attributed, at least in part, to peripheral endocrine factors (Rogers et al., 1996). The dorsal vagal complex (DVC) lacks a blood brain barrier, which renders this site susceptible to humoral signals such as cytokines and hormones. Since the DVC influences parasympathetic activity associated with digestive processes, cytokines and ensuing hormonal changes may exert effects on feeding through indirect signals acting on parasympathetic functioning.

Anorexia and Anhedonia

Regardless of whether cytokines, vagal innervations, or a combination of the two mediate the behavioral responses to endotoxin challenge, a note of caution must be interjected concerning the processes aligned with the anorexic symptoms. When describing the “sickness behavior” associated with immune activation, most investigators have concluded that compounds such as LPS induce a reduction in feeding and also decrease motivation to consume food. On the one hand, the reduced motivation may reflect diminished appetite secondary to illness, but on the other hand it may reflect a diminution in the rewarding attributes ordinarily ascribed to feeding.

It has recently been suggested that activation of the immune system results in physiological and behavioral changes that resemble the characteristics of depression. Indeed, it has been demonstrated that in some cases, depression is associated with immune activation, characterized by an increase in plasma levels of cytokines such as IL-1 and IL-6, and dysregulation of the HPA axis. The loss of appetite, reduced body weight, increased somnolence and anorexia which follow cytokine or endotoxin administration in animals are all symptoms of depression. In addition, animal models of depression include the attenuation of sucrose consumption and place-preference paradigms which are used to

study the rewarding properties of food and drugs (Willner, Towell, Sampson, Sophokleous & Muscat 1987; Papp, Willner & Muscat, 1991; Suzuki et al., 1994). In effect, the view can be taken that the reduced food consumption associated with endotoxin administration may be a reflection of the potential anhedonic and depressionogenic effects of this treatment. Indeed, it was demonstrated that peripheral pretreatment with LPS abolished the reinforcing effects of cocaine in the place-preference paradigm (Suzuki et al., 1994).

Consistent with the view that LPS induces depressive-like effects, Yirmiya (1996) demonstrated that a single dose of LPS suppressed sexual behavior in male rats, and reduced their preference for, and consumption of, a saccharin solution. Furthermore, repeated treatment with clinically effective antidepressant agents (eg. imipramine) effectively eliminated the anorexic effects ordinarily induced by LPS treatment.

Purpose

In view of the observation that the proinflammatory cytokines may have synergistic actions with respect to hormonal functioning, it was of interest to establish whether these cytokines would also have such effects on behaviors associated with illness. In this context, the present investigation was undertaken to determine the individual and conjoint actions of IL-1, IL-6 and TNF- α on the consumption of a highly palatable food, as well as the variations of plasma corticosterone levels. Inasmuch as few studies have assessed these cytokine actions, despite their recent clinical usage (Meyers & Valentine, 1995), a dose response curve was determined for LPS and for each of the cytokines, after which the conjoint actions of suboptimal doses of the cytokines were evaluated.

Experiments 1-4

The initial Experiments were conducted in order to evaluate the anorexic effects, as well as the corticosterone variations induced by LPS, IL-1, IL-6 and TNF- α . Dose-response curves were established for these compounds, since sub-optimal doses had to be determined to evaluate potential synergistic actions of the components. As well, the initial experiments sought to establish whether the behavioral effects of the treatments paralleled their potential hormonal actions.

Materials and Methods

Subjects

Male CD-1 mice, 8-12 weeks of age were obtained from Charles River Inc, Laprairie Quebec. After arrival at the facility, mice were housed in groups of four and acclimated to the laboratory for at least 2 weeks. Room temperature was maintained at 21 $^{\circ}$ C. lighting was maintained on a 12 hr light/dark cycle (lights on at 0800). One week prior to the beginning of the experiments, mice were separated into individual polypropylene cages with wire mesh lids and maintained ad libitum on pellet mouse chow (5075 Rodent diet Autoclaved, Ralston Purina), and tap water.

Prior to LPS or cytokine administration, mice were given free access to chocolate milk (Sealtest, 1% partly skimmed) for one hour each day during the light phase (bottles in at 10:00). Bottles were weighed at the beginning and end of the hour in order to determine consumption (weights were converted to volume). Following the establishment of a steady rate of drinking (3 consecutive days on which consumption varied by less than 10%), mice received an acute intraperitoneal (i.p.) injection of either vehicle or LPS, at one of several doses. 90 min prior to receiving the chocolate milk. On the day following drug administration, consumption was again measured in order to determine rate of recovery.

Likewise, in Experiments 2-4, following establishment of baseline consumption, mice received an acute i.p. injection of either vehicle or IL-1 β , IL-6 or TNF- α (at one of

several doses) and 45 min (in the case of IL-1 and IL-6) or 30 min (in the case of TNF- α) afterward, consumption was measured.

Drugs

LPS (from *Escherichia coli* serotype 026:B6 L-3755 Sigma) was dissolved in sterile saline (9%) and administered i.p. 90 minutes prior to receiving milk. Each mouse received a single dose of 0.5, 0.75 or 1.0 μg ($n=10$ per group) All drugs were administered in a volume of 0.4 ml. Vehicle animals were injected i.p. with 0.4 ml of sterile saline.

IL-1 β was kindly provided by Dr. Craig Reynolds (Biological Response Modifiers Program, National Cancer Institute, Frederick, Md. USA. Produced by E.I. Dupont De Nemours. specific activity 1.81×10^4 units/ μg). TNF- α (specific activity= 1.1×10^5 units/mg) and IL-6 (specific activity= 7.0×10^3 units/ μg) were obtained from R&D Systems. All drugs were suspended in sterile saline (9%). IL-1 and IL-6 were administered i.p. 45 minutes prior to receiving milk, whereas TNF- α was administered i.p. 30 minutes prior to receiving milk. IL-1 was administered in a single dose of 0.025, 0.05, 0.1, 0.4, or 0.8 μg ($n=10$ per group) in a volume of 0.4 ml. IL-6 was administered i.p. in a single dose of 0.1, 0.2, 0.8 or 1.6 μg ($n=10$ per group). TNF- α was administered i.p. in a single dose of 1, 2 or 4 μg ($n=10$ per group). Vehicle animals in all groups ($n=10$ per group) were injected i.p. with 0.4 ml sterile saline.

In parallel experiments mice received i.p. administration of either the LPS¹, IL-1, IL-6 TNF- α or their respective vehicles, and then either 120 minutes (in the case of LPS) or 60 minutes afterward (in the case of the cytokines), mice were decapitated and trunk blood was collected in tubes containing 10 μl EDTA. The blood was centrifuged at

¹Mice in the LPS study were also used to evaluate the anxiogenic effects of endotoxin treatment. Thus, mice had received a 5 min exposure to a light-dark box at 90 min following LPS treatment. As the endotoxin was without effect on light-dark performance, it was deemed unnecessary to conduct an additional experiment to evaluate the already well documented hormonal actions of LPS.

3500 rpm. for 15 minutes, and the plasma frozen and stored at -70^o C. The plasma corticosterone concentrations were determined, in duplicate, by radioimmunoassay using kits obtained from ICN Biomedicals Inc². These assays were found to yield less than 10% intra- and interassay variability.

Results and Discussion

Variations of consumption of palatable diet

Figures 1 and 2 show the variations of chocolate milk consumption over a 1 hr period as a function of the treatment mice received.

LPS

On test day, an acute i.p. injection of LPS 90 min prior to testing reduced consumption of chocolate milk (See Figure 1). A repeated-measures analysis of variance revealed a significant Treatment x Sampling period interaction $F(6.72)=6.829$, $p<.01$. Newman-Keuls multiple comparisons of the means comprising the simple effects of this interaction ($\alpha = .05$) indicated that among vehicle treated mice consumption did not differ over any of the three sampling periods. In contrast, all doses of LPS provoked a significant reduction in consumption compared to baseline levels. Consumption of mice retested 24 hr after LPS treatment returned to baseline levels. Between group comparisons indicated that on Test day, consumption in LPS-treated animals at each dosage was significantly reduced relative to vehicle treated mice.

Insert Figure 1 about here

IL-1

IL-1 administration reduced consumption of chocolate milk (See Figure 2-Upper panel), and the analysis of variance indicated a significant Cytokine x Sampling period

²Owing to the expense of the assays, the effects of only some of the doses were determined. The specific doses for each drug were based on pilot studies.

Figure 1. Mean ($\pm$ S.E.M.) consumption (ml) of a highly palatable food among mice that received peripheral lipopolysaccharide (LPS) or saline. Various doses of LPS (0.5 μ g, 0.75 μ g or 1 μ g) were administered intraperitoneally to male CD-1 mice and consumption of chocolate milk was measured over a 1 hr. period commencing 90 minutes following injection. Baseline values were calculated by averaging consumption over the three day period immediately prior to testing. Recovery data were collected 24 hr. following testing.

interaction $F(10.108) = 5.435$, $p < .01$. Multiple comparisons within groups revealed that mice treated with 0.05, 0.1, 0.2 or 0.8 μg of IL-1 45 minutes prior to testing reduced consumption of chocolate milk relative to baseline levels. Consumption in all animals tested returned to baseline levels 24 hr following treatment. Among vehicle treated mice or those that received the lowest IL-1 dose (0.025 μg) consumption did not vary over the sampling periods. Between group comparisons indicated that following cytokine treatment, mice in the 0.05 - 0.8 μg groups also consumed significantly less chocolate milk than saline treated animals.

 Insert Figure 2 about here

TNF- α

Analysis of variance of chocolate milk consumption in the TNF- α treated mice yielded a significant Cytokine x Sampling period interaction, $F(6, 72) = 5.978$, $p < .01$. Multiple comparisons of the simple effects comprising the interaction indicated that among the vehicle treated mice consumption did not differ over the 3 sampling periods. In contrast, within each of the three TNF- α groups a significant decline from baseline values was evident on the day of cytokine treatment, followed by a significant increase 24 hr later. Between-group comparisons confirmed that the groups did not differ at baseline or 24 hr after cytokine treatment. The TNF- α was found to reduce consumption of the chocolate milk relative to saline-treated mice, but this difference only reached statistical significance among mice treated with the 4.0 μg dose (see Figure 2-Middle panel).

IL-6

In contrast to the effects of IL-1 and TNF- α , systemic administration of IL-6 did not significantly affect consumption (See Figure 2-Lower panel). Although a small decrease in chocolate milk intake was seen on the day of treatment in mice receiving either 0.1 or 0.2 μg of IL-6, this decrease did not approach statistical significance. Indeed, at the

Figure 2 Mean ($\pm$ S.E.M.) consumption (ml) of a highly palatable food among mice receiving peripheral interleukin-1 β (IL-1), tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6) or saline. Various doses of IL-1 (0.025 μ g, 0.05 μ g, 0.1 μ g, 0.2 μ g, or 0.8 μ g--Upper panel), TNF- α (1 μ g, 2 μ g, or 4 μ g--Middle panel), or IL-6 (0.1 μ g, 0.2 μ g, 0.8 μ g or 1.6 μ g--Lower panel) were administered intraperitoneally to male CD-1 mice and consumption of chocolate milk was measured over a 1 hr. period commencing either 45 minutes (for IL-1 and ILK-6) or 30 minutes (for TNF- α) following injection. Baseline values were calculated by averaging consumption over the three day period immediately prior to testing. Recovery data were collected 24 hr. following testing.

higher doses tested (0.8 μg and 1.6 μg), a modest increase in consumption was detected relative to baseline levels. Rates of consumption among vehicle treated mice did not differ over the three sampling periods.

Summarizing, the results of Experiments 1-4 indicate that systemic administration of IL-1, TNF- α , and LPS (which induces the synthesis and release of IL-1, IL-6 and TNF- α) dose dependently suppressed consumption of a palatable substance. In contrast, IL-6 administration was without effect. Although these findings are consistent with previous reports of cytokine- and/or LPS-induced anorexia (Van der Meer, Sweep, Pesman, Borm & Hermus, 1995; Plata-Salaman, 1995; Sonti, Ilyin & Plata-Salaman, 1996), the possibility remains that the behavioral effects observed in the present investigation may not simply reflect an anorexic response. Indeed, it has recently been argued that the administration of LPS (Yirmiya, 1996) and cytokines (Anisman et al., 1996) results in behavioral alterations that may reflect anhedonia, and in the case of LPS the behavioral changes were antagonized by chronic administration of an antidepressant. Thus, it ought to be considered that the reduction in consumption of a palatable diet following LPS or cytokine administration may be characteristic of an anhedonic as well as an anorexic response.

Hormonal Variations

Figures 3 and 4 show the variations of plasma corticosterone levels as a function of the treatment mice received.

LPS

Consistent with earlier reports (Mefford et al., 1991; Dunn, 1992a; Johnson et al., 1992), treatment with LPS was found to dose-dependently increase plasma corticosterone concentrations (See Figure 3). Analysis of variance revealed that corticosterone levels varied as a function of the LPS Treatment, $F(2, 23) = 47.763$, $p < .01$, and testing. Newman-Keuls multiple comparisons indicated that at all doses of LPS tested, plasma

corticosterone levels were significantly increased relative to saline-treated animals ($\alpha = .05$).

 Insert Figure 3 about here

IL-1

When administered i.p. one hour prior to testing, the behaviorally active doses of IL-1 increased plasma corticosterone levels in a dose-dependent manner (See Figure 4-Upper panel). Analysis of variance revealed a significant effect of Treatment $F(3,36)=10.3$, $p<.01$ and multiple comparisons indicated that in mice treated with 0.05 and 0.1 μg of IL-1 corticosterone levels were significantly increased relative to saline-treated animals. Although treatment with 0.025 μg of IL-1 produced a modest increase in plasma corticosterone levels relative to saline-treated mice, this increase did not approach statistical significance.

 Insert Figure 4 about here

TNF- α

As in the case of the IL-1 treatment, analysis of variance indicated that TNF- α administered one hour prior to testing significantly increased plasma corticosterone levels $F(3,36)=16.087$, $p<.01$. Multiple comparisons revealed that plasma corticosterone levels in mice treated with 4 μg of TNF- α were significantly increased when compared to mice treated with saline, 1, or 2 μg of TNF- α . The lower doses, however, yielded corticosterone levels that did not differ from those of control mice (See Figure 4-Middle panel).

Figure 3. Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g/dl}$) among mice that received peripheral lipopolysaccharide (LPS) or saline. Various doses of LPS (0.5 μg or 1 μg) were administered intraperitoneally to male CD-1 mice and corticosterone concentrations were measured 2 hr later (* $p < .01$ relative to controls).

Figure 4. Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/\text{dl}$) in mice receiving peripheral interleukin-1 β (IL-1), tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6) or saline. Various doses of IL-1 (0.025 μg , 0.05 μg or 0.1 μg -Upper panel), TNF- α (1 μg , 2 μg or 4 μg -Middle panel), or IL-6 (0.1 μg or 0.8 μg -Lower panel) were administered intraperitoneally to male CD-1 mice and plasma corticosterone concentrations were measured 1 hr later (* $p < .05$ relative to controls).

IL-6

In view of the observation that no significant behavioral effects of IL-6 were detected, only two doses were chosen in order to assess the effects of the cytokine on HPA activation. Consistent with earlier reports (Dunn, 1992a; Zalcman et al., 1994), the analysis of variance confirmed that there was no difference between plasma corticosterone levels in mice treated with 0.1, or 0.8 μ g of IL-6 and saline-treated animals (See Figure 4-Lower panel).

Taken together, it appears that LPS, IL-1 and TNF- α , each of which influenced consumption, also had the effect of increasing plasma corticosterone concentrations. In contrast, treatment with IL-6, which failed to affect food consumption, likewise was without effect on plasma corticosterone concentrations. It is of considerable interest that in each experiment, behaviorally active doses of LPS and the cytokines were associated with increases in HPA activity. Moreover, those doses of TNF- α and IL-6 which did not significantly alter consumption were also without effect on plasma corticosterone levels. It is conceivable that the correspondence between the changes in consumption of a palatable substance and the HPA effects induced by LPS or cytokine administration reflects a similarity in the mechanism(s) that underlie both the anorexic and the hormonal responses to peripheral cytokine administration.

Experiments 5-7

As indicated earlier, there is reason to expect that the pro-inflammatory cytokines may have synergistic effects with respect to the behavioral and hormonal variations. Experiments 5-7 assessed the synergistic actions of these cytokines. Sub-optimal doses (in terms of behavior and endocrine effects) of the cytokines were chosen to assess the synergistic effect of co-administration of IL-1 and IL-6, IL-1 and TNF- α , and IL-6 and TNF- α on consumption of a palatable substance and plasma corticosterone levels.

Methods

A total of 40 experimentally naive, male, CD-1 mice were used in each of Experiments 5-7. The subject characteristics and the housing conditions were identical to those described in the preceding Experiments.

In 3 independent studies, mice received the treatments shown in Table 1. In each instance the compounds were administered in two successive i.p. injections, in a total volume of 0.4 ml. The IL-1 was administered in a dose of 0.025 μ g, IL-6 in a dose of 0.1 μ g and TNF- α in a dose of 1.0 μ g. Behavioral testing, as described in the preceding Experiments, was conducted 1 hour following drug treatment.

Insert Table 1 about here

Results and Discussion

Variations in consumption of a palatable diet

Figure 5 shows the variations in consumption of a palatable diet as a function of the treatment mice received.

Insert Figure 5 about here

IL-1 + IL-6

Figure 5 (Upper panel) shows the chocolate milk consumption as a function of the IL-1 and IL-6 treatments. The analysis of variance of the consumption yielded a significant effect of the IL-1 x Sampling period interaction, $F(2,72)=8.257$, $p<.01$ Newman-Keuls multiple comparisons of the main effects that comprised the interaction indicated that on Test day there was a significant reduction ($\alpha=.05$) in the amount of chocolate milk consumed by mice treated with .025 μ g IL-1 + Vehicle, followed by a significant increase to baseline levels 24 hours later. There was no reduction in consumption evident among

Table 1

Drug Treatments Administered in Experiments 5-7

<u>Experiment 5</u>	<u>Experiment 6</u>	<u>Experiment 7</u>
Veh + Veh	Veh + Veh	Veh + Veh
IL-1 + Veh	IL-6 + Veh	TNF- α + Veh
Veh + IL-6	Veh + TNF- α	Veh + IL-1
IL-1 + IL-6	IL-6 + TNF- α	TNF- α + IL-1

Figure 5. Effect of intraperitoneal administration of 0.025 μ g IL-1 + 0.1 μ g IL-6 (Upper panel), 0.1 mg IL-6 + 1 μ g TNF- α (Middle panel) or 0.025 μ g IL-1 + 1 μ g TNF- α (Lower panel) on mean ($\pm$ S.E.M.) consumption (mls) of a highly palatable food. Male CD-1 mice were injected with the cytokine combinations or 0.025 μ g IL-1 + Veh, 0.1 μ g IL-6 + Veh, 1 μ g TNF- α + Veh or Veh + Veh, and consumption of chocolate milk was measured over a 1 hr. period commencing 1 hr following injection. Baseline values were calculated by averaging consumption over the three day period immediately prior to testing. Recovery data were collected 24 hr. following testing.

vehicle treated animals. In contrast to the IL-1 treatment, the analysis of variance revealed that neither the main effect of IL-6, nor the interactions involving this variable approached significance.

IL-6 + TNF- α

The combination of 0.1 μ g IL-6 + 1 μ g TNF- α did not act synergistically to reduce chocolate milk consumption (See Figure 5-Middle panel). A repeated measures ANOVA revealed a significant TNF- α x Sampling period interaction $F(2,72) = 5.807$, $p < .01$, and multiple comparisons indicated that on Test day injection of 1 μ g TNF- α + Vehicle significantly reduced the consumption of chocolate milk relative to baseline levels and vehicle treated mice. Treatment with IL-6 did not influence consumption, and likewise the interaction between TNF- α and IL-6 was not significant.

IL-1 + TNF- α

Figure 5 (Lower panel) shows the consumption of chocolate milk as a function of the IL-1 and TNF- α treatments. Analysis of variance of the consumption scores indicated a significant interaction between IL-1, TNF- α and Sampling period, $F(2,72)=5.175$, $p < .01$. Multiple comparisons of the simple effects comprising this interaction confirmed that among vehicle treated animals consumption was stable over sampling periods. As expected, the low doses of IL-1 and TNF- α did not provoke changes of consumption. However, the combination of these treatments markedly reduced consumption of chocolate milk relative to baseline scores as well as in comparison with that of animals that received only one of the treatments. Regardless of the treatment mice received, the consumption exhibited 24 hr afterward was comparable to the baseline scores.

The results of the present investigation indicated that the co-administration of IL-6 with either IL-1 or TNF- α was no more effective in suppressing the consumption of chocolate milk than the administration of IL-1 or TNF- α alone. Of course, the possibility

cannot be excluded that a synergism would have appeared had other doses or time frames been assessed. It is particularly interesting, however, that the combination of sub-effective doses of IL-1 and TNF- α markedly suppressed consumption in a synergistic fashion. These results are consistent with recent reports indicating a synergistic effect between IL-1 and TNF- α on feeding in rats (Yang, Koseki, Meguid, Gleason & Debonis, 1994; Van der Meer et al., 1995). The latter studies suggested that IL-1 and TNF- α act synergistically to provoke anorexia, as reflected by the suppression of feeding in rats given free access to lab chow.

Hormonal variations

Figure 6 shows the variations in plasma corticosterone levels as a function of the treatment mice received.

Insert Figure 6 about here

IL-1 + IL-6

Analysis of variance of plasma corticosterone levels following the co-administration of 0.025 μ g of IL-1 + 0.1 μ g of IL-6 revealed that the effects of IL-6 did not interact with those of IL-1. In contrast, treatment with IL-1 significantly increased circulating corticosterone concentrations, $F(1,36) = 39.82$, $p < .01$, relative to control animals. Treatment with IL-6 was ineffective in affecting the levels of corticosterone (see Figure 6-Upper panel)

IL-6 + TNF- α

Treatment with TNF- α increased plasma corticosterone levels, $F(1, 36) = 19.79$, $p < .01$, whereas IL-6 was again found to be without effect. As well, IL-6 treatment did not augment the actions of TNF- α (See Figure 6-Middle panel). Thus, it seems that the

Figure 6. Effect of intraperitoneal administration of 0.025 μ g IL-1 + 0.1 μ g IL-6 (Upper panel), 0.1 μ g IL-6 + 1 μ g TNF- α (Middle panel) or 0.025 μ g IL-1 + 1 μ g TNF- α (Lower panel) on mean ($\pm$ S.E.M.) plasma corticosterone concentrations (μ g/dl). Male CD-1 mice were injected with the cytokine combinations or 0.025 μ g IL-1 + Veh, 0.1 μ g IL-6 + Veh, 1 μ g TNF- α , or Veh + Veh, and plasma corticosterone levels were measured 1 hr following injection (*synergistic increase relative to control animals $p < .05$).

combination of these two cytokines was not more effective in provoking an increase in corticosterone levels than the administration of TNF- α + Vehicle.

IL-1 + TNF- α

As observed in the behavioral study, the co-administration of 0.025 μ g IL-1 and 1 μ g TNF- α resulted in a synergistic increase in plasma corticosterone levels (See Figure 6-Lower panel). Analysis of variance revealed a significant IL-1 $\times$ TNF- α interaction, $F(1, 36) = 4.01$, $p < .01$. The separate administration of either IL-1 or TNF- α also significantly increased levels of corticosterone ($F(1,36) = 21.126$, $p < .01$ and $F(1,36) = 9.598$, $p < .01$ respectively) relative to saline-treated animals, however, multiple comparisons indicated that when the cytokines were administered jointly, the levels of corticosterone exceeded those seen in mice that received either treatment alone ($\alpha = .05$).

It appears that in mice treated with low doses of IL-1 and TNF- α , synergistic effects may be induced with respect to consumption of a highly palatable substance and circulating corticosterone levels. In contrast, neither the combination of IL-1 and IL-6, nor that of IL-6 and TNF- α , was associated with such synergisms. It is important to note that the alterations in consumption of a palatable food observed in the behavioral studies appear to correspond to the hormonal variations. Previous investigators have reported a synergism between IL-1 and TNF- α on HPA activity (Van der Meer et al., 1995). As was the case in the present investigation, the reductions in food intake in the Van der Meer study corresponded to the increases in corticosterone levels. While it is premature to conclude that the behavioral and hormonal variations observed are causally linked, it is interesting to note that over the course of the three day infusion period in the Van der Meer study, both feeding and HPA activity returned to baseline levels at the same time.

General Discussion

Numerous mechanisms have been elucidated which attempt to describe development of anorexia, including modifications of neurotransmitter levels, alterations in hormone and/or peptide levels, and modulation of hypothalamic or gastrointestinal

hormone and/or peptide levels, and modulation of hypothalamic or gastrointestinal functioning. Feeding can be disrupted by satiety signals (e.g., leptin, CCK, bombesin) (Gibbs, 1985; Smith, Jerome & Norgren, 1985; Edwards, Ladenheim & Ritter, 1986; Corwin, Gibbs & Smith, 1991; Pelleymounter et al., 1995; Campfield, Smith, Guisez, Devos & Burn, 1995; Halaas et al., 1995; Sarraf et al., 1997; Plamadon & Merali, 1997), but it should not be surprising to find that this behavior is also influenced by factors unrelated to satiety per se. For instance, it is well documented that feeding is reduced by illness (Kent et al., 1996), aversive stimulation (Griffiths, Shanks, & Anisman, 1992), and psychological factors such as stress and depression (Willner, 1985). At least some of these events may reflect biologically adaptive responses. For instance, the reduced feeding associated with aversive events may be important so that organisms in stress situations focus on eliminating the aversive stimuli, rather than becoming involved in food gathering. Similarly, it has been suggested that the anorexia associated with illness is essential to direct energy resources to immune functioning, rather than to divert energy to gastrointestinal processes (Hart, 1988; Kent et al., 1992b). Thus, it was suggested that alterations in cytokine levels promote various illness behaviors, which may include the behaviorally adaptive response of diminished feeding (De Kloet, Oitzl & Schobitz, 1994; Kent et al., 1996).

Experimental paradigms involving both humans and infrahuman species have demonstrated that acute or chronic administration of cytokines, such as IL-1, IL-6 and TNF- α can result in anorexia (Plata-Salaman, Oomura & Kai, 1987; Socher et al., 1988; Chance & Fischer, 1991; Plata-Salaman & Borkoski, 1993; Myers et al., 1994). Consistent with these observations, the results of Experiments 1-4 indicated that (a) in the CD-1 mouse peripheral administration of LPS, which induces IL-1, IL-6 and TNF- α release from macrophages, effectively provoked dose-dependent reductions in the consumption of a palatable food, and (b) acute i.p. administration of IL-1 β or TNF- α

provoked a dose-dependent reduction in the consumption of a palatable food, whereas IL-6 treatment was without effect.

The finding that i.p. IL-6 did not suppress feeding is consistent with previous reports that demonstrated that peripheral administration of IL-6 is associated with modest reductions in consumption even with doses approaching pharmacological levels (Plata-Salaman, 1988). Thus, although central IL-6 treatment is reliably associated with anorexia (Plata-Salaman, 1988; Schobitz et al., 1995), the effect of peripheral administration is much less pronounced even at high doses (Espat et al., 1996). The lack of effect in the present investigation may therefore be attributable to the relatively low doses used. However, it is also significant that in the present investigation consumption of a highly palatable substance presented during the light cycle was assessed, whereas in the Plata-Salaman study, free feeding volume was determined nocturnally. Thus, while Plata-Salaman might have been assessing anorexia, consumption in the present investigation may have been an index of anhedonia, rather than anorexia per se. Of course, the procedures used in the present investigation do not permit dissociation of the anorexic and the anhedonic effects of the cytokine treatments and such a conclusion is highly provisional.

It will be recalled that LPS induces the release of several cytokines, which have the potential to operate synergistically in determining hormonal and behavioral changes. Indeed, it was demonstrated that IL-1 and IL-6 had interactive effects in the provocation of corticosterone (Zhou et al., 1996) and ACTH secretion (Perlstein, Mougey, Jackson & Neta, 1991; Matta et al., 1992), while IL-1 and TNF- α synergistically decreased blood glucose levels (Vogel, Henricson & Neta, 1991), and induced anorexia (Yang et al., 1994). In the present investigation IL-1 and IL-6 were not found to have synergistic actions with respect to chocolate milk consumption, nor did the combination of TNF- α and IL-6 have effects beyond those elicited by TNF- α alone. However, the reduction of consumption in mice that received the combination of low doses of IL-1 and TNF- α was appreciably greater than the additive effects of the two treatments. In effect, it seemed that

these cytokines acted synergistically in suppressing palatable food consumption. It is of considerable interest that previous investigations (Yang et al., 1994, Van der Meer et al., 1995) reporting the synergistic effects of IL-1 and TNF- α on food consumption found that the most significant decline in food intake (Purina rat chow) occurred during nocturnally associated ingestion intervals. The reduction in food intake diurnally, although significant, was less conspicuous. In the present investigation, the palatable diet was provided during the light-phase, since it was of interest to establish the effects of the cytokines on consumption of a highly palatable food in sated animals. Under such conditions IL-1 and TNF- α still interacted synergistically to produce a profound suppression of consumption, a finding consistent with the proposition that the treatment lead to an anorexia independent of effects on satiety per se.

It must be underscored that in the present investigation a limited number of test doses were used, and consumption of chocolate milk was assessed from 1-2 hr following administration of the cytokines, and plasma corticosterone was assessed 1 hr after administration. It is certainly conceivable that synergistic actions of other cytokine combinations would have been detected at other doses or at other post-administration intervals. In fact, Zhou et al. (1996) found that a 20 ng intravenous (iv) dose of both IL-1 and IL-6 synergistically increased ACTH secretion in rats 30 minutes following administration, followed by a return to baseline levels by 60 minutes. Co-administration of 100 ng of IL-1 and IL-6 also produced maximal effects 30 minutes after administration, although at 60 minutes ACTH levels were still significantly elevated. Matta et al. (1992) have likewise reported that when injected iv, 100 ng of IL-1 and IL-6 produced a maximal effect on ACTH levels which occurred soon after administration (20 min). Further to this point, Perlstein et al. (1991) found that when administered i.p., 10 ng of IL-1 α combined with 1 μ g of IL-6 produced a synergistic increase in the early ACTH response (30-60 minutes later), while at 2-3 hr post-injection the ACTH response was similar to that elicited by IL-1 alone. In effect, it seems that the synergistic actions of the two treatments may not

plasma ACTH level occurred 120 minutes following the coadministration (i.p.) of 1 μ g TNF- α and 10 ng IL-1 α , whereas the combination of 1 μ g TNF- α with 1.25 μ g of IL-6 produced a peak synergistic effect on ACTH levels from 30-60 minutes following administration. In light of these time-dependent synergistic effects of the cytokines on ACTH variations, it is premature at this juncture to conclude that cytokine combinations other than IL-1 and TNF- α do not engender synergistic actions with respect to either palatable food intake or hormonal variations.

Hormonal changes associated with the administration of LPS and the cytokines indicate that, in almost every experiment, elevations of corticosterone levels occurred contemporaneously with the anorexic and/or anhedonic effects. Indeed, in Experiments 1-3, the doses of TNF- α , IL-1 and LPS which produced significant reductions in consumption of a palatable food, were also associated with significant increases of circulating corticosterone levels. Moreover, those doses of TNF- α and IL-6 which did not produce significant reductions in chocolate milk consumption were also without effect on corticosterone levels. It might be noted at this juncture that although variations of HPA activity have typically been associated with aversive events, appetitive stimuli may influence CRH, ACTH and corticosterone levels (McIntosh, Kent, & Merali, 1996), and conversely alterations of HPA hormonal activity may impact on feeding (Strack, Sebastian, Schwartz & Dallman, 1995). Thus, there is reason to suppose that an association exists, albeit not necessarily a causal one, between the effects of the cytokines on anorexia (and/or anhedonia) and on HPA functioning.

It may be added parenthetically at this juncture that Gelin, Moldawer, Iresjo and Lundholm (1993) reported that in mice with intact adrenal glands, TNF- α and IL-1 (450 and 40 ng/day, respectively) did not influence food consumption. However, in adrenalectomized animals, these treatments reduced consumption significantly, and a further decline was provoked by the combination of the treatments. In effect, adrenalectomy potentiated the anorexic response to the cytokines. Interestingly,

further decline was provoked by the combination of the treatments. In effect, adrenalectomy potentiated the anorexic response to the cytokines. Interestingly, pretreatment with pharmacological doses of hydrocortisone (2.5 mg) abolished the anorexic response to the individual or combined doses of IL-1 and TNF- α . Taken together, these data suggest that the anorexic effect of TNF- α and IL-1 (or their combination) is probably not a direct result of the corticosterone changes induced by the treatments. Clearly, if hormonal factors were solely responsible for the anorexia, then adrenalectomy should have antagonized the effects of the cytokines.

As in the behavioral study, there was no evidence of an increase in plasma corticosterone levels following peripheral IL-6 administration. Although previous studies reported increases in ACTH levels following central IL-6 treatment (e.g., Matta et al., 1996), it appears that peripheral IL-6 treatment, at least at the doses selected in the present investigation, does not have a comparable influence on corticosterone concentrations. It should be underscored that in rodents, IL-6 does not appear to stimulate HPA activity to the same degree as IL-1 or TNF- α (Matta et al., 1992; Dunn, 1992a; Perlstein et al., 1993; Van Haasteren et al., 1994). Indeed, only moderate increases in ACTH or corticosterone levels are seen after administration of large (i.e., 100 ng icv) doses of IL-6. The results of the present investigation are thus consistent with studies showing that low/moderate doses of IL-6 are insufficient to produce significant changes in levels of corticosterone.

While it does appear that IL-6 can directly activate the HPA axis, there is some evidence that it may be more important for sustaining, rather than initiating, HPA activation as a result of inflammation (Zhou et al., 1996). Moreover, Fattori et al. (1994) suggested activation of the HPA axis can and does occur even in the absence of IL-6. They demonstrated that IL-6 deficient mice are capable of mounting a normal inflammatory response to systemic tissue damage generated by the i.p. administration of LPS. Both wild-type and IL-6-deficient mice showed similar induction of corticosterone and mild anorexia

following LPS, suggesting that the physiological contribution of IL-6 to the HPA response in the case of systemic inflammation is negligible.

Commensurate with such findings, it was demonstrated in the present investigation that the increases of corticosterone following the co-administration of either IL-1 and IL-6 or TNF- α and IL-6, were not appreciably greater than those evident following administration of either IL-1 or TNF- α . It is possible that a synergistic effect would have been observed following the administration of higher doses of the cytokines, or if endocrine measures had been taken at a different time following treatment. Nevertheless, results such as these suggest that peripheral treatment with a low dose of IL-6 did not activate unique corticosterone secretagogues in addition to those influenced by the separate administration of IL-1 and/or TNF- α (Matta et al., 1992).

The co-administration of IL-1 and TNF- α , as indicated earlier, produced a synergistic increase in plasma corticosterone levels measured one hour following treatment. These results were not unexpected, given the dramatic synergistic effects of the two cytokines on the behavior measured in this, and previous investigations (Long et al., 1992; Sonti et al., 1996; Bluthé et al., 1994). It will be recalled that Van der Meer et al (1995) reported that the co-administration of peripheral IL-1 and TNF- α acted synergistically in inducing anorexia, and increasing plasma ACTH and corticosterone levels in rats. Thus, the results of the present investigation support the suggestion that low doses of peripheral cytokines can act synergistically to influence HPA activity and feeding in the mouse. Given the overlapping biological functions of IL-1 and TNF- α it is not surprising that these two cytokines can act in a synergistic fashion, although the reason for the existence of two such similar, and apparently redundant, mechanisms is not clear. Perhaps, as suggested by Van der Meer et al. (1995), the two systems co-exist in order to increase the host's sensitivity to subtle inflammatory stimuli and to maximize the ability to respond to such threats in an appropriate manner.

Although the co-administration of IL-1 and TNF- α has been shown to have synergistic effects on a number of behavioral (e.g., reduction in food intake and social exploration) and metabolic measures (e.g., increased plasma corticosterone levels, depression of blood glucose levels), it must be emphasized at this point, that the synergistic actions of cytokine combinations do not necessarily extend to all behaviors or hormonal actions (Vogel et al., 1991; Perlstein et al., 1993; Bluthé et al., 1994; Van der Meer et al., 1995). It would be premature to conclude, for instance, that the lack of a synergistic effect of the combination of IL-1 and IL-6 on food consumption or plasma corticosterone levels would preclude a synergistic effect on social exploration or blood glucose levels. Moreover, as indicated earlier, it would be essential to evaluate the hormonal and behavioral actions of the cytokine combinations across a wide range of post-administration intervals.

The view was offered that cytokine-induced anorexia was a centrally mediated effect, since icv administration of low doses of cytokines (in the nanogram range) effectively reduced feeding (Plata-Salaman et al., 1987; Chance & Fischer, 1991; Myers et al., 1994; Plata-Salaman, Sonti, Borkoski, Wilson & Ffrench-Mullen, 1996). Reductions in food intake were not seen following peripheral administration of cytokines, unless relatively high doses (in the microgram range) were administered (McLaughlin et al., 1992). Further, the anti-pyretic, indomethacin, blocks both the anorexia and fever provoked by cytokine or LPS treatment, a finding consistent with the position that such effects are mediated by a common, central mechanism (Langhans, Harlacher & Scharrer 1989; Crestani, Seguy & Dantzer, 1991). Moreover, the anorexic effects of centrally administered cytokines could be blocked by pre-treatment with the appropriate receptor antagonists applied icv, supporting the contention that the suppression of feeding was mediated by direct action in the CNS (Plata-Salaman & Ffrench-Mullen, 1992).

It was recently shown, however, that peripheral mechanisms may also contribute to cytokine-induced anorexia. In particular, Kent et al. (1996), demonstrated that food-

motivated behavior in rats was reduced to a comparable extent following either i.p. or icv IL-1 treatment. Peripherally administered IL-1 required a much larger dose to reduce food-motivated behavior, but decreased the latency to reduced responding relative to that seen following icv administration. Thus these data provisionally suggest that peripheral mechanisms may also be involved. Consistent with this conclusion, it was demonstrated that the anorexic effects of i.p. IL-1 were blocked by pretreatment with an i.p. (but not an icv) injection of IL-1ra. Kent et al. (1992a) showed that cytokine-induced anorexia could be dissociated from the febrile response, by using central IL-1ra to block the pyrogenic, but not the anorexic effects of icv IL-1 treatment. Moreover, Bret-Dibat, Kent, Couraud, Creminon & Dantzer (1994) reported that in addition to reducing food-motivated behavior, peripheral LPS treatment increased levels of substance P, neurokinin A and calcitonin gene-related product in spinal cord afferents. It would thus appear that both peripheral and central mechanisms subserve the anorexia induced by cytokines.

It has been suggested that peripheral cytokine signalling to the central nervous system is mediated by the vagal afferent fibres (Bluthe et al., 1994; Laye et al., 1995; Gaykema et al., 1995; Kent et al., 1995). In particular, subdiaphragmatic vagotomy has been shown to attenuate or block the behavioral effects of i.p. administration of either LPS or IL-1 (Bluthe et al., 1994; Bluthe, Michaud, Kelley & Dantzer 1996a; 1996b). Moreover, such a treatment attenuated the increased plasma ACTH and corticosterone secretion ordinarily observed following i.p. administration of either IL-1 or LPS (Gaykema et al., 1995; Fleshner et al., 1995; Kapcala et al., 1996). The alterations in ACTH secretion following subdiaphragmatic vagotomy appear to be specific to immune-related stressors, as hypoglycemic stimulation of ACTH secretion was unaffected by sectioning of the vagus (Kapcala et al., 1996). It is important to note as well, that the effects of vagotomy on ACTH and corticosterone levels do not appear to reflect a generalized reduction in HPA responsivity, but instead are likely related specifically to immune activation. In particular, vagotomy does not affect increases in plasma corticosterone levels produced by electric

shock (see discussion in Watkins, Maier & Goehler, 1995b). Moreover, while subdiaphragmatic vagotomy completely blocks PVN c-fos induction following central or peripheral LPS administration, it potentiates PVN c-fos induced by footshock in rats (Wan, Wetmore, Sorensen, Greenberg & Nance, 1994).

The findings of the aforementioned investigations have led to the suggestion that the influence of subdiaphragmatic vagotomy is limited to those functions which are mediated by the transmission of messages from peripheral cytokines to the brain (Watkins, Maier & Goehler, 1995b). Indeed, Bluthé et al. (1996a; 1996b) demonstrated that vagotomy attenuates the behavioral effects of peripheral, but not subcutaneous, intravenous, or central administration of IL-1. Presumably, sectioning of the vagus prevents the activation of vagal afferents ordinarily stimulated by peripheral cytokines. Thus, such a treatment would be expected to preclude the essential factor that would otherwise lead to increased brain cytokine levels that ultimately mediate illness responses, including fever and anorexia. It should be emphasized that while the vagus may be an important route for cytokine-brain communication, such innervation does not preclude the existence of alternate communication pathways for immune signalling of the CNS.

In addition to a potential role for immune-brain communication, vagal afferent fibres have also been implicated in the mediation of feeding. For example, it was reported that selective sectioning of vagal afferents, but not efferents, blocked the satiating effects of peripherally administered CCK (Smith, Jerome, Cushin, Eterno & Simansky, 1981; Smith et al., 1985). South and Ritter (1988), and Bluthé et al. (1996a; 1996b) subsequently demonstrated that direct application of capsaicin to subdiaphragmatic vagal afferents also attenuated CCK induced suppression of food intake. Likewise, capsaicin influenced variations of feeding ordinarily provoked by bombesin treatment (Kent & Merali, 1996; McIntosh & Merali, 1996). It was further demonstrated that, in mice, vagotomy attenuated the decrease in responding for food reward following peripheral administration of IL-1 or LPS (Bret-Dibat et al., 1994; Kent et al., 1996). Such findings are not unexpected in view

of the observation that vagal afferent fibres project to the paraventricular nucleus of the hypothalamus, which appears to be an integral area in the mediation of feeding. It should also be considered that the activity of glucose sensitive neurons in the lateral hypothalamus (LH) is suppressed following iontophoretic application of IL-1 and TNF- α , whereas glucose-sensitive neurons in the ventromedial hypothalamus (VMH) are excited following the application of IL-1 (Plata-Salaman et al., 1988; Kuriyama, Hori, Mori & Nakashima, 1990). Alterations in the activity of both the LH and VMH have been associated with changes in the microstructure of feeding behavior, including decreases in meal frequency and meal size, as well as increases in post-prandial intermeal intervals (Plata-Salaman, 1997). Similar microstructural alterations in feeding patterns are evident following subdiaphragmatic vagotomy (Davis et al., 1994; 1995).

In view of the proposed relationship between immune activation of vagal afferents and feeding, it was hypothesized that destruction of these afferent fibres would attenuate the reduced consumption of a palatable food induced by i.p. LPS treatment. As outlined in Appendix A, capsaicin administered to neonatal mice subcutaneously selectively destroys approximately 95% of C fiber afferents (Holzer, 1991). Accordingly, it was expected that neonatal capsaicin administration would antagonize the anorexia otherwise induced by LPS (See Appendix A). The results of this supplementary experiment failed, however, to support this hypothesis. The consumption of chocolate milk by mice treated with capsaicin did not differ significantly from that of vehicle treated animals following an i.p. injection of LPS. In addition, there were no significant differences in corticosterone levels between either group of mice.

Several possibilities, in addition to non-involvement of vagal afferents in post-LPS feeding, exist to account for the observed outcome. The most parsimonious explanation for the lack of behavioral differentiation among capsaicin and vehicle treated mice is inadequate vagal interruption. In the present investigation, capsaicin treated neonatal mice exhibited mild respiratory distress and cutaneous vasodilation for a brief period immediately

following drug administration. In contrast, there was no evidence of either physical or respiratory discomfort among vehicle treated mice. Nonetheless, the results of the eye-wipe test conducted at 30 days of age (data not shown), which is used as an index of afferent fibre destruction, failed to reveal any differences in the response of either the capsaicin or vehicle treated animals. It is therefore conceivable that the failure to provoke effects on LPS-induced feeding and corticosterone levels may be attributable to incomplete destruction of the afferent fibres following capsaicin administration.

An alternative explanation for the apparent lack of effect among capsaicin treated mice is that the reduction in consumption of chocolate milk induced by LPS was related to activation of central mechanisms, which precluded the appearance of putative peripheral effects of capsaicin. This hypothesis is not a likely one since the low dose of LPS employed (0.5 μg) would have primarily influenced peripheral mechanisms. Indeed, preliminary studies revealed that this dose of LPS elicited only a moderate decline in the consumption of chocolate milk in otherwise untreated mice, accompanied by a modest increase of corticosterone levels. Furthermore, Dunn (1992b; Dunn & Chuluyan, 1994; Lavicky & Dunn, 1995) demonstrated that when LPS was administered i.p., the minimal effective dose which increased cerebral catecholamine metabolism was 1 μg . with the peak effect occurring between 2-8 hours following drug administration. Thus, it is likely that a 0.5 μg dose of LPS would not be sufficient to trigger centrally mediated changes in HPA activity.

The possibility that capsaicin did not effectively disrupt vagal afferents cannot be readily dismissed. As indicated earlier, injection of capsaicin resulted in immediate behavioral changes consistent with vagotomy. Nevertheless, it should be emphasized that, as adults, these mice did not show a disturbed corneal sensitivity as assessed by the eyewipe test. Interestingly, a very recent study conducted by Bret-Dibat et al. (1997) also revealed that, in contrast to surgical vagotomy, capsaicin did not block the decline in food-motivated behavior ordinarily produced by LPS or IL-1 treatment. In this study a series of

7 i.p. capsaicin injections (75 mg/kg), administered on successive days, was used to destroy C-fiber afferents in adult mice, and the effectiveness of the capsaicin treatment was verified by alterations in corneal and pain sensitivity, and by attenuation of CCK-induced satiety. It has likewise been reported (Tilders et al., 1994) that capsaicin treatment in rats did not attenuate the ACTH response to a 2.5 mg/kg i.p. dose of LPS. Clearly, the findings of the present investigation concerning the lack of an effect of capsaicin on chocolate milk consumption and plasma corticosterone levels are consistent with both of these reports. It is possible, as suggested by Bret-Dibat et al. (1997), that the signalling pathway that transmits information regarding peripheral cytokines to the brain is not exclusively comprised of capsaicin-sensitive C-afferents.

The question remains as to whether the reduction in consumption of a palatable food, such as the chocolate milk, represents an anorexic effect, or would be more appropriately considered a reflection of anhedonia. It will be recalled that several differences can be identified between the feeding behavior assessed in this investigation and feeding measured in other laboratories (e.g., Yang et al., 1994; Van Der Meer et al., 1995; Plata-Salaman et al., 1996; Sonti et al., 1996; Bret-Dibat et al., 1997). In the present investigation, mice were given *ad libitum* access to lab chow. The chocolate milk was only presented during the light-cycle, shortly after the lights were turned on. Diurnal associated feeding would be expected to be minimal following the relative hyperphagia which occurs nocturnally (Plata Salaman et al. 1996; Sonti et al., 1996; Yang et al., 1996). Indeed, mice would not be expected to be 'hungry'. Despite these factors, sated mice consumed a substantial amount of chocolate milk during a one hour period, implying a strong motivation to consume a palatable substance. Indeed, it has been argued that the consumption of palatable diets under some circumstances (e.g., following stressor exposure) may represent an ideal method of assessing anhedonic effects associated with such treatments (Willner et al., 1987; Papp et al., 1991). While not dismissing the potential effects of cytokines on anorexia, it is possible that the reduction in consumption of

chocolate milk observed in the present investigation following the administration of LPS, TNF- α and IL-1 may be a reflection of a decrease in sensitivity to rewards, similar to that observed following stressors.

A very recent study conducted by Swiergiel, Smagin & Dunn (1997), assessed the effect of infection with influenza virus, LPS and IL-1 on the ingestive behavior of mice. In this study, both intake of food pellets and the consumption of sweetened condensed milk were measured following acute i.p. injections of LPS or IL-1, or intranasal inoculation with influenza virus. It was observed that LPS, IL-1 and infection with a lethal dose of the virus were associated with a reduction in both food pellet intake and consumption of milk. In contrast, exposure to sublethal doses of influenza virus caused a lesser reduction in food pellet intake, but was without effect on milk consumption. Thus it would appear that mice infected with sublethal doses of influenza virus were more motivated to drink the highly palatable milk than to eat food pellets. Based on these results, it might be argued that the reduction in food pellet intake reflects anorexia, while the reduction in the consumption of condensed milk reflects anhedonia. Indeed, in the same study, pretreatment with indomethacin significantly attenuated the reduction in both food pellet and milk intake caused by IL-1 and LPS. In contrast, in mice infected with a lethal dose of influenza virus, chronic indomethacin attenuated the reduction in food pellet intake, but was without effect on the reduction of milk intake.

Anhedonic-like behaviors have previously been demonstrated following the administration of cytokines or LPS. Peripheral LPS administration markedly reduced hedonic behavior in male rats, as reflected by a decrease in consumption of saccharin solution, and a suppression of sexual behavior (Yirmiya, 1996). Anisman et al. (1996), showed that, in rats, peripheral IL-2 treatment disrupted responding for rewarding brain stimulation, accompanied by decreased DA release from the nucleus accumbens. Dopamine activity in this area has been implicated in motivational disturbances associated with goal-directed responding to positively reinforcing stimuli. In contrast to the effects of IL-2,

however, rats treated with IL-1 showed proficient responding for rewarding brain stimulation, and normal accumbal DA efflux, although there was evidence of increased accumbal 5-HT utilization in these animals. Interestingly, peripheral administration of IL-6 (which had no influence on feeding in the present investigation), also disrupted accumbal DA efflux, although it did not affect responding for rewarding brain stimulation (Anisman, Kokkinidis, Borowski, & Merali, 1997). Together, these data suggest that the effects of IL-1 are distinct from those of IL-2 and IL-6. Furthermore, the behavioral and neurochemical profiles associated with IL-1 administration are not consistent with treatments believed to have anhedonic action (e.g., stressors, neuroleptics). In effect, when coupled with the data of the present investigation, it would appear likely that the actions of IL-1 would be more comfortably interpreted in terms of the potential anorexic effects of such a treatment. Of course, this does not imply that the effects of TNF- α are independent of anhedonic effects. Indeed, it is conceivable that treatments such as LPS, which promote release of IL-1 and TNF- α , suppress feeding owing to both anorexic and anhedonic effects. It remains to be determined whether systemic administration of TNF- α influences responding for rewarding stimuli that do not have an appetitive component (e.g., rewarding brain stimulation).

Finally, it ought to be underscored that the potential hormonal and behavioral consequences of cytokine administration is only a newly evolving research area, and understandably, the available data are limited. Complete dose response curves, and time-response curves, have not been reported with respect to the behavioral actions of the cytokines. As a result, data such as those of Anisman et al. (1997) must be considered as provisional, at best, pending more detailed elaboration of cytokine actions. Inasmuch as the conclusion of the present investigation is derived, in part, from the limited literature available, the notion that the cytokine-induced feeding effects involve anorexic rather than anhedonic effects, must likewise be construed as tentative.

References

- Anisman, H., Kokkinidis, L., Borowski, T. & Merali, Z. (1997). Differential effects of interleukin (IL)-1 β , IL-2 and IL-6 on responding for rewarding lateral hypothalamic stimulation. Brain Research, in press.
- Anisman, H., Kokkinidis, L. & Merali, Z. (1996). Interleukin-2 decreases accumbal dopamine efflux and responding for rewarding lateral hypothalamic stimulation. Brain Research, 731, 1-11.
- Anisman, H., Zalcman, S. & Zacharko, R.M. (1993). The impact of stressors on immune and central neurotransmitter activity: Bidirectional communication. Reviews in Neuroscience, 4, 147-180.
- Arzt, E., Sauer, J., Pollmacher, T., Labeur, M., Holsboer, F., Reul, J.M.H.M. & Stalla, G.K. (1994). Glucocorticoids suppress interleukin-1 receptor antagonist synthesis following induction by endotoxin. Endocrinology, 134, 672-677.
- 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.
- 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, 524-526.
- Berkenbosch, F., Wolvers, D.A.W. & Derijk, R. (1991). Neuroendocrine and immunological mechanisms in stress-induced immunomodulation. Journal of Steroid Biochemistry and Molecular Biology, 40, 639-647.
- Bernstein, I.L. (1995). Neural mediation of food aversions and anorexia induced by tumor necrosis factor and tumors. Neuroscience and Biobehavioral Reviews, 20, 177-181.

- Besedovsky, H., del Rey, A., Sorkin, E., Da Prada, M., Burri, R. & Honnegger, C. (1983). The immune response evokes changes in 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.O., Sorkin, E., Felix, D. & Haas, H. (1977). Hypothalamic changes during the immune response. European Journal of Immunology, 7, 323-325.
- Blalock, J.E. (1994). The syntax of immune-endocrine communication. Immunology Today, 15, 504-544.
- Blalock, J.E. (1984). The immune system as a sensory organ. Journal of Immunology, 132, 1067-1070.
- Blalock, J.E. & Smith, E.M. (1985). A complete regulatory loop between the immune and neuroendocrine systems. Federation Proceedings FASEB, 44, 108-111.
- Bluthe, R.M., Dantzer, R., & Kelley, K.W. (1992). Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rat. Brain Research, 575, 318-320.
- Bluthe, R.M., Michaud, B., Kelley, K., & Dantzer, R. (1996a). Vagotomy attenuates behavioral effects of interleukin-1 injected peripherally but not centrally. NeuroReport, 7, 1485-1488.
- Bluthe, R.M., Michaud, B., Kelley, K., & Dantzer, R. (1996b). Vagotomy blocks behavioral effects of interleukin-1 injected via the intraperitoneal route but not via other systemic routes. NeuroReport, 7, 2823-2827.
- Bluthe, R.M., Pawlowski, M., Suarez, S., Parnet, P., Pittman, Q., Kelley, K.W. & Dantzer, R. (1994). Synergy between tumor necrosis factor α and interleukin-1 in the induction of sickness behavior in mice. Psychoneuroendocrinology, 19, 197-207.

- Bluthe, R.M., Walter, V., Parnet, P., Laye, S., Lestage, J., Verrier, D., Poole, S., Stenning, B.E., Kelley, K.W. & Dantzer, R. (1994). Lipopolysaccharide induces sickness behavior in rats by a vagal mediated mechanism. Life Sciences, 317, 499-503.
- Bret-Dibat, J.L., Creminon, C., Couraud, J.Y., Kelley, K.W., Dantzer, R., & Kent, S. (1997). Systemic capsaicin pretreatment fails to block the decrease in food-motiated behavior induced by lipopolysaccharide and interleukin-1 β Brain Research Bulletin, 42, 443-449.
- Bret-Dibat, J.L., Kent, S., Couraud, J.Y., Creminon, C., & Dantzer, R. (1994). A behaviorally active dose of lipopolysaccharide increases sensory neuropeptides levels in mouse spinal cord. Neuroscience Letters, 173, 205-209.
- Buttini, M. & Boddeke, H. (1995). Peripheral lipopolysaccharide stimulation induces interleukin-1 β messenger RNA in rat brain microglial cells. Neuroscience, 65, 523-530.
- Campfield, L.A., Smith, F.J., Guisez, Y., Devos, R. & Burn, P. (1995). Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science, 269, 546-549.
- 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 Behavior Immunology, 1, 52-63.
- Chance, W.T. & Fischer, J.E. (1991). Aphagic and adipsic effects of interleukin-1. Brain Research, 568, 261-264.
- Coelho, M.M., Souza, G.E.P. & Pela, I.R. (1992). Endotoxin-induced fever is modulated by endogenous glucocorticoids in rats. American Journal of Physiology, 263, R423-R427.
- Corwin, R.L., Gibbs, J. & Smith, G.P. (1991). Increased food intake after type A but not type B cholecystokinin receptor blockade. Physiology and Behavior, 50, 255-258.

- Crestani, F., Seguy, F. & Dantzer R. (1991). Behavioural effects of peripherally injected interleukin-1: role of prostaglandins. Brain Research, 542, 330-335.
- Dantzer, R. & Kelley, K.W. (1989). Stress and immunity: An integrated view of relationships between the brain and the immune system. Life Sciences, 44, 1995-2008.
- Davis, J.D., Smith, G.P. & Kung, T.M. (1994). Abdominal vagotomy alters the structure of the ingestive behavior of rats ingesting liquid diets. Behavioral Neuroscience, 108, 767-779.
- Davis, J.D., Smith, G.P. & Kung, T.M. (1995). Abdominal vagotomy attenuates the inhibiting effect of mannitol on the ingestive behavior of rats. Behavioral Neuroscience, 109, 161-167.
- De Kloet, E.R., Oitzl, M.S., & Schobitz, B. (1994). Cytokines and the brain corticosteroid receptor balance: Relevance to pathophysiology of neuroendocrine-immune communication. Psychoneuroendocrinology, 19, 121-134.
- Derijk, R., Van Rooijen, N., Tilders, F.J.H., 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. Endocrinology, 128, 330-338.
- 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. Endocrinology, 136, 897-902.
- Dunn, A.J. (1988). Systemic interleukin-1 administration stimulates hypothalamic norepinephrine metabolism paralleling the increased plasma corticosterone. Life Sciences, 43, 429-435.
- Dunn, A.J. (1990). Interleukin-1 as a stimulator of hormone secretion. Progress in Neurology, Endocrinology and Immunology, 3, 26-34.

- Dunn, A. (1992a). The role of interleukin-1 and tumor necrosis factor α in the neurochemical and neuroendocrine responses to endotoxin. Brain Research Bulletin, 29, 807-812.
- Dunn, A. (1992b). Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: Comparison with interleukin-1. The Journal of Pharmacology and Experimental Therapeutics, 261, 964-969.
- Dunn, A.J. (1995). Interactions between nervous system and immune system: Implications of psychopharmacology. In F.E. Bloom and D.J. Kupfer (Eds.), Psychopharmacology: A 4th Generation of Progress. New York: Raven Press. pp. 719-732.
- Dunn, A.J. & Chuluyan, H.E. (1994). Endotoxin elicits normal tryptophan and indolamine responses but impaired catecholamine and pituitary-adrenal responses in endotoxin-resistant mice. Life Sciences, 54, 847-853.
- Dunn, A.J., Powell, M.L., & Gaskin, J.M. (1987). Virus-induced increases in plasma corticosterone: A technical comment on Smith, Meyer and Blalock. Science, 238, 1423-1424.
- Dunn, A.J., Powell, M.L., Meitin, C. & Small, P.A. (1989). Virus infection as a stressor: Influenza virus elevates plasma corticosterone, and brain tryptophan concentrations of MHPG and tryptophan. Physiology & Behavior, 45, 591-594.
- Dunn, A.J. & Vickers, S.L. (1994). Neurochemical and neuroendocrine responses to Newcastle disease virus administration in mice. Brain Research, 645, 103-112.
- 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. American Journal of Physiology 266, E986-E992.
- Edwards, G., Ladenheim, E.E. & Ritter, R. (1986). Dorsomedial hindbrain participation in cholecystikinin-induced satiety. American Journal of Physiology, 251, R971-977.

- Espat, J.J., Auffenberg, T., Rosenberg, J.J., Rogy, M., Martin, D., Fang, C.H., Hasselgren, P.O., Copeland, E.M., & Moldawer, L.L. (1996). Ciliary neurotrophic factor is catabolic and shares with IL-6 the capacity to induce an acute phase response. American Journal of Physiology, *271*, R185-R190.
- Exton, M.S., Bull, D.F. & King, M., G. (1995). Behavioral conditioning of lipopolysaccharide-induced anorexia. Physiology and Behavior, *57*, 401-405.
- Fantino, M. & Wieteska, L. (1992). Evidence for a direct central anorectic effect of tumor-necrosis-factor- α in the rat. Physiology & Behavior, *53*, 477-483.
- Fantuzzi, G., Di Santo, E., Sacco, S., Benigni, F. & Ghezzi, P. (1995). Role of the hypothalamus-pituitary-adrenal axis in the regulation of TNF production in mice. Effect of stress and inhibition of endogenous glucocorticoids. The Journal of Immunology, *155*, 3552-3555.
- Fattori, E., Cappelletti, M., Costa, P., Sellitto, C., Cantoni, L., Carelli, M., Faggioni, R., Fantuzzi, G., Ghezzi, P. & Poli, V. (1994). Defective inflammatory response in interleukin 6-deficient mice. Journal of Experimental Medicine, *180*, 1243-1250.
- Felten, S.Y. & Felten, D.L. (1991). Innervation of lymphoid tissue. In R. Ader, D.L. Felten and N. Cohen (Eds.), Psychoneuroimmunology, San Diego: Academic Press. pp. 27-72.
- 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 vagally mediated. Brain Research Bulletin, *37*, 605-610.
- 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 Research, *624*, 291-294.
- Gaykema, R.P.A., Dijkstra, I. & Tilders, F.J.H. (1995). Subdiaphragmatic vagotomy suppresses endotoxin-induced activation of hypothalamic corticotropin-releasing hormone neurons and ACTH secretion. Endocrinology, *136*, 4717-4720.

- Gibbs, J. (1985). Effect of bombesin on feeding behavior. Life Sciences, 37, 147-153.
- Goujon, E., Parnet, P., Aubert, A., Goodall, G. & Dantzer, R. (1995). Corticosterone regulates behavioral effects of lipopolysaccharide and interleukin-1 β in mice. American Journal of Physiology, 269, R154-R159.
- Griffiths, J., Shanks, N., & Anisman, H. (1992). Strain-specific alterations in consumption of a palatable diet following repeated stressor exposure. Pharmacology, Biochemistry and Behavior, 42, 219-227.
- Hallas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K. & Friedman, J.M. (1995). Weight-reducing effects of the plasma protein encoded by the obese gene. Science, 269, 543-546.
- Hart, B. (1988). Biological basis of the behavior of sick animals. Neuroscience and Biobehavioral Reviews, 12, 123-137.
- Harbuz, M.S., Stephanou, A., Sarlis, N. & Lightman, S.L. (1992). The effects of recombinant human interleukin (IL)-1 α , IL- β , or IL-6 on hypothalamo-pituitary-adrenal axis activation. Journal of Endocrinology, 133, 349-355.
- Holzer, P. (1991). Capsaicin: Cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacology Review, 43, 143-201.
- Johnson, R.W., Propes, M.J. & Shavit, Y. (1996). Corticosterone modulates behavioral and metabolic effects of lipopolysaccharide. American Journal of Physiology, 270, R192-R198.
- 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. Endocrinology, 133, 815-821.
- Kapcala, L.P., He, J.R., Gao, Y., Pieper, J.O. & DeTolla, L.J. (1996). Subdiaphragmatic vagotomy inhibits intra-abdominal interleukin-1 β stimulation of adrenocorticotropin secretion. Brain Research, 728, 247-254.

- Keller, S., 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 and N.Cohen (Eds.), Psychoneuroimmunology, San Diego: Academic Press. pp. 771-789.
- Kent, P. & Merali, Z. (1996). Does bombesin (BN) mediate stress responses through CRF release? Effects of bombesin on brain CRF, plasma ACTH, corticosterone and catecholamines. Summer Neuropeptide Conference, Edgartown, Mass.
- Kent, S., Bluthé, R.M., Dantzer, R., Hardwick, A.J., Kelley, K., Rothwell, N. & Vannice, J.L. (1992a). Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin-1. Proceedings of the National Academy of Science, 89, 9117-9120.
- Kent, S., Bluthé, R.M., Kelley, K.W. & Dantzer, R. (1992b). Sickness behavior as a new target for drug development. Trends in Pharmacological Science, 13, 24-28.
- Kent, S., Bret-Dibat, J.L., Kelley, K.W. & Dantzer, R. (1996). Mechanisms of sickness induced decreases in food-motivated behavior. Neuroscience and Biobehavioral Reviews, 20, 171-175.
- Klir, J.J., McClellan, J.L., Kozak, W., Szelenyi, Z., Wong, G.H.W. & Kluger, M. (1995). Systemic but not central administration of tumor necrosis factor- α attenuates LPS-induced fever in rats. American Journal of Physiology, 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. American Journal of Physiology, 265, R512-R517.
- Kuriyama, K., Hori, T., Mori, T., & Nakashima, T. (1990). Actions of Interferon- α and interleukin-1 β on the glucose-sensitive neurons in the ventromedial hypothalamus. Brain Research Bulletin, 24, 803-810.
- Lacosta, S., Merali, Z., Zalcman, S. & Anisman, H. (1995). Time-dependent in vivo mesolimbic dopamine variations following antigenic challenge. Brain Research, 664, 225-230.

- Langhans, W., Harlacher, R. & Scharrer, E. (1989). Verapamil and indomethacin attenuate endotoxin-induced anorexia. Physiology and Behavior, **46**, 535-539.
- Langhans, W., Savoldelli, D & Weingarten, S. (1993). Comparison of the feeding responses to bacterial lipopolysaccharide and interleukin-1 β . Physiology and Behavior, **53**, 643-649.
- Lavicky, J. & Dunn, A.J. (1995). Endotoxin administration stimulates cerebral catecholamine release in freely moving rats as assessed by microdialysis. Journal of Neuroscience Research, **40**, 407-413.
- Laye, S., Bluthé, R.M., Kent, S., Combe, C., Medina, C., Parnet, P., Kelley, K. & Dantzer, R. (1995). Subdiaphragmatic vagotomy blocks induction of IL-1 β mRNA in mice brain in response to peripheral LPS. American Journal of Physiology, **268**, R1327-R1331.
- Long, N.C., Morimoto, A., Nakamori, T. & Murakami, N. (1992). Systemic injection of TNF- α attenuates fever due to IL-1 β and LPS in rats. American Journal of Physiology, **263**, R987-R991.
- Long, N.C., Otterness, I., Kunkel, S.L., Vander, A.J. & Kluger, M.J. (1990). Roles of interleukin 1 β and tumor necrosis factor in lipopolysaccharide fever in rats. American Journal of Physiology, **259**, R724-R728.
- Madden, K.S. & Livnat, S. (1991). Catecholamine action and immunologic reactivity. In R. Ader, D.L. Felten and N. Cohen (Eds.), Psychoneuroimmunology. San Diego: Academic Press. pp.283-310.
- 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. Psychoneuroendocrinology, **19**, 189-196.
- 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 . Neuroendocrinology, **56**, 516-525.

- McCarthy, D.O., Kluger, M.J. & Vander, A. (1986). Effect of centrally administered interleukin-1 and endotoxin on food intake of fasted rats. Physiology & Behavior, 36, 745-749.
- McClellan, J.L., Klir, J.J., Morrow, L.E. & Kluger, M.J. (1994). Central effects of glucocorticoid receptor antagonist RU-38486 on lipopolysaccharide and stress-induced fever. American Journal of Physiology, 267, R7705-R711.
- McLaughlin, C.L., Rogan, G.J., Tou, J., Baile, 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. Physiology and Behavior, 52, 1155-1160.
- McIntosh, J., Kent, P. & Merali, Z. (1996). Microdialysis reveals that both food ingestion and stress enhance CRF release at the central amygdaloid nucleus. Society for Neuroscience Abstracts, 22, 1408
- McIntosh, J. & Merali, Z. (1996). Immobilization stress is associated with increased release of CRF and bombesin-like peptides at the central nucleus of the amygdala. Summer Neuropeptide Conference, Edgartown, Mass.
- 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 Research, 247, 327-330.
- Melik Parsadaniantz, S.M., Levin, N., Lenoir, V., Roberts, J.L. & Kerdellhue, B. (1994). Human interleukin 1 β : Corticotropin releasing factor and ACTH release and gene expression in the male rat: In vivo and in vitro studies. Journal of Neuroscience Research, 37, 675-682.
- Merali, Z., Lacosta, S. & Anisman, H. (1997). Effects of IL-1 β and mild stress on alteration of norepinephrine, dopamine and serotonin neurotransmission: A regional microdialysis study. Brain Research, in press.

- Michie, H.R., Sherman, M.L., Spriggs, D.R., Rounds, J., Christie, M. & Wilmore, D. (1989). Chronic TNF infusion causes anorexia but not accelerated nitrogen loss. Annals of Surgery, 209, 19-24.
- Morrow, L.E., McClellan, J.J., Conn, C.A. & Kluger, M.J. (1993). Glucocorticoids alter fever and IL-6 responses to psychological stress and to lipopolysaccharide. American Journal of Physiology, 264, R1010-R1016.
- Munck, A., Guyre, P.M. & Holbrook, N.J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Reviews, 5, 25-44.
- Myers, R.D., Lopez-Valpuesta, F.J., Minano, F.J., Wooten, M.H., Barwick, V.S. & Wolpe, S.D. (1994). Fever and feeding in the rat: Actions of intrahypothalamic interleukin-6 compared to macrophage inflammatory protein-1 β (MIP-1 β) Journal of Neuroscience Research, 39, 31-37.
- Myers, C.A. & Valentine, A.D. (1995). Neurological and psychiatric adverse effects of immunological therapy. CNS Drugs, 3, 56-68.
- Nagy, J.L., Iversen, L.L., Goedert, M., Chapman, D. & Hunt, S.P. (1983). Dose-dependent effects of capsaicin on primary sensory neurons in neonatal rat. Journal of Neuroscience, 3, 399-406.
- Oldenburg, H.S.A., Rogy, M.A., Lazarus, D.D., Van Zee, K.J., Keeler, B.P., Chizzonite, R.A., Lowry, S.F. & Moldawer, L.L. (1993). Cachexia and the acute-phase protein response in inflammation are regulated by interleukin-6. European Journal of Immunology, 23, 1889-1894.
- 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. Physiology and Behavior, 42, 287-291.
- Papp, M., Willner, P. & Muscat, R. (1991). An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. Psychopharmacology, 104, 255-259.

- Pelleymounter, M.A., Cullen, M.J., Baker, M.B., Hecht, R., Winters, D., Boone, T. & Collins, F. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. Science, 269, 540-543.
- Perlstein, R.S., Moughey, 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 and Cytokine Research, 10, 141-146.
- Perlstein, R.S., Whitnall, M.H., Abrams, J.S., Moughey, E.H. & Neta, R. (1993). Synergistic roles of interleukin-6, interleukin-1, and tumor necrosis factor in the adrenocorticotropin response to bacterial lipopolysaccharide in vivo. Endocrinology, 132, 946-952.
- Pezeshki, G., Pohl, T. & Schobitz, B. (1996). Corticosterone controls interleukin-1 β and sickness behavior in the rat. Journal of Neuroendocrinology, 8, 129-135.
- Plamondon, H. & Merali, Z. (1997). Spontaneous meal is associated with distinct changes in the endogenous levels of bombesin-like peptides and corticotropin releasing factor in specific brain nuclei. American Journal of Physiology, 272, R268-R274.
- Plata-Salaman, C.R. (1988). Food intake suppression by immunomodulators. Neuroscience Research Communications, 3, 159-165.
- Plata-Salaman, C.R. (1994). Meal patterns in response to the intracerebroventricular administration of interleukin-1 β in rats. Physiology and Behavior, 55, 727-733.
- Plata-Salaman, C.R. (1995). Cytokines and feeding suppression: An integrative view from neurologic to molecular levels. Nutrition, 11(Supp), 674-677.
- Plata-Salaman, C.R. (1996). Cytokine action in the nervous system at pathophysiological versus pharmacological concentrations. In Friedman (Ed.). AIDS, Drugs of Abuse, and the Neuroimmune Axis. N.Y: Plenum pp. 191-197.
- Plata-Salaman, C.R. (1997). Anorexia during acute and chronic disease: Relevance of neurotransmitter-peptide-cytokine interactions. Nutrition, 13, 159-160.

- Plata-Salaman, C.R. & Borkoski, J.P. (1993). Centrally administered bacterial lipopolysaccharide depresses feeding in rats. Pharmacology Biochemistry and Behavior, 46, 787-791.
- Plata-Salaman, C.R. & Ffrench-Mullen, J.M.H. (1992). Intracerebroventricular administration of a specific IL-1 receptor antagonist blocks food and water intake suppression induced by interleukin-1 β . Physiology and Behavior, 51, 1277-1279.
- Plata-Salaman, C.R., Oomura, Y. & Kai, Y. (1988). Tumor necrosis factor and interleukin-1 β : suppression of food intake by direct action in the central nervous system. Brain Research, 448, 106-114.
- Plata-Salaman, C.R., Sonti, G., Borkoski, J.P., Wilson, C.D., & Ffrench-Mullen, J.M.H. (1996). Anorexia induced by chronic central administration of cytokines at estimated pathophysiological concentrations. Physiology and Behavior, 60, 867-875.
- 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. Endocrinology, 125, 2800-2805.
- Rogers, R.C., McTigue, D.M. & Hermann, G.E. (1996). Vagal control of digestion: Modulation by central neural and peripheral endocrine factors. Neuroscience and Biobehavioral Reviews, 20, 57-66.
- Rothwell, N. J., & Luheshi, G. (1994). Pharmacology of interleukin-1 actions in the brain. Advances in Pharmacology, 25, 1-20.
- Sapolsky, R., Rivier, C., Yimamoto, G., Plotsky, P. & Vale, W. (1987). Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science, 238, 522-524.
- Sarraf, P., Frederich, R.C., Turner, E.M., Ma, G., Jaskowiak, N.T., Rivet D.J., Flier, J.S., Lowell, B.B., Fraker, D.L & Alexander, H.R. (1997). Multiple cytokines and acute inflammation raise mouse leptin levels: Potential role in inflammatory anorexia. The Journal of Experimental Medicine, 185, 171-175.

- 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. Progress in Neurobiology, 44, 397-432.
- Schobitz, B., Pezeshki, G., Pohl, T., Hemmann, U., Heinrich, P.C., Holsboer, F., & Ruel, J.M.H.M. (1995). Soluble interleukin-6 (IL-6) receptor augments central effects of IL-6 in vivo. FASEB Journal, 9, 659-664.
- Schotanus, K., Tilders, F.J.H. & Berkenbosch, F. (1993). Human recombinant interleukin-1 receptor antagonist prevents adrenocorticotropin, but not interleukin-6 responses to bacterial endotoxin in rats. Endocrinology, 133, 2461-2468.
- Shapiro, R.E. Miselis, R.R. (1985). The central organization of the vagus nerve innervating the stomach of the rat. The Journal of Comparative Neurology, 238, 473-488.
- Shimomura, Y., Shimizu, H., Takahashi, M., Uehara, Y., Negishi, M., Sato, N., Inukai, T., Kobayashi, I. & Kobayashi, S. (1990). Effects of peripheral administration of recombinant interleukin-1 on feeding behavior of the rat. Life Sciences, 47, 2185-2192.
- Smith, E.M., Meyer, W.J. & Blalock, J.E. (1982). Virus-induced corticosterone in hypophysectomized mice: A possible lymphoid adrenal axis. Science, 218, 1311-1312.
- Smith, G.P., Jerome, C., Cushin, B.J., Eterno, R. & Simansky, K.J. (1981). Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science, 213, 1036-1037.
- Smith, G.P., Jerome, C. & Norgren, R. (1985). Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. American Journal of Physiology, 249, R638-641.

- Socher, S.H., Friedman, A. & Martinez, D. (1988). Recombinant human tumor necrosis factor induces acute reductions in food intake and body weight in mice. Journal of Experimental Medicine, 167, 1957-1962.
- Sonti, G., Ilyin, S.E., & Plata-Salaman, C. (1996). Anorexia induced by cytokine interactions at pathophysiological concentrations. American Journal of Physiology, 270, R1394-1402.
- South, E.H., & Ritter, R.C. (1988). Capsaicin application to central or peripheral vagal fibres attenuates CCK satiety. Peptides, 9, 601-612.
- Strack, A.M., Sebastian, R.J., Schwartz, M.W. & Dallman, M.F. (1995). Glucocorticoids and insulin: Reciprocal signals for energy balance. American Journal of Physiology, 268, R142-R149
- Suzuki, T., Funada, M., Sugano, Y., Misawa, M., Okutomi, T., Soma, G. & Mizuno, D. (1994). Effects of lipopolysaccharide from *Pantoea agglomerans* on the cocaine-induced place preference. Life Sciences, 54, 75-80.
- Swiergiel, A.H., Smagin, G. N. & Dunn, A.J. (1997). Influenza virus infection of mice induces anorexia: Comparison with endotoxin and interleukin-1 and the effects of indomethacin. Pharmacology, Biochemistry and Behavior, 57, 389-396.
- Tilders, J.J.H., DeRijk, R.H., Van Dam, A.M., Vincent, V.A.M., Schotanus, K. & Persoons, J.H.A. (1993). Activation of the hypothalamus-pituitary-adrenal axis by bacterial endotoxins: Routes and intermediate signals. Psychoneuroendocrinology, 19, 209-232.
- Tracey, KJ., Wei, H., Manogue, K.R., Fong, Y., Hesse, D.G., Nguyen, H.T., Kuo, G.C., Beutler, B., Cotran, R.S., Cerami, A. & Lowry, S.F. (1988). Cachectin/tumor necrosis factor induces cachexia, anemia, and inflammation. Journal of Experimental Medicine, 167, 1211-1227.
- Turnbull, A.V. & Rivier, C. (1995). Regulation of the HPA axis by cytokines. Brain, Behavior and Immunity, 9, 253-275.

- 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 Research, 588, 291-296.
- Van der Meer, M.J.M., Sweep, C.G.J.F., Pesman, G.J., Borm, G.F., & Hermus, R.M.M. (1995). Synergism between IL-1 β and TNF- α on the activity of the pituitary-adrenal axis and on food intake of rats. American Journal of Physiology, 268, E551-E557.
- Van Haasteren, G.A.C., Van der Meer, M.J.M., Hermus, A.R.M.M., Linkels, E., Klootwijk, W., Kaptein, E., Van Toor, H., Sweep, C.G.J., Visser, T.J. & De Greef, W.J. (1994). Different effects of continuous infusion of interleukin-1 and interleukin-6 on the hypothalamic-hypophyseal-thyroid axis. Endocrinology, 135, 1336-1345.
- Vogel, S.N., Henricson, B.E. & Neta, R. (1991). Roles of interleukin-1 and tumor necrosis factor in lipopolysaccharide-induced hypoglycemia. Brain, Behavior and Immunity, 59, 2494-2498.
- Wan, W., Wetmore, L., Sorensen, C.M., Greenberg, A.H. & Nance, D.M. (1994). Neural and biochemical mediators of endotoxin and stress-induced c-fos expression in the rat brain. Brain Research Bulletin, 34, 7-14.
- Watkins, L.R., Goehler, L.E., Relton, J.K., Tartaglia, N., Silbert, L., Martin, D. & Maier, S.F. (1994). Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. Neuroscience Letters, 183, 27-31.
- Watkins, L.R., Maier, S.F. & Goehler, L.E. (1995a). Cytokine-to-brain communication: A review & analysis of alternative mechanisms. Life Sciences, 57, 1011-1026.
- Watkins, L.R., Maier, S.F. & Goehler, L.E. (1995b). Immune activation: the role of pro-inflammatory cytokines in inflammation, illness responses and pathological pain states. Pain, 63, 289-302.

- Wehrenberg, W.B., Saudan, P., Corder, R., Voltz, D.M., Glustina, 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 Journal, 2, 273-277.
- Willner, P. (1985). Depression: A psychobiological synthesis. N.Y.: Wiley
- Willner, P., Towell, A., Sampson, D., Sophokleous, S. & Muscat, R. (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology, 93, 358-364.
- Yang, Z., Koseki, M., Meguid, M. M., Gleason, J.R., & Debonis D. (1994). Synergistic effect of rhTNF- α and rhIL-1 α in inducing anorexia in rats. American Journal of Physiology, 267, R1056-R1064.
- Yang, Z., Ratto, C., Gleason, J.R., Bellatone, R., Crucitti, F. & Meguid, M.M. (1992). Influence of anterior subdiaphragmatic vagotomy and TPN on rat feeding behavior. Physiology and Behavior, 51, 919-926.
- Yirmiya, R. (1996). Endotoxin produces a depressive-like episode in rats. Brain Research, 711, 163-174.
- Zalcman, S., Green-Johnson, J.M., 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 Research, 643, 40-49.
- Zalcman, S., Shanks, N. & Anisman, H. (1991). Time-dependent variations of central norepinephrine and dopamine following antigen administration. Brain Research, 557, 69-76.
- Zanetti, G., Heusmann, D., Gerain, J., Kohler, J., Abbet, P., Barras, C., Lucas, R., Glauser, M & Baumgartner, J. (1992). Cytokine production after intravenous or peritoneal gram negative bacterial challenge in mice. The Journal of Immunology, 6, 1890-1897.

Zhou, D., Shanks, N., Riechman, S.E., Liang, R., Kusnecov, A.W. & Rabin, B.S. (1996). Interleukin 6 modulates interleukin-1 and stress-induced activation of the hypothalamic-pituitary-adrenal axis in male rats. Neuroendocrinology, 63, 227-236.

Appendix A

Experiment 8

In order to further investigate the involvement of vagal afferent fibres in the mediation of the behavioral and endocrine effects following LPS-induced immune activation, capsaicin was used to eliminate afferent fibres in neonatal CD-1 mice. As indicated earlier, subdiaphragmatic vagotomy severs both afferent and efferent vagal fibers. Thus, the effects of this treatment on LPS-induced immune or endocrine variations may reflect the actions of the endotoxin on messages to the CNS or, alternatively, the effects of negative feedback mechanisms on endocrine or behavioral processes (Tilders et al., 1993). Unlike vagotomy, neonatal treatment with capsaicin has been shown to selectively eliminate afferent vagal fibers, while sparing efferent fibers (Holzer, 1991). Thus, effects of this treatment on LPS-induced immune, endocrine or behavioral effects can be ascribed more directly to the interference of messages from the peripheral to central nervous system. Experiment 8 was conducted to assess the effects of capsaicin-elicited vagal disturbances on the behavioral and hormonal changes exerted by LPS treatment.

Materials and Methods

Subjects

Neonatal male CD-1 mice from an in-house breeding colony were used. Mice were injected subcutaneously with capsaicin at 2 days of age and then returned to their mother. Following weaning (at 21 days) mice were separated into groups of 4 and housed under the conditions previously described. At 60 days of age mice were individually housed for one week prior to testing.

Drugs

Capsaicin was suspended in 10% Tween 80, 90% distilled water and 10% ethanol (95%). Mice were administered an acute 50 mg/kg subcutaneous injection of capsaicin or vehicle (Tween 80, distilled water, 95% ethanol). This procedure, which has been used by

Dr. Z. Merali, has been shown to eliminate up to 95% of vagal afferent fibres (Nagy, Iversen, Goedert, Chapman, & Hunt, 1983).

Method

At 60 days of age mice in the capsaicin and vehicle groups (n=12 per group) were separately housed for one week prior to being trained in the chocolate milk paradigm, and as in Experiment 1, tested following treatment with LPS or saline. On the day of the test, mice were given a single intraperitoneal injection of 0.5 μ g LPS or saline 90 minutes prior to receiving milk, and consumption was measured and compared to baseline levels as previously described. Consumption was measured again 24 hours following the test period in order to determine rate of recovery. In a parallel study the effects of capsaicin on the plasma corticosterone changes elicited by the endotoxin were assessed. The dose of LPS, based on Experiment 1, was one that produced submaximal effects on behavior and hormone factors, thus precluding the possibility of a lack of effect owing to a dose that was too high.

Results and Discussion

Variations in consumption of a palatable food

Analysis of variance of chocolate milk consumption indicated that there was a significant LPS x Sampling period interaction $F(2,88)=11.082$, $p<.01$, as well as a significant main effect of LPS treatment $F(1,44)=10.916$, $p<.01$. In contrast, neither the main effect nor any of the interactions involving capsaicin treatment reached statistical significance. As seen in Figure 7, in both vehicle- and capsaicin-treated groups there was a significant decline in consumption of chocolate milk, relative to control animals, following a single i.p. injection of 0.5 μ g of LPS 90 minutes prior to testing. In both capsaicin- and vehicle-treated groups, the rate of consumption of control mice was similar across sampling periods. Post-hoc multiple comparisons, in fact, confirmed that there was no difference in the reduction of milk consumption by capsaicin-treated mice that received

LPS, relative to vehicle-treated mice that received LPS. Rates of consumption returned to baseline or near baseline levels 24 hours following treatment with LPS.

 Insert Figure 7 about here

Hormonal variations

Analysis of variance of plasma corticosterone levels following treatment with 0.5 μ g of LPS indicated that there were no differences between capsaicin- and vehicle-treated mice (See Figure 8). Following the administration of LPS, plasma corticosterone levels in capsaicin- and vehicle-treated mice were significantly increased relative to capsaicin- or vehicle-treated mice that received saline $F(1,16)=80.802$ $p<.01$. Post-hoc multiple comparisons indicated that there was no difference between corticosterone levels in capsaicin-treated mice following LPS administration, relative to levels in vehicle-treated mice that received LPS.

 Insert Figure 8 about here

The results of Experiment 8 indicated that neonatal capsaicin treatment did not have a significant effect on either consumption of chocolate milk or elevations in plasma corticosterone levels following peripheral administration of a low dose of endotoxin. Because the dose of LPS (0.5 μ g) used in the experiment was exceptionally low, it is unlikely that centrally mediated alterations in HPA activity would account for the increased corticosterone levels observed. It is, however, possible that the capsaicin treatment did not completely destroy vagal afferent fibres, and thus did not effectively eliminate the communication pathway from the periphery to the CNS.

Figure 7. Mean ($\pm$ S.E.M.) consumption (in mls) of a highly palatable food by capsaicin- and vehicle-treated mice following intraperitoneal administration of lipopolysaccharide (LPS) or saline. Neo-natal CD-1 mice were treated with subcutaneous capsaicin or vehicle at 2 days of age. At 60 days of age both groups of mice were injected with 0.5 μ g LPS or saline, and consumption of chocolate milk was measured over a 1 hr. period commencing 90 minutes following injection. Baseline values were calculated by averaging consumption over the three day period immediately prior to testing. Recovery data were collected 24 hr. following testing.

Figure 8. Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g/dl}$) in capsaicin- or vehicle-treated mice receiving intraperitoneal lipopolysaccharide (LPS) or saline. Neo-natal male CD-1 mice were treated with subcutaneous capsaicin or vehicle at 2 days of age. At 60 days of age both groups of mice were injected with 0.5 μg LPS or saline and plasma corticosterone concentrations were measured 2 hr later.

Appendix B

ANOVA Summary Tables for Experiment 1

Experiment 1: Lipopolysaccharide (LPS) Dose Response Curve (chocolate milk consumption)

Two-Factor repeated Measures ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Dose (A)	3	12.726	4.242	1.37	.2674
Subj. w. Groups	36	111.446	3.096		
Days (B)	2	18.649	9.324	27.155	.0001
AB	6	14.07	2.354	6.829	.0001
Error	72	24.723	.343		

LPS Dose Response Curve (plasma corticosterone concentrations)

One-factor ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Dose (A)	2	3154.662	1577.331	47.763	.0001
Error	21	693.501	33.024		
Total	23	3848.162			

Appendix C

ANOVA Summary Tables for Experiment 2

Experiment 2: Interleukin-1 (IL-1) Dose Response Curve (chocolate milk consumption)

Two-factor Repeated Measures ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Dose (A)	5	18.731	3.746	1.169	.3363
Subj. w. Groups	54	173.005	3.204		
Days (B)	2	27.321	13.661	38.495	.0001
AB	10	19.286	1.929	5.435	.0001
Error	108	38.326	.355		

IL-1 Dose Response Curve (plasma corticosterone concentrations)

One-factor ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Dose (A)	3	1439.669	479.89	10.3	.0001
Error	36	1677.333	46.593		
Total	39	3117.002			

Appendix D

ANOVA Summary Tables for Experiment 3

Experiment 3: Tumor necrosis factor-alpha (TNF- α) Dose Response Curve (chocolate milk consumption)

Two-factor Repeated Measures ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Dose (A)	3	3.299	1.1	.42	.74
Subj. w. Groups	36	94.341	2.621		
Days (B)	2	11.222	5.611	19.082	.0001
AB	6	10.547	1.758	5.978	.0001
Error	72	21.171	.294		

TNF- α Dose Response Curve (plasma corticosterone concentrations)

One-factor ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Dose (A)	3	1030.215	343.405	16.087	.0001
Error	36	768.48	21.347		
Total	39	1798.695			

Appendix E

ANOVA Summary Tables for Experiment 4

Experiment 4: Interleukin-6 (IL-6) Dose Response Curve (chocolate milk consumption)

Two-factor Repeated Measures ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Dose (A)	4	18.601	4.65	1.678	.1717
Subj. w. Groups	45	124.712	2.771		
Days (B)	2	2.103	1.051	4.098	.0198
AB	8	2.321	.29	1.131	.3506
Error	90	23.088	.257		

IL-6 Dose Response Curve (plasma corticosterone concentrations)

One-factor ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Dose (A)	2	44.022	22.011	.535	.5936
Error	21	864.44	41.164		
Total	23	908.467			

Appendix F

ANOVA Summary Tables for Experiment 5

Experiment 5: Interleukin-1 (IL-1) + Interleukin-6 (IL-6) Co-administration (chocolate milk consumption)

Three-factor Repeated Measures ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Sal/IL-1 (A)	1	5.105	5.105	2.096	.1563
Sal/IL-6 (B)	1	.257	.257	.105	.7473
AB	1	.196	.196	.08	.7783
Subj. w. Groups	36	2.436	2.436		
Sampling period (C)	2	3.156	3.156	6.659	.0022
AC	2	3.914	3.914	8.257	.0006
BC	2	.042	.042	.088	.9157
ABC	2	1.021	1.021	2.153	.1235
C x subj w. Groups	72	.474	.474		

IL-1 + IL-6 Co-administration (plasma corticosterone concentrations)

Two-factor ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
IL-1 (A)	1	3097.424	3097.424	39.824	.0001
IL-6 (B)	1	41.027	41.027	.527	.4724
AB	1	77.312	77.312	.994	.3254
Error	36	2800.001	77.778		

Appendix G

ANOVA Summary Tables for Experiment 6

Experiment 6: Interleukin-6 (IL-6) + Tumor necrosis factor-alpha (TNF- α) Co-administration (chocolate milk consumption)

Three-factor Repeated Measures ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Sal/IL-6 (A)	1	.406	.406	.134	.7167
Sal/TNF- α (B)	1	11.857	11.857	3.908	.0558
AB	1	.571	.571	.188	.6669
Subj. w. Groups	36	109.231	3.034		
Sampling period (C)	2	1.357	.678	2.292	.1084
AC	2	.144	.072	.243	.7852
BC	2	3.437	1.718	5.807	.0046
ABC	2	.696	.348	1.176	.3144
C x subj w. Groups	72	21.307	.296		

IL-6 + TNF- α Co-administration (plasma corticosterone concentrations)

Two-factor ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
IL-6 (A)	1	604.506	604.506	19.795	.0001
TNF- α (B)	1	15.055	15.055	.493	.4871
AB	1	61.951	61.951	2.029	.163
Error	36	1099.359	30.538		

Appendix H

ANOVA Summary Tables for Experiment 7

Experiment 7: Interleukin-1 (IL-1) + Tumor necrosis factor-alpha (TNF- α) Co-administration (chocolate milk consumption)

Three-factor Repeated Measures ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Sal/IL-1 (A)	1	5.039	5.039	2.444	.1267
Sal/ TNF- α (B)	1	3.37	3.37	1.634	.2093
AB	1	.038	.038	.018	.893
Subj. w. Groups	36	74.231	2.062		
Sampling period (C)	2	4.455	2.228	6.404	.0028
AC	2	6.236	3.118	8.964	.0003
BC	2	12.638	6.319	18.165	.0001
ABC	2	3.601	1.8	5.175	.0079
C x subj w. Groups	72	25.046	.348		

IL-1 + TNF- α Co-administration (plasma corticosterone concentrations)

Two-factor ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
IL-1 (A)	1	969.24	969.24	21.126	.0001
TNF- α (B)	1	440.365	440.365	9.958	.0038
AB	1	183.784	183.784	4.006	.0529
Error	36	1651.64	45.879		

Appendix I

ANOVA Summary Tables for Experiment 8

Experiment 8: Effect of neo-natal capsaicin treatment on response to lipopolysaccharide (LPS) administration (chocolate milk consumption)

Three-factor Repeated Measures ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Veh/Cap (A)	1	.137	.137	.125	.7254
Sal/ LPS (B)	1	11.96	11.96	10.916	.0019
AB	1	1.174	1.174	1.071	.3063
Subj. w. Groups	44	48.207	1.096		
Sampling period (C)	2	4.885	2.443	9.4	.0002
AC	2	.299	.149	.574	.5651
BC	2	5.579	2.88	11.082	.0001
ABC	2	.018	.009	.034	.9666
C x subj w. Groups	88	22.866	.26		

Effect of neo-natal capsaicin treatment on response to LPS administration (plasma corticosterone concentrations)

Two-factor ANOVA

Source	df	Sum of Squares	Mean Square	F-test	p-value
Veh/Cap (A)	1	17.541	17.541	.413	.5296
Sal/LPS (B)	1	3432.968	3432.986	80.802	.0001
AB	1	.003	.003	.00006	.9938
Error	16	679.78	42.486		

IMAGE EVALUATION TEST TARGET (QA-3)

APPLIED IMAGE, Inc.
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989

© 1993, Applied Image, Inc., All Rights Reserved