1

PM-1 3½"x4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGETS

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

**The Influence of Stressor and Cytokine Treatment on
Plasma Corticosterone and Central Biogenic Amines**

**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
© copyright
1994, Leslie Drummond**

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

THE AUTHOR HAS GRANTED AN
IRREVOCABLE NON-EXCLUSIVE
LICENCE ALLOWING THE NATIONAL
LIBRARY OF CANADA TO
REPRODUCE, LOAN, DISTRIBUTE OR
SELL COPIES OF HIS/HER THESIS BY
ANY MEANS AND IN ANY FORM OR
FORMAT, MAKING THIS THESIS
AVAILABLE TO INTERESTED
PERSONS.

L'AUTEUR A ACCORDE UNE LICENCE
IRREVOCABLE ET NON EXCLUSIVE
PERMETTANT A LA BIBLIOTHEQUE
NATIONALE DU CANADA DE
REPRODUIRE, PRETER, DISTRIBUER
OU VENDRE DES COPIES DE SA
THESE DE QUELQUE MANIERE ET
SOUS QUELQUE FORME QUE CE SOIT
POUR METTRE DES EXEMPLAIRES DE
CETTE THESE A LA DISPOSITION DES
PERSONNE INTERESSEES.

THE AUTHOR RETAINS OWNERSHIP
OF THE COPYRIGHT IN HIS/HER
THESIS. NEITHER THE THESIS NOR
SUBSTANTIAL EXTRACTS FROM IT
MAY BE PRINTED OR OTHERWISE
REPRODUCED WITHOUT HIS/HER
PERMISSION.

L'AUTEUR CONSERVE LA PROPRIETE
DU DROIT D'AUTEUR QUI PROTEGE
SA THESE. NI LA THESE NI DES
EXTRAITS SUBSTANTIELS DE CELLE-
CI NE DOIVENT ETRE IMPRIMES OU
AUTREMENT REPRODUITS SANS SON
AUTORISATION.

ISBN 0-315-98645-X

Canada

Name Leslie Ann Drummond

Dissertation Abstracts International is arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation. Enter the corresponding four-digit code in the spaces provided.

Psychology - Psychobiology

SUBJECT TERM

0349

SUBJECT CODE

U·M·I

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS

Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION

General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523

Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS

Language 0679
General 0289
Ancient 0290
Linguistics 0291
Modern 0401
Literature 0294
General 0295
Classical 0297
Comparative 0298
Medieval 0316
Modern 0391
African 0305
American 0352
Asian 0355
Canadian (English) 0593
Canadian (French) 0311
English 0312
Germanic 0315
Latin American 0313
Middle Eastern 0314
Romance 0370
Slavic and East European 0372

PHILOSOPHY, RELIGION AND THEOLOGY

Philosophy 0422
Religion 0318
General 0321
Biblical Studies 0319
Clergy 0320
History of 0322
Philosophy of 0469
Theology 0323

SOCIAL SCIENCES

American Studies 0324
Anthropology 0326
Archaeology 0327
Cultural 0310
Physical 0272
Business Administration 0770
General 0454
Accounting 0338
Banking 0385
Management 0501
Marketing 0503
Canadian Studies 0505
Economics 0509
General 0510
Agricultural 0511
Commerce-Business 0538
Finance 0366
History 0351
Labor 0578
Theory 0578
Folklore 0366
Geography 0351
Gerontology 0578
History 0578
General 0578

Ancient 0579
Medieval 0581
Modern 0582
Black 0328
African 0331
Asia, Australia and Oceania 0332
Canadian 0334
European 0335
Latin American 0336
Middle Eastern 0333
United States 0337
History of Science 0585
Law 0398
Political Science 0615
General 0616
International Law and Relations 0617
Public Administration 0814
Recreation 0452
Social Work 0626
Sociology 0627
General 0938
Criminology and Penology 0631
Demography 0628
Ethnic and Racial Studies 0629
Individual and Family Studies 0630
Industrial and Labor Relations 0700
Public and Social Welfare 0344
Social Structure and Development 0709
Theory and Methods 0999
Transportation 0453
Urban and Regional Planning 0453
Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES

Agriculture 0473
General 0285
Agronomy 0475
Animal Culture and Nutrition 0476
Animal Pathology 0359
Food Science and Technology 0478
Forestry and Wildlife 0479
Plant Culture 0480
Plant Pathology 0617
Plant Physiology 0777
Rangeland Management 0746
Wood Technology 0306
Biology 0287
General 0308
Anatomy 0309
Biostatistics 0379
Botany 0329
Cell 0353
Ecology 0369
Entomology 0793
Genetics 0410
Limnology 0397
Microbiology 0317
Molecular 0416
Neuroscience 0433
Oceanography 0821
Physiology 0778
Radiation 0472
Radiation 0821
Veterinary Science 0786
Zoology 0760
Biophysics 0425
General 0996
Medical 0996

Geodesy 0370
Geology 0372
Geophysics 0373
Hydrology 0388
Mineralogy 0411
Paleobotany 0345
Paleoecology 0426
Paleontology 0418
Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES

Environmental Sciences 0768
Health Sciences 0566
General 0300
Audiology 0992
Chemotherapy 0567
Dentistry 0350
Education 0769
Hospital Management 0758
Human Development 0982
Immunology 0564
Medicine and Surgery 0347
Mental Health 0569
Nursing 0570
Nutrition 0380
Obstetrics and Gynecology 0354
Occupational Health and Therapy 0381
Ophthalmology 0571
Pathology 0419
Pharmacology 0572
Pharmacy 0382
Physical Therapy 0573
Public Health 0574
Radiology 0575
Recreation 0575

Speech Pathology 0460
Toxicology 0383
Home Economics 0386

PHYSICAL SCIENCES

Pure Sciences 0485
Chemistry 0749
General 0486
Agricultural 0487
Analytical 0488
Biochemistry 0488
Inorganic 0738
Nuclear 0490
Organic 0491
Pharmaceutical 0494
Physical 0495
Polymer 0754
Radiation 0405
Mathematics 0605
Physics 0986
General 0606
Acoustics 0608
Astronomy and Astrophysics 0748
Atmospheric Science 0607
Atomic 0798
Electronics and Electricity 0759
Elementary Particles and High Energy 0609
Fluid and Plasma 0610
Molecular 0752
Nuclear 0756
Optics 0611
Radiation 0463
Solid State 0346
Statistics 0984

Applied Sciences

Applied Mechanics 0346
Computer Science 0984

Engineering 0537
General 0538
Aerospace 0539
Agricultural 0540
Automotive 0541
Biomedical 0542
Chemical 0543
Civil 0544
Electronics and Electrical 0348
Heat and Thermodynamics 0545
Hydraulic 0546
Industrial 0547
Marine 0794
Materials Science 0548
Mechanical 0743
Metallurgy 0551
Mining 0552
Nuclear 0549
Packaging 0765
Petroleum 0554
Sanitary and Municipal 0790
System Science 0428
Geotechnology 0796
Operations Research 0795
Plastics Technology 0994
Textile Technology 0994

PSYCHOLOGY

General 0621
Behavioral 0384
Clinical 0622
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

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

Leslie Drummond

in partial fulfilment of the requirements for the degree of

Master of Science

.....
Chair, Department of Psychology

.....
Thesis Supervisor

**Carleton University
December, 1994**

ABSTRACT

A series of experiments was conducted to assess the central neurochemical and plasma corticosterone consequences of systemic interleukin-1 (IL-1) and interleukin-2 (IL-2) treatment. Where IL-1 provoked a marked increase of plasma corticosterone, this was not the case with respect to IL-2. In addition, IL-1 and IL-2 were found to increase the utilization of hypothalamic NE as gauged by the increase accumulation of the NE metabolite, MHPG. However, given that IL-2 did not influence corticosterone levels, it may be the case that the central effects of IL-1, but not IL-2 involve alterations of the hypothalamic-pituitary-adrenal (HPA) axis. Further to the hypothalamic NE changes, variations of DA were noted in prefrontal cortex in IL-1 treated animals. Moreover, although nucleus accumbens DA was affected by IL-1, these variations were inconsistent across experiments. Finally, treatment with IL-1 and stressor exposure, while affecting hypothalamic NE and mesocorticolimbic DA, did not have synergistic effects. Furthermore, stressor exposure did not markedly enhance the endocrine or central neurochemical consequences of subsequent IL-1 treatment. While there seemed to be some enhancement of IL-1 effect on central amines, this may have reflected the neurochemical changes engendered by reexposure to stressor related cues rather than a genuine sensitization effect. Although it seems that IL-1 has some central neurochemical effects similar to those elicited by stressors, it is premature to assume that cytokine action is being interpreted as a stressor.

ACKNOWLEDGEMENTS

I would like to thank Dr. Hymie Anisman, without whose support and guidance, this thesis would not have been possible. Additionally, I extend my gratitude to Jerzey Kulczycki who spent countless hours analyzing tissue and blood samples for this thesis.

I would also like to thank everyone in the lab who lent, at times, much needed moral support, ideas and some great laughs over the years; Marilee, Glenda, Cindy, Rachel, Paul, Monique, Andrea, Sue, Yael and Judy.

Very special thank you's are extended to my family, who constantly served as a source of strength and motivation for me. This thesis would not have been possible without them.

This research was supported by grant number 8043 from the Natural Sciences and Engineering Council of Canada.

TABLE OF CONTENTS

Title	i
Acceptance Page	ii
Abstract	iii
Acknowledgements	iv
Table of Contents	v
Glossary	vi
List of Figures	ix
List of Tables	xii
I. Introduction	1
II. Acute Stressor Provoked Alterations of Neurotransmitters	2
III. Chronic Stressor Effects on Neurotransmitters	5
IV. Sensitization Effects	6
V. Bidirectional Communication - Immune/Brain Interaction	8
VI. Summary	13
Experiment 1. Neurochemical Effects of IL-1 Administration	14
Experiment 2. Neurochemical Effects of IL-2 Administration	29
Experiment 3. Neurochemical Sensitization in a Stress to Stress Paradigm	42
Experiment 4. Neurochemical Alterations Induced by the Combination of Interleukin-1 and Stressor Administration	58
Experiment 5. Neurochemical Sensitization of Stressor Exposure to Subsequent IL-1 Administration	70
General Discussion	83
References	95

GLOSSARY

Adrenocorticotrophic Hormone (ACTH): a hormone secreted by the anterior pituitary gland that acts primarily on the adrenal cortex, stimulating its growth and its secretion of corticosteroids, which can affect immune functioning. The production of ACTH is increased during times of stress.³

Antibody: A protein formed in response to immunization.²

Antigen: A substance which causes the induction of antibodies, and reacts in some specific manner with the antibodies so induced. Antigens may be soluble substances such as toxins and foreign proteins, or particulate such as bacteria and tissue cells.³

Corticotropin Releasing Factor (CRF): A neuropeptide produced by the hypothalamus which stimulates the immune system and the pituitary gland to produce adrenocorticotrophic hormone (ACTH). CRF is also known as corticotropin releasing hormone (CRH).³

Cytokine: A group of antigen-nonspecific glycoproteins produced by lymphocytes (lymphokines) or monocytes (monokines) which are involved in signalling between cells of the immune system. Those cytokines which participate in cellular communication between leukocytes are referred to as interleukins.²

Interleukins: A group of antigen-nonspecific glycoproteins involved in lymphocyte activation and differentiation.

Interleukin-1 (IL-1) is produced by monocytes and macrophages; it promotes T cell proliferation.

Interleukin-2 (IL-2) is produced by activated T cells. It is necessary for the long term proliferation of T cells and can also act on previously stimulated B cells.²

Lymphocyte: A small, spherical cell with a large round nucleus, but virtually no cytoplasm. The term lymphocyte is essentially a morphological one that is used to refer to cells associated with all aspects of specific immunity, some (B lymphocytes) being associated with humoral immunity, and others (T lymphocytes) with cell-mediated immunity.⁴

B cells: Cells that are derived from bone marrow without passing through the thymus. B lymphocytes play a major role in humoral immunity. Upon

stimulation by an antigen, they differentiate into antibody-forming plasma cells.⁴

T cells: Cells that are derived from the thymus. T lymphocytes play a major role in the response against viruses, bacteria, and fungi. Several subsets of these cells develop into:

T-Helper cells: A subset of T-lymphocytes which stimulate antibody production.

T-Cytotoxic cells: A subset of T-lymphocytes which directly lyse virus-infected cells, tumor cells, and transplanted tissue.

T-Suppressor cells: A subset of T-lymphocytes capable of regulating the magnitude of immune effector cell responding.²

Macrophage: A large phagocytic cell of the mononuclear series. They are long-lived cells, and may remain in the tissues for years. Other macrophages recirculate through the secondary lymphoid organs, spleen, and lymph nodes where they may function as antigen-presenting cells.⁵

Mitogen: Molecule which induces differentiation and division (mitosis) of cells.

A few are commonly used by immunologists to induce lymphocyte activation including:

Concavalin A (Con-A): A T cell mitogen which stimulates both primed and unprimed cells.

Phytohaemagglutinin (PHA): A T cell mitogen which can induce cell functions and lymphocyte production.

Pokeweed Mitogen (PWM): Stimulates both T and B cells, and can induce IgM synthesis by B cells.⁵

Natural Killer (NK) Cells: Large, granular, lymphocyte-like killer cells which are capable of killing a number of virally-infected and transformed target cells with which they have had no prior interaction.²

Sensitization: A phenomena which may be observed in the nervous, endocrine and immune systems. It refers to the enhanced response of a system to a stimulus following prior exposure to a stimulus of similar or greater intensity.¹

Synergism: Cooperative action of discrete factors such that the total effect is greater than the sum of the two effects taken independently.

- ¹Antelman, S.M. Time-dependent sensitization as the cornerstone for a new approach to pharmacotherapy: drugs as foreign/stressful stimuli. Drug Dev. Res. 14, 1-30.
- ²Benjamini, E. & Leskowitz, S. Immunology: A Short Course. New York: A. John Wiley and Sons Publications Inc., 1991.
- ³Dorland's Illustrated Medical Dictionary. Twenty-fifth Edition. Philadelphia: W.B. Saunders Company., 1965.
- ⁴Herbert, W.J. & Wilkinson, P.C. A Dictionary of Immunology. Second Edition. Philadelphia: Blackwell Scientific Publications Ltd., 1977.
- ⁵Male, D. Immunology: An Illustrated Outline. St. Louis: The C.V. Mosby Company., 1987.

LIST of FIGURES

- Figure 1.** Mean ($\pm$ S.E.M.) norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-1 (0.4 μ g), or saline, (0.4 ml).
- Figure 2a.** Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-1 (0.4 μ g), or saline (0.4 ml).
- Figure 2b.** Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-1 (0.4 μ g), or saline (0.4 ml).
- Figure 3.** Mean ($\pm$ S.E.M.) serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-1 (0.4 μ g) or saline (0.4 ml).
- Figure 4.** Mean ($\pm$ S.E.M.) norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-2 (0.4 μ g), or saline (0.4 ml).
- Figure 5a.** Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-2 (0.4 μ g), or saline (0.4 ml).
- Figure 5b.** Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-2 (0.4 μ g), or saline (0.4 ml).

- Figure 6.** Mean ($\pm$ S.E.M.) serotonin (5-HT) and 5-hydroxyindoloacetic acid (5-HIAA) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-2 (0.4 μ g) or saline (0.4 ml).
- Figure 7.** Mean ($\pm$ S.E.M.) norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) concentrations (ng/ml protein) in animals that had originally been exposed to No Treatment (NT) or Shock (SH) and then one week later, reexposed to either NT, Apparatus (APP) or SH.
- Figure 8a.** Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) in animals that had originally been exposed to No Treatment (NT) or Shock (SH) and then one week later, reexposed to either NT, Apparatus (APP) or SH.
- Figure 8b.** Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) in animals that had originally been exposed to No Treatment (NT) or Shock (SH) and then one week later, reexposed to either NT, Apparatus (APP) or SH.
- Figure 9.** Mean ($\pm$ S.E.M.) serotonin (5-HT) and 5-hydroxyindoloacetic acid (5-HIAA) concentrations (ng/ml protein) in animals that had originally been exposed to No Treatment (NT) or Shock (SH) and then one week later, reexposed to either NT, Apparatus (APP) or SH.
- Figure 10.** Mean ($\pm$ S.E.M.) norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) concentrations (ng/ml protein) in mice injected with either IL-1 (0.4 μ g) or saline (0.4 ml), followed fifteen minutes later by 1.1 hours of NT, APP or SH.

- Figure 11. Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) in mice injected with either IL-1 (0.4 μ g) or saline (0.4 ml), followed fifteen minutes later by 1.1 hours of NT, APP or SH.
- Figure 12. Mean ($\pm$ S.E.M.) serotonin (5-HT) and 5-hydroxyindoloacetic acid (5-HIAA) concentrations (ng/ml protein) in mice injected with either IL-1 (0.4 μ g) or saline (0.4 ml), followed fifteen minutes later by 1.1 hours of NT, APP or SH.
- Figure 13. Mean ($\pm$ S.E.M.) norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) concentrations (ng/ml protein) in mice that initially received either NT or SH followed one week later by systemic injection of either saline (0.4 ml), IL-1 (0.2 μ g) or IL-1 (0.4 μ g).
- Figure 14. Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) in mice that initially received either NT or SH followed one week later by systemic injection of either saline (0.4 ml), IL-1 (0.2 μ g) or IL-1 (0.4 μ g).
- Figure 15. Mean ($\pm$ S.E.M.) serotonin (5-HT) and 5-hydroxyindoloacetic acid (5-HIAA) concentrations (ng/ml protein) in mice that initially received either NT or SH followed one week later by systemic injection of either saline (0.4 ml), IL-1 (0.2 μ g) or IL-1 (0.4 μ g).

LIST of TABLES

- Table 1.** Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml}$) at one or two hours following systemic injection of IL-1 β (0.4 μg), or sterile saline (0.4ml).
- Table 2.** Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml}$) at one or two hours following systemic injection of IL-2 (0.4 μg), or sterile saline (0.4 ml).
- Table 3.** Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml}$) in mice that initially had been given No Treatment (NT) or Shock (SH) and then one week later, were reexposed to either NT, Apparatus (APP) or SH.
- Table 4.** Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml}$) in mice injected with either 0.4 μg of IL-1 β or 0.4 ml of sterile saline, followed fifteen minutes later by 1.1 hrs. of NT, APP or SH.
- Table 5.** Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml}$) in mice given either NT or SH followed one week later by injection with either 0.4 ml of sterile saline, 0.2 μg of IL-1 β or 0.4 μg of IL-1 β .

INTRODUCTION

Although it was long thought that the immune and central nervous systems acted independently of one another, it has become apparent that these systems, together with the sympathetic nervous system and various endocrine systems, operate in an integrated fashion. Indeed, it seems that multidirectional communication exists between the immune and each of these other systems (Carr & Blalock, 1991; Dunn, 1990). Blalock (1984), went so far as to suggest that the immune system acts as a sensory organ, informing the brain of immune challenge. Subsequently, the position was offered (Anisman, Shanks, Zalcman & Zacharko, 1991; Dunn, 1990) that the brain may be interpreting immune signals much as if they were stressors. On the one hand, such a response may serve in an adaptive capacity, giving rise to endocrine and neurotransmitter changes, as well as behavioural styles, that limit the impact of the challenge or "stressor". On the other hand, excessive neuroendocrine/neurotransmitter alterations, or reduction in the levels of transmitters, may give rise to psychopathological outcomes (Miller, Spencer, McEwen & Stein, 1993).

It has been demonstrated on several occasions that immunological challenge will provoke central neurochemical changes (Besedovsky, Sorkin, Felix & Haas, 1977; Carlson, Felten, Livnat & Felten, 1987; Zalcman, Shanks & Anisman, 1991). For the most part, these neurochemical alterations were reported to occur in the hypothalamus, although there have been indications of amine alterations within the hippocampus (Carlson et al., 1987) as well as mesolimbic sites (Zalcman et al., 1991; Lacosta, Merali & Anisman, 1993). Moreover, it has been suggested that cytokines released from activated immune cells, may be the carrier of messages to the central nervous system (Dunn, 1990). One purpose of the present investigation was to assess whether cytokines, such as interleukin (IL)-1 and IL-2, would influence the activity of central norepinephrine (NE), dopamine

(DA) and serotonin (5-HT). Moreover, if the effects of interleukins are interpreted as a stressor, then one might expect that the profile of neurochemical changes (i.e., the specific transmitter alterations induced and the brain regions in which these occur) would be similar to that induced by stressors. Finally, in the present investigation we sought to establish whether the response to interleukins would be enhanced by prior exposure to uncontrollable stressors. A hallmark of stressors is that such treatments proactively influence the response to later stressors, such that neurochemical alterations are greatly enhanced (sensitization) (Anisman & Sklar, 1979; Antelman, 1988; Cassens, Roffman, Kuruc, Orsulak & Schildkraut, 1980; Doherty & Gratton, 1992; Roth, Tam, Ida, Yang & Deutch, 1988; Abercrombie & Jacobs, 1988). Likewise, cross-stressor sensitization has been reported, as well as sensitization between catecholamine stimulating drugs and stressors. Thus, it was of interest to determine whether stressful events would similarly result in a sensitization effect wherein the response to later interleukin treatment would be enhanced.

ACUTE STRESSOR PROVOKED ALTERATIONS OF NEUROTRANSMITTERS

It has been demonstrated that stressors reliably influence central neurochemical functioning. Among other things, environmental insults induce alterations in the levels and activity of NE, DA and 5-HT (Tanaka, Kohno, Nakagawa, Ida, Takeda, & Nagasaki, 1982; Anisman, Zalcman, Shanks & Zacharko, 1991; Weiss, Goodman, Losito, Corrigan, Charry, & Bailey, 1981; Deutch, Tam & Roth, 1985; Sherman & Petty, 1980). The impact of stressors on amine concentrations depends upon factors such as the type of stressor and its chronicity, prior stressor experience, organismic variables such as age, sex and strain of the animal and the

brain region being investigated (See review in, Anisman, Zalcman & Zacharko, 1993).

When an organism encounters a stressful situation it reacts with a series of behaviours which are believed to help the animal cope with the stressor. In addition, several neurochemical changes occur which may be necessary for behavioural coping efforts and may also serve to diminish the physical and psychological effects of the stressor. However, circumstances may arise wherein the organism is unable to adapt or cope with the environmental demands. When this occurs, neurochemical reserves may be depleted, leaving the organism vulnerable to physical and psychological disease states (Anisman, Zalcman, Shanks & Zacharko, 1991).

Investigations of the impact of stressors on NE have demonstrated that NE synthesis and utilization may be altered by a variety of stressful situations. If the stressor is acute and of a moderate severity, then utilization of NE is increased and this is met with increased NE synthesis. However, if the stressor is unpredictable and uncontrollable, NE utilization may exceed synthesis resulting in a net decrease of the tissue transmitter levels (Anisman, Pizzino & Sklar, 1980; Weiss et al., 1981). Typically, stressors of moderate severity readily provoke changes in amine concentrations within the hypothalamus and locus coeruleus. With more severe stressors, amine variations are also noted within the hippocampus and frontal cortex (Tanaka et al., 1982). These NE alterations persist for a relatively short period of time (a few hours). However, with more severe stressors NE changes may last as long as 48 to 72 hours (Weiss et al., 1981). Likewise, the longevity of the neurochemical changes may be influenced by organismic factors. For instance, the NE depletion in young adult rats persists for only 1-3 hours, whereas in aged animals the NE reduction may be evident as long as 24-48 hours (Ritter & Pelzer, 1978).

In addition to the changes of NE turnover, stressors appear to influence DA levels, but such effects are restricted to specific brain regions. Increased mesocortical and mesolimbic DA utilization is readily provoked, as evidenced by an increase in the accumulation of the metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) (See review in, Anisman et al., 1993). For instance, acute stressors have been shown to provoke very marked increases of DA utilization in prefrontal cortex and modest alterations of DOPAC accumulation within nucleus accumbens (Thierry, Tassin, Blanc & Glowinski, 1976; Deutch et al., 1985). In contrast, alterations of DA within the substantia nigra or caudate are limited (Abercrombie, Keefe, DeFrischia & Zigmond, 1989;1990), but may be evident after stressors that involve cold exposure (Dunn & File, 1983), possibly reflecting thermoregulatory effects as opposed to genuine stressor actions.

Stressors also provoke 5-HT alterations, although these appear to be restricted to some brain regions. Following stressor exposure, increases of 5-HT concentrations are apparent within the pons, medulla, septum and striatum, whereas decreases are observed in the midbrain and posterior hypothalamus (Hellhammer, Hingtgen, Wade, Shea & Aprison, 1983). It appears that 5-HT alterations are not induced as readily as that of NE or DA and a more severe stressor is necessary to elicit such an effect (See review in, Anisman et al., 1991). Dunn (1988) has also observed decreases in 5-HT concentrations within the prefrontal cortex and hypothalamus in CD-1 mice following 15 minutes of footshock, but these decreases were not apparent following 30 minutes of footshock. Concentrations of 5-HIAA were increased in prefrontal cortex after 15 minutes of footshock and also increased in prefrontal cortex and hypothalamus following 15 or 30 minutes of exposure to this stressor. It was suggested that the 5-HT neuronal activity may actually be exquisitely sensitive to stressors. The transient 5-HT decreases were caused by an increase in 5-HT release prompted by

the footshock, but with further stress 5-HT is readily replenished by increased availability of systemic tryptophan (Dunn, 1988).

CHRONIC STRESSOR EFFECTS ON NEUROTRANSMITTERS

Where acute stressors have the overall effect of temporarily reducing amine concentrations within specific brain regions, chronic stressor regimens may engender different patterns of neurochemical change (Anisman et al., 1991). In contrast to the decrease of NE following acute stressors, chronic stressor exposure results in NE levels comparable to that of nonstressed animals. Interestingly, accumulation of MHPG is greatly enhanced following a chronic stressor, indicating increased utilization. It seems that chronic stressors induce a compensatory increase of synthesis thereby precluding the NE depletion observed during acute stress (Zackarko & Anisman, 1989; Herman, Guillonneau, Dantzer, Scatton, Semerdjian-Rouquier & LeMoal, 1982).

As in the case of NE, it has been demonstrated that DA changes associated with acute stressors are appreciably modified in animals exposed to chronic stressor regimens or a protracted single stressor session (Deutch et al., 1985). As in the case of NE, the DA reductions are not evident but this largely stems from moderation of excessive DA utilization ordinarily induced by acute stressors (Herman et al., 1982; Anisman et al., 1991). It is significant to note that the course of these changes are brain region specific. For instance, within 10 minutes of restraint stress, DOPAC accumulation is increased and DA reduced in prefrontal cortex, and adaptation is present within 30 minutes. In the nucleus accumbens in contrast, DA changes are not evident until animals received 20-30 minutes of restraint and the adaptation does not begin to appear until 1 hour of stressor exposure (Deutch et al., 1985). Thus one can imagine that the behavioural sequelae of protracted stressors might also vary as an inverted U shape function of

duration of stressor exposure, as well as the brain region subserving a specific behavioural outcome (Anisman et al., 1993). It might be noted that if the stressor is an unpredictable one (ie., varying in time or in the form of the stressor applied), then neurochemical adaptation is slower to develop (Anisman et al., 1986, 1991, 1993; Molina, Volosin, Cancela, Keller, Murua & Basso, 1990).

SENSITIZATION EFFECTS

Sensitization is a phenomena which may be observed in the nervous, endocrine and immune systems. It refers to the enhanced response of a system to a stimulus following prior exposure to a stimulus of similar or greater intensity (Antelman, 1988). For instance, acute stressors may proactively influence the NE response to subsequently applied stressors. In particular, cues associated with previously presented stressors or reexposure to a milder stressor results in increased utilization of NE and DA within the hypothalamus and mesocortical structures (Anisman & Sklar, 1979; Cassens et al., 1980; Doherty & Gratton, 1992; Herman et al., 1982; Roth, Tam, Ida, Yang & Deutch, 1988; Abercrombie & Jacobs, 1988; Abercrombie et al., 1986). Sensitization of this nature has been shown to occur following a diverse number of stimuli, including natural and man-made disaster (Archibald & Tuddenham, 1965; Silverman, 1986), laboratory stressors (Antelman & Chido, 1983; Kandel, 1983) immunological challenge (Cooper, 1980), industrial chemicals (Karol, Stadler & Magreni, 1985), hormones (Tache et al., 1976; Robinson, Angus & Becker, 1987) and pharmacological agents (Antelman, Kocan, Edwards & Knopf, 1987; Post, 1980). The key factors involved in sensitization appear to be the potential threat to the organism's well being and the intermittency of the stimulus presentations (Antelman, 1988; Post, 1980). Additionally, it seems that passage of time may be essential for the development of sensitization effects (Antelman, 1988). The appearance of a sensitization to various stimuli including

immunological and pharmacological challenge as well as stressors seems to be more pronounced weeks after exposure to stress than shortly after the primary stressor exposure (Antelman, Kocan, Edwards, Knopf, Perel & Stiller, 1986; Archibald & Tuddenham, 1965).

In addition to the within stressor sensitizing effects that have been reported, cross-stimulus sensitization has been observed. For instance, numerous studies have demonstrated sensitizing effects of catecholamine stimulants such as amphetamine (AMPH) and cocaine, as well as DA agonists like bromocriptine and apomorphine (Antelman & Eichler, 1979; Herman, Stinus & LeMoal, 1984; Robinson et al., 1987). Moreover, cross-sensitization has been shown to occur such that the response to stressors may be enhanced in animals previously treated with psychostimulant drugs (Antelman, Eichler, Black & Kocan, 1980). Amphetamine, which is known to mimic stressor effects (Antelman & Chiodo, 1983), has been shown to induce sensitization with respect to subsequent tail pinch-induced eating (Antelman & Eichler, 1979). Likewise, tail-pinch (Antelman et al., 1980), footshock (Antelman & Chiodo, 1983) and restraint (Antelman & Chiodo, 1983) have all been shown to induce sensitization of the polydipsic, anorectic and sniffing responses to AMPH administration.

As in the case of acute stressors, chronic stressor regimens may also proactively influence the neurochemical response to a later stressor exposure. For instance, in chronically stressed animals, a subsequently applied stressor results in an increase of MHPG without a reduction of NE. It has been suggested that where an acute stressor provokes the sensitization of mechanisms involved with amine release, chronic stressor exposure also results in sensitization of mechanisms involved with amine synthesis (Irwin, Ahluwalia & Anisman, 1986). Not unlike NE sensitization effects, it appears that prior exposure to a stressor may have sensitizing effects upon DA activity. Presentation of cues associated with a prior

stressor resulted in increased mesocortical DOPAC accumulation (Herman et al., 1982; Deutch et al., 1985). Moreover, cues associated with previous stressors enhance DA release within the prefrontal cortex and VTA but not within the nucleus accumbens, amygdala or olfactory tubercle (Herman et al., 1982; Roth et al., 1988). As well, pharmacological stimulation of DA receptors following prior stress exposure caused enhanced behavioural effects (Antelman et al., 1980).

A chronic stressor regimen may provoke the sensitization of neurochemical systems to a subsequently applied stressor of a different nature. For instance, Nisenbaum et al, (1991) demonstrated an enhanced release of NE in animals exposed to tailshock which had previously been exposed to cold stress. It will be recalled from the previous discussion that when animals exposed to chronic stressor regimens involving different stressors, applied on an unpredictable schedule, the amine response may be more profound than after acute stressors. In effect, such an outcome may reflect the sensitization of neurochemical processes to meet the demands of a changing and threatening environment.

BIDIRECTIONAL COMMUNICATION- IMMUNE/BRAIN INTERACTION

Bidirectional communication appears to exist between the central nervous system (CNS) and the immune system. Alterations of immune functioning influences CNS and neuroendocrine activity, just as CNS manipulations influence the immune response (Carr & Blalock, 1991; Dunn, Powell, Meitin & Small, 1989). In effect, the possibility exists that immunological challenges may affect the CNS, thus influencing behaviour and psychological states. Conversely, CNS manipulations could contribute to immunological disturbances (See reviews in Anisman et al., 1993; Black, 1994 a,b).

Electrolytic lesions of the anterior hypothalamus have been shown to influence natural killer (NK) cell activity, T-cell proliferation in response to a mitogen as well as antibody production (Cross et al., 1980, 1982, 1986; Roszman & Carlson, 1991). In addition, pharmacological manipulations of central neurotransmitters may also influence immune functioning. Catecholamine reductions following intracisternal 6-OHDA administration reduce the antibody response (Cross et al., 1986) suggesting that cortical areas, brain stem nuclei and forebrain manipulations may also affect immunocompetence. In contrast, lesions of the lateral septum, amygdala and dorsal hippocampus result in an increase of cell proliferation in response to a T-cell mitogen (Cross et al., 1982; Nance, Rayson & Carr, 1987).

The proposition that immune functioning could influence CNS activity follows from findings that sheep red blood cell (SRBC) administration provokes alterations in neuronal activity within the hypothalamus (Besedovsky, Sorkin, Felix & Haas, 1977). Moreover, changes of NE turnover have been observed in the hypothalamus, and these alterations corresponded temporally with the time of the peak immune response (Besedovsky, Del Rey, Sorkin, Da Prada, Burri & Honegger, 1983; Dunn et al., 1989; Zalcman, Shanks & Anisman, 1991). Serotonin reductions have also been observed in the hypothalamus, notably within the paraventricular and supraoptic nuclei. Additionally, 5-HT levels were reduced within the hippocampus and increased in the nucleus tractus solitarius two days following SRBC administration (Carlson et al., 1987). Dopamine changes were found within the prefrontal cortex and nucleus accumbens, and these alterations also corresponded to the time course of the immune response (Zalcman et al., 1991). Indeed, using in-vivo microdialysis increases of DA release within the nucleus accumbens were demonstrated at a time corresponding to the peak immune response to SRBC administration (Lacosta, Merali, Zalcman & Anisman,

in press). Nigrostriatal regions, in contrast, did not show any alteration in DA levels or activity following immunological challenge (Zalcman et al., 1991).

Commensurate with the effects of SRBC, administration of lipopolysaccharide (LPS) or pokeweed mitogen also provoked an increase of NE, DA and 5-HT turnover within the hypothalamus (Mefford & Heyes, 1990). Dunn et al. (1989) also observed increases in NE turnover within the hypothalamus and increased tryptophan concentrations in hypothalamus and parietal cortex in animals infected with influenza virus PR8(H1N1). Taken together, these data suggest that neurochemical changes following systemic administration of antigens are due to immunological changes which occur over a period of days rather than the immediate stress of injection or handling of the animal.

As indicated earlier, Blalock (1984) has suggested that, among other things, the immune system acts as a sensory organ informing the brain of immune activation. Interestingly, the profile of neurochemical alterations following immunological challenge are reminiscent of those engendered by environmental stressors. The similarity between the effects of stressors and immunological challenge on CNS neurotransmitters has prompted the suggestion that the immune message is translated by the brain as if it were a stressor (Dunn, 1990; Anisman, Shanks, Zalcman & Zacharko, 1991).

It has been postulated that endocrine factors may act as a messenger between the immune system and CNS. Indeed, immunologic challenge causes increases in ACTH and glucocorticoid secretion (Carr, 1992). Besedovsky et al., (1975) demonstrated that the increased corticosterone levels following SRBC administration, peaked at a time corresponding to the peak immune response. However, numerous investigators have failed to reproduce these findings (See review in, Anisman et al. 1993; Rivier, 1993). Subsequently, Blalock (1984) reported that immune activation was associated with an increase of an ACTH-like

product even in hypophysectomized animals. Thus, it was suggested that immune cells secrete an ACTH-like substance which may be involved in informing the brain of immune challenge. However, later studies demonstrated that the Blalock findings might have been due to incomplete hypophysectomy, and when the pituitary was removed in its entirety, immune challenge was not associated with an increase in plasma ACTH (Dunn, 1992).

As an alternative to ACTH, the proposition has been put forth that cytokines, such as IL-1, may act as the messenger between the immune and central nervous systems. Interleukins are cytokines released from activated immune cells such as monocytes, macrophages and lymphocytes. Both systemic and intracerebroventricular injection of IL-1 will cause increases in CRF release, plasma ACTH and corticosterone, growth hormone, leutenizing hormone and thyroid stimulating hormone (Berkenbosch, van Oers, Del Rey, Tolders & Besedovsky, 1987; Dunn, 1990; Rivier, 1991; Sapolsky, Rivier, Yamamoto, Plotsky & Vale, 1987). It seems that IL-1 does not directly influence secretion of pituitary hormones, but rather does so by stimulating CRF secretion (Rivier, 1993).

Given IL-1's ability to increase the release of certain pituitary hormones, it was proposed that IL-1 exerts its actions at the level of the pituitary gland. However, IL-1 containing neurons have been recognized to impinge upon the paraventricular nucleus (PVN) of the hypothalamus, and the cytokine acts to promote the release of CRF (Breder, Cinarello & Saper, 1988). In particular, it has been demonstrated that IL-1 provoked increases of ACTH and corticosterone secretion could be blocked by prior immunization with CRF antisera (Sapolsky et al., 1987; Berkenbosch et al., 1987; 1990). Furthermore, CRF but not IL-1 was capable of promoting the release of ACTH from pituitary cells in vitro (Sapolsky et al., 1987). Taken together, these findings suggest IL-1 acts at the level of the

hypothalamus to promote release of CRF, rather than acting directly upon the pituitary.

Although peripheral interleukins do not have ready access to the brain, the possibility exists that they may gain access through the circumventricular organs (CVOs) such as the organum vasculosum, area postrema, pineal gland, subfornical organ and tuber cinereum. Additionally, peripheral interleukins may activate a lipophilic factor which does cross the blood brain barrier hence stimulating interleukins endogenous to brain (Koenig, 1991). Binding sites for IL-1 and IL-2, have been identified within the hypothalamus, hippocampus and dentate gyrus, and IL-2 binding sites and synthesis have also been noted within the striatum (Araumo, Lapchak, Collier & Quirion, 1989; Farrar, Kilian, Ruff, Hill & Pert, 1987; Lapchak, 1992; Lapchak, Araumo, Quirion & Beaudet, 1991; Farrar et al., 1987).

Commensurate with the view that cytokines might act as a messenger between the immune and CNS, a number of investigators have now demonstrated that systemic IL-1 β administration increases hypothalamic NE activity (Dunn, 1988; Kabiersch, Del Rey, Honegger & Besedovsky, 1988; Zalcman et al., 1994). Using a dose of 10 μ g, Mefford and Heyes (1990) reported that the prostaglandin inhibitor, indomethacin, prevented the catecholamine changes induced by IL-1. Thus, these investigators suggested that the amine changes might be secondary to the pyrogenic action of IL-1. However, several investigations demonstrated that the amine changes occurred at subpyrogenic doses (1 μ g or less) (Dunn, 1988; Kabersh et al., 1988; Zalcman et al., 1994).

Given the interplay between cytokines and endocrine factors, most of the attention concerning system interaction has focused on the HPA axis, with particular reference to endocrine alterations following cytokine administration. Indeed it is now well documented that central administration of interleukins will profoundly influence hypothalamic NE which in turn may be responsible for CRF

release and consequent ACTH changes (Rivier, 1993). In addition, however, it seems that interleukins may affect forebrain regions that are more commonly associated with mood alterations. For example, like the effects of SRBC, systemic administration of IL-1 was shown to influence NE and or DA activity within the prefrontal cortex, nucleus accumbens and hippocampus (Zalcman et al., 1994). However, it should be underscored that the effects of IL-1 could be distinguished from that of SRBC. As mentioned earlier, SRBC induces alterations of extracellular DA in freely moving animals and these effects were maximal at the time of the peak immune response. In contrast, in vivo, IL-1 did not effect accumbal DA release, although 5-HIAA accumulation was enhanced (Lacosta et al., 1994). In as much as brain amine changes are not only induced by IL-1, but have been reported following IL-2 and IL-6, the possibility remains that cytokines other than IL-1 contribute to immune/brain communication (Zalcman, 1993).

Summarizing, it appears that immunological challenge, as well as cytokine administration, induce endocrine and central neurotransmitter alterations. These data are consistent with the contention that interplay exists between immune, endocrine and central nervous systems. Moreover, it seems that the brain regions in which the neurotransmitter and endocrine changes are observed parallel those induced by stressors. As discussed earlier, these data were taken to support the contention that the activation of the immune system is interpreted by the brain as if it were a stressor. Moreover, it is likely that interleukins act as messengers between the immune activation and central neurochemical processes. To date, however, the data regarding the impact of cytokine challenge on central transmitters have (a) been limited to assessment of acute cytokine challenge and, (b) in the main, been restricted to a small number of reports showing brain transmitter changes in the hypothalamus. Additionally, there is no information

available as to whether the response to interleukin is influenced by the stimulus context in which the cytokine is administered.

It will be recalled that a hallmark of central neurochemical alterations associated with stressors is that the NE and DA alterations are subject to a sensitization effect (including amine changes upon challenge with pharmacological agents which stimulate catecholamine release). The present investigation was aimed at (a) determining some of the central NE, DA and 5-HT alterations induced by IL-1 and IL-2, in hypothalamus and in mesolimbic regions, (b) assessing whether the amine changes elicited by IL-1 and IL-2 are reminiscent of the effects of stressors, and (c) determining whether stressful events will enhance the immediate response to interleukins and whether stressors proactively influence the response to later interleukin administration.

EXPERIMENT 1	Neurochemical Effects of IL-1 Administration
EXPERIMENT 2	Neurochemical Effects of IL-2 Administration

We assessed in Experiments 1 and 2 whether IL-1 and IL-2 would influence brain neurotransmitter levels and activity, and whether the profile of chemical changes (ie., with respect to the specific changes induced by interleukins and the brain regions in which these occurred) paralleled those induced by stressors.

Experiment 1

Method

Subjects:

A total of 30, experimentally naive, male, CD-1 mice, aged approximately 6 weeks, were obtained from Charles River, LaPrairie, Quebec. Mice were acclimatized to the laboratory conditions for 6 weeks prior to being used as subjects in the experiment. During this period, mice were housed in standard polypropylene cages in groups of four and maintained on a twelve hour light/dark

cycle (lights on 0700h and 1900h). All mice were given ad libitum access to water and standard Purina Laboratory Chow.

Procedure

Mice were randomly assigned to one of four groups (n=9/group) and treated with either IL-1 β (0.4 μ g in 0.1 ml of saline, ip.) or an equivalent volume of saline and then returned to individual holding cages. The IL-1 β was obtained from R&D Systems and had an ED₅₀ of 5-10 pg/mL and an endotoxin content of <0.1 ng per 1 μ g of the cytokine. Aliquots of 1.0 μ g were made up in phosphate buffered saline and were stored at - 82°C. The IL-1 β was thawed immediately prior to being used. Either 1 or 2 hours later, mice were decapitated, brains removed and sectioned onto a glass slide and then placed on dry ice.

Brain tissue was sectioned using an aluminum dissecting block with 0.5 mm thick templates separated by brass plates of 0.25 mm thickness. The slots created by the brass plates served as guides for single edged razor blades which slice the brain. Each slice was then removed from the blade and placed on a glass microscope slide (Canlab Cat. #M6145) and immediately frozen on dry ice. Using a glass petri dish containing dry ice, the prefrontal cortex, nucleus accumbens (Nas), caudate, dorsal hippocampus, ventral tegmental area (VTA), substantia nigra (SN) and locus coeruleus (LC) were illuminated by a fiber optic cold light source. Hollow microdissection needles ranging in gauge from 16 to 22 were used to punch various brain regions. The hypothalamus was removed in its entirety prior to sectioning of the remaining brain. All brain regions were stored in round-welled titer trays (Falcon #3910, Baxter Corp.) at -82° C.

Brain tissue was analyzed using high performance liquid chromatography (HPLC) for norepinephrine (NE), its metabolite 3-methoxy,4-hydroxyphenylethylene (MHPG), dopamine (DA), its metabolites; 3,4-

dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) and for serotonin (5-HT) and its metabolite 5-hydroxyindoloacetic acid (5-HIAA). HPLC analysis with coulometric detection using a modified procedure of Seegal, Brosch & Bush (1986) was used to determine amine and metabolite concentrations in all microsectioned brain regions.

Frozen brain microsections were sonified in 0.3 M monochloroacetic acid containing 0.1 mM disodium ethylenediamine tetraacetate (EDTA - Fisher cat. #SS311-500). The protein content of each sample was measured colorimetrically following the method of Lowry, Rosebrough, Farr and Randall (1951). Brain homogenates were centrifuged at 1500 g (approximately 13,000 r.p.m.) and the supernatants were filtered through 0.2 μ m of regenerated cellulose fiber. Aliquots of each filtrate were injected onto a C18 reverse-phase column (Chromatography Sciences Company Inc.) in a high performance liquid chromatography system (Waters Co. #T12-WISP) equipped with a coulometric detector (ESA). The output signals from the detector were plotted and measured using a Hewlett-Packard HP-3390A plotting integrator. The mobile phase used for separation of all catecholamines and their metabolites was 1.3 g/L heptanesulfonic acid, HSA (Fisher #0-3013), 7.3 g/L triethylamine (Fisher #04884), 0.1 g/L disodium ethylene tetraacetate, EDTA (Fisher #S311) and 60 ml/L acetonitrile (Fisher #998B). The pH of the mobile phase was adjusted to 2.45 with phosphoric acid (Fisher #H260). Prior to use, the mobile phase was filtered with a 0.22 μ m filter paper. The flow rate of the mobile phase was 1.5 ml/minute. For NE, MHPG, DA, DOPAC, and 3-MT, working standards were used at a 50 ng/ml concentration and for 5-HT, 5-HIAA and HVA working standards of 100 ng/ml were used.

Trunk blood was collected and centrifuged at 1300 g. Plasma samples were frozen and stored at -82 °C. Plasma corticosterone levels were determined using

rat ^3H corticosterone radioimmunoassay kits (ICN Chemicals, Montreal, Quebec, CAT #07-120002).

Results and Discussion

Analyses of variance were conducted independently for each neurotransmitter and its metabolites. A number of samples were lost during the tissue retrieval process or during HPLC analyses, hence leading to differing degrees of freedom across brain regions.

Norepinephrine

Figure 1 shows the concentrations of NE and MHPG for each brain region as a function of the treatment mice received. Commensurate with earlier findings suggesting that systemic IL-1 increases hypothalamic NE utilization (Zalcman et al., 1994, Dunn, 1988; Kabiersch et al., 1988), the analysis of variance indicated that this cytokine significantly increased MHPG accumulation, $F(1,24)=6.86$, $p<.05$, and reduced hypothalamic NE levels, $F(1, 24)=13.5$, $p<.01$. Paralleling these results, within the locus coeruleus IL-1 administration significantly decreased NE levels, $F(1, 21)=11.44$, $p<.01$. However, no corresponding MHPG increases were detected in this brain region. Within the prefrontal cortex, NE levels were significantly decreased in IL-1 treated subjects, $F(1,25)=8.38$, $p<.01$, again without an accompanying rise in MHPG accumulation. No significant effect of IL-1 administration upon hippocampal NE or MHPG was detected.

 Insert Figure 1 about here

Dopamine

As previously reported by Zalcman et al., (1994) mesocorticolimbic DA was influenced by IL-1 (see Figure 2a). Specifically, administration of IL-1 increased

accumulation of DOPAC within the prefrontal cortex, $F(1, 25)=4.88$, $p<.05$. Curiously, IL-1 also significantly decreased concentrations of the extracellular metabolite, 3-MT, $F(1, 25)=6.35$, $p<.05$. In contrast to the results observed in prefrontal cortex, 3-MT levels within the nucleus accumbens were significantly affected by the time of injection relative to sacrifice regardless of treatment group, $F(1, 26)=5.27$, $p<.05$. Specifically, levels of 3-MT were lower one hour after injection than after two hours. No other significant DA alterations were observed within the nucleus accumbens. Finally, IL-1 administration also had the effect of decreasing DA concentrations within the caudate, $F(1,24)=5.93$, $p<.05$, an outcome not previously demonstrated with this cytokine (see Figure 2b).

 Insert Figure 2a and 2b about here

Serotonin

Hypothalamic 5-HIAA levels varied as a function of the Time x Treatment interaction, $F(2, 23)=7.10$, $p<.05$. Newman-Keuls multiple comparisons of the simple effects comprising this interaction indicated that relative to saline treated mice, 5-HIAA was significantly increased in IL-1 subjects sacrificed at 2 hours post-injection (see Figure 3). In contrast to the findings of Zalcman et al. (1994), IL-1 treatment was also found to significantly increase 5-HT concentrations, $F(1,25)=7.82$, $p<.01$ and 5-HIAA accumulation, $F(1, 25)=4.62$, $p<.05$ within the prefrontal cortex. Within the hippocampus, 5-HT levels were significantly altered as a result of time of injection relative to sacrifice, $F(1, 26)=4.71$, $p<.05$. Subjects sacrificed at one hour post-injection had increased levels of 5-HT when compared to subjects sacrificed at two hours.

Insert Figure 3 about here

Corticosterone

In accordance with several earlier studies, (Berkenbosch et al., 1987; Dunn, 1990; Rivier, 1991, Sapolsky et al., 1987), IL-1 treatment significantly increased corticosterone levels, $F(1, 26)=32.71$, $p<.01$. As seen in Table 1, at one hour post-injection, a six-fold increase over saline subjects was detected in IL-1 subjects, and by 2 hours post-injection, this increase had risen eight-fold over saline subjects.

Insert Table 1 about here

Figure 1. Mean ($\pm$ S.E.M.) norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-1, .4 μ g, or saline, .4 ml.

* = $p < .05$

Figure 2a. Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-1, .4 μ g, or saline, .4 ml.

* = $p < .05$

Figure 2b. Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-1, .4 μ g, or saline, .4 ml.

* = $p < .05$

Figure 3. Mean ($\pm$ S.E.M.) serotonin (5-HT) and 5-hydroxyindoloacetic acid (5-HIAA) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-1, .4 μ g or saline, .4 ml.

* = $p < .05$

Table 1. Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml}$) at one or two hours following systemic injection of IL-1 β (.4 μg), or sterile saline (.4ml).

Treatment	Time Following Injection	
	<u>1 Hour</u>	<u>2 Hours</u>
	<u>Saline</u>	
	3.43 $\pm$.64	2.76 $\pm$.35
	<u>IL-1β</u>	
	12.94 $\pm$ 1.78	17.73 $\pm$ 2.91

EXPERIMENT 2 Neurochemical Effects of IL-2 Administration

Method

The subject characteristics and procedure of Experiment 2 were the same as Experiment 1. However, Interleukin-2 (0.4 μ g) was used in place of IL-1 β . IL-2 was obtained from R&D Chemicals and had an ED₅₀ of .25 to .5 ng/mL and an endotoxin content of <0.1 ng per 1 μ g of the cytokine. Aliquots of 1.0 μ g were made up in sterile PBS to a quantity of 1 μ g/ml and were stored at -82°C.

Results and Discussion

Norepinephrine

As reported by Zalcman et al. (1994), analysis of variance indicated that MHPG levels within the hypothalamus varied as a function of the Time x Treatment interaction, $F(2, 32)=5.39$, $p<.05$. Newman-Keuls multiple comparisons ($\alpha=.05$) of the simple effects comprising this interaction indicated that IL-2 treated subjects had increased MHPG accumulation at two hours post-injection (see Figure 4). In contrast to these results, MHPG levels within the locus coeruleus varied significantly as a result of time of injection, $F(1, 29)=4.48$, $p<.05$. The MHPG levels were greater in both the saline and IL-2 groups sacrificed at 2 hours post-injection relative to that seen 1 hour post-injection. However, it is important to note that in saline treated mice, two subjects demonstrated unusually high MHPG levels thus increasing the group mean. Thus, although subjects treated with IL-2 and sacrificed at 2 hours had the highest levels of MHPG accumulation, they did not differ significantly from the saline controls. Finally, hippocampal MHPG accumulation was also significantly increased as a result of IL-2 administration, $F(1, 31)=11.82$, $p<.01$. Subjects in both the one and two hour IL-2 groups showed increases in MHPG when compared to saline treated controls.

 Insert Figure 4 about here

Dopamine

There was no significant effect of IL-2 treatment upon DA within any brain region (see Figure 5a). However, several significant effects were observed in the accumulation of various metabolites. Within the prefrontal cortex, concentrations of the extracellular metabolite, HVA, varied as a function of the Time x Treatment interaction, $F(2, 32)=6.33$, $p<.05$. Newman - Keuls multiple comparisons of simple effects comprising the interaction indicated that HVA levels were significantly higher in saline subjects sacrificed one hour following injection. There was no significant difference between the remaining groups. Like the effects in prefrontal cortex, HVA levels within the caudate varied as a function of the Time x Treatment interaction, $F(2, 29)=7.33$, $p<.01$. Newman-Keuls multiple comparisons of the simple effects indicated that HVA concentrations were significantly lower one hour following IL-2 administration. However, by two hours, the HVA levels had risen significantly and did not differ from those of saline treated mice (see Figure 5b).

 Insert Figure 5a and 5b about here

Serotonin

There was no effect of IL-2 treatment upon serotonin or its metabolite within the hypothalamus or hippocampus. However, 5-HT levels within the prefrontal cortex varied as a function of the Time x Treatment interaction $F(1, 32)=5.03$, $p<.05$, (see Figure 6). Newman-Keuls multiple comparisons indicated that 5-HT levels were significantly higher in saline treated subjects sacrificed at one hour

post-injection. Curiously, a significant effect of time of injection was also observed within the prefrontal cortex when 5-HIAA concentrations were analysed, $F(1, 30)=8.28$, $p<.01$. Irrespective of treatment group, 5-HIAA levels were significantly higher one hour post-treatment than at the two hour interval.

 Insert Figure 6 about here

Corticosterone

As previously reported, IL-2 did not have a significant effect upon corticosterone levels (Zalcman et al., 1994). However, a significant effect of time of injection was observed, $F(1, 29)=4.64$, $p<.05$. Corticosterone levels were slightly, but significantly increased one hour post-injection in both the saline and IL-2 subjects (see Table 2). It is tempting to suppose that the heightened corticosterone levels observed after treatment is a reflection of the stress associated with injection. However, even relatively potent stressors that produce many fold increases of corticosterone, tend to persist for only about one hour (Shanks et al., 1989). Thus it would be surprising if the elevated corticosterone levels in the present investigation were simply a reflection of stress associated with injection. Further, in as much as all animals were assessed within a small window of time, factors such as diurnal variations were likely not responsible for the observed effects. Finally, although statistically significant, the proportion of increase of corticosterone levels at one hour versus two hours was not particularly marked. Taken together, it is more likely that the observed outcome was a spurious one. Yet, the fact remains that the time dependent variations observed were not restricted to corticoids, but were also evident in terms of NE, DA and 5-HT variations in several brain regions. On the one hand, such an outcome could arise as a result of unfortunate sampling (eg. more emotionally reactive animals in one

group than another). On the other hand, the time-dependent effects may reflect extraneous factors such as effects of isolating animals between time of injection and decapitation or other events that animals may have experienced during that interval.

Insert Table 2 about here

Figure 4. Mean ($\pm$ S.E.M.) norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-2, .4 μ g, or saline, .4 ml.

* = $p < .05$

Figure 5a. Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-2, .4 μ g, or saline, .4 ml

* = $p < .05$

Figure 5b. Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-2, .4 μ g, or saline, .4 ml

* = $p < .05$

Figure 6. Mean ($\pm$ S.E.M.) serotonin (5-HT) and 5-hydroxyindoloacetic acid (5-HIAA) concentrations (ng/ml protein) at one or two hours following systemic injection of IL-2, .4 μ g or saline, .4 ml.
* = $p < .05$

Table 2. Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml}$) at one or two hours following systemic injection of IL-2 ($.4\mu\text{g}$), or sterile saline ($.4\text{ ml}$).

		Time Following Injection	
		<u>1 Hour</u>	<u>2 Hours</u>
Treatment	<u>Saline</u>	4.24 $\pm$.51	3.08 $\pm$.6
	<u>IL-2</u>	5.51 $\pm$ 1.2	3.45 $\pm$.53

EXPERIMENT 3 Neurochemical Sensitization in a Stress to Stress Paradigm

As discussed earlier, stressor reexposure provokes a more pronounced change of amines relative to that seen in animals stressed for the first time. Experiment 3 was conducted to confirm that such an effect would be evident in the CD-1 strain of mouse. Additionally, it was of interest to establish whether a similar sensitization would be evident with respect to 5-HT.

Method

Subjects:

Sixty-six, male, CD-1 mice of approximately 150 days of age served as subjects. Mice were obtained from Charles River at 6 weeks of age and allowed to acclimatize to the laboratory for a period of 6-7 weeks. Housing conditions were the same as described in Experiment 1.

Apparatus

The apparatus used to deliver footshock consisted of a black Plexiglas chamber measuring 30.0 x 14.0 x 15.0 cm. Stainless steel plates lined the insides of the chamber and were connected in series with the grid floor. The chamber floor consisted of twenty-eight 0.32 cm. stainless steel rods spaced 1.0 cm apart (centre to centre) connected in series by neon bulbs. The chamber was covered by a red, translucent Plexiglas lid, preventing illumination. Shock (300 μ A) was delivered to the chamber floor through a 3000-V source, thereby providing relatively constant current.

Procedure

Mice were randomly assigned to either a No Treatment (NT) or Footshock (SH) condition. The initial shock treatment consisted of subjects being placed in the apparatus for 1.1 hrs. during which footshock (300 μ A; 2 sec duration) was delivered at 9 sec intervals, (# = 360 shocks). This shock intensity has been shown previously in this laboratory to reliably induce stressor related neurochemical alterations. Nonshocked mice were handled and then placed individually in holding cages. Mice in each of these groups were individually housed and one week later exposed to either No Treatment, Apparatus (APP) or Shock (300 μ A). Shocked animals were placed in the apparatus for 10 min. during which they received (60 shocks of 2 sec duration, 300 μ A, at 9 sec intervals. The apparatus exposed animals were placed in the shock chambers for 10 min. without shock being delivered. The nontreated animals were not be placed in any apparatus. Immediately thereafter, mice were sacrificed and brains removed, sectioned and frozen for subsequent amine determinations. Trunk blood was collected for analysis of plasma corticosterone levels. All HPLC and corticosterone analysis were performed as outlined in Experiment 1.

Results and Discussion

Norepinephrine

Commensurate with earlier reports showing that stressors provoke increased NE turnover, analysis of variance revealed that hypothalamic MHPG accumulation was increased as a result of Initial Shock exposure, $F(1, 61)=11.51$, $p<.01$, and also varied as a function of the Subsequent Stressor treatment, $F(2, 61)=4.23$, $p<.01$. Newman-Keuls multiple comparisons ($\alpha=.05$) of the means of the second stressor treatment indicated that shocked subjects had significantly increased levels of MHPG in comparison to subjects exposed to Apparatus. Intermediate MHPG

levels were evident in nonshocked subjects, such that the concentration of MHPG did not differ from that of either of the other two groups (see Figure 7). Although no significant interaction of Initial stressor experience x Subsequent stressor encounter was evidenced, multiple comparisons were performed since a priori predictions had been made concerning this interaction. Newman-Keuls multiple comparisons indicated that animals who received Shock during the first stressor session and No Treatment thereafter, exhibited higher MHPG concentrations than animals who received no stressor treatment whatsoever. The second stressor session itself provoked a modest increase of MHPG concentrations, but this effect did not reach statistical significance. However, among mice stressed during both sessions, MHPG concentrations were greater than among mice stressed only on the second occasion. Curiously, MHPG concentrations in the former condition did not significantly exceed those of mice who received shock only during the first session. Thus it appears that the initial stress session may have long-term consequences on NE utilization, and to some degree this effect was enhanced by stressor reexposure.

Paralleling the MHPG results, NE levels within the hypothalamus were significantly affected by both the Initial shock treatment $F(1,61)=5.80$, $p<.01$ and by Subsequent stressor exposure, $F(2, 61)=7.33$, $p<.01$. Newman-Keuls multiple comparisons of the second stressor experience indicated that subjects exposed to Shock had significantly lower levels of NE when compared to the No Shock or Apparatus groups. Once again, although no significant interaction existed between the two stressor experiences, multiple comparisons were performed since a priori prediction had been made concerning this interaction. Newman-Keuls multiple comparisons indicated that subjects originally given No Treatment and then exposed to Apparatus, had higher levels of NE when compared to subjects given No Treatment and then exposed to Shock or to those given Shock treatment

originally and then reexposed to the Apparatus. Finally, within the locus coeruleus, a main effect of the second stressor experience was evidenced with respect to MHPG accumulation, $F(2, 58)=3.66$, $p<.05$. Newman-Keuls multiple comparisons revealed that subjects exposed to the shock treatment had significantly increased MHPG accumulation when compared to No Shock or Apparatus groups, which did not differ from one another. No significant interaction of the two stressor experiences was demonstrated. However, multiple comparisons were conducted based upon a priori predictions of such an interaction. These comparisons revealed subjects originally exposed to shock and then subsequently reexposed to the stressor, had greater MHPG accumulation relative to all other groups.

 Insert Figure 7 about here

Dopamine

The levels of DA, DOPAC, HVA and 3-MT for each of the groups are shown in Figures 8a and 8b. There was no significant effect of Initial or Subsequent Stressor exposure upon DA or its metabolites within the prefrontal cortex. However, as observed in earlier studies indicating that stressors provoke increases of DOPAC accumulation within the nucleus accumbens (see Anisman et al., 1991), analysis of variance indicated that the initial Shock treatment increased DOPAC accumulation within this brain region, $F(1, 57)=6.17$, $p<.01$. Likewise, 3-MT levels in the nucleus accumbens varied as a function of the Initial Stressor x Stressor reexposure interaction, $F(2, 56)=7.66$, $p<.01$. Newman-Keuls multiple comparisons of the simple effects confirmed that subjects that were originally not shocked and then subsequently exposed to a Shock stressor had significantly higher levels of 3-MT when compared to animals originally given No Treatment

and then exposed to Apparatus or No Treatment, as well as those subjects originally given Shock and then reexposed to the Shock stressor. Additionally, subjects originally exposed to a Shock stressor and then either reexposed to the Apparatus or to Shock treatment, had significantly higher levels of 3-MT than subjects originally exposed to Shock and then given No Treatment.

Within the caudate, a main effect of the second stressor experience upon dopamine levels was observed, $F(2, 61)=5.78$, $p<.01$. Newman-Keuls multiple comparisons indicated that subjects exposed to shock showed a moderate decline in DA levels in comparison to the No Treatment subjects. In contrast, Apparatus subjects demonstrated a moderate increase in DA levels when compared to No Treatment groups. Additionally, DOPAC accumulation was significantly affected by the initial shock treatment, $F(1, 59)=6.65$, $p<.01$, such that subjects within the shock groups had lower levels of DOPAC in comparison to the nonstressed groups.

A significant interaction of initial stressor experience and second stressor application was evidenced in DOPAC accumulation within the substantia nigra, $F(2, 56)=3.65$, $p<.05$. Newman-Keuls multiple comparisons of the simple effects comprising this interaction revealed that subjects that originally received No Treatment and were then exposed to the Apparatus, had higher levels of DOPAC when compared to subjects that either had initially been exposed to shock and then reexposed to Apparatus or to subjects that received No Treatment and then exposed to footshock. Typically, stressors do not affect nigrostriatal DA or DOPAC. This fact, coupled with the unusual pattern of differences, makes it likely that the aforementioned DA alterations were spurious.

 Insert Figure 8a and 8b about here

Serotonin

There was no significant effect of Initial or Subsequent Stressor experience upon serotonin or 5-HIAA within the hypothalamus. However, within the hippocampus, 5-HIAA levels differed significantly as a result of initial shock treatment, $F(1, 58)=5.17, p<.05$. Subjects exposed to a shock stressor had significantly higher levels of 5-HIAA in comparison to the No Treatment groups. Levels of serotonin did not vary as a function of initial shock exposure, although the effects of the second stressor encounter approached significance, $F(2, 59)=3.04, p=.055$. Subjects exposed to shock had higher levels of 5-HT than those exposed to Apparatus or No Treatment. Neither 5-HT nor 5-HIAA varied as a function of the treatments in the remaining brain regions (see Figure 9).

 Insert Figure 9 about here

Corticosterone

Commensurate with earlier findings suggesting that stressor exposure increased plasma corticosterone concentrations, a main effect of the second stressor experience, (immediately prior to blood collection) was evidenced, $F(2, 30)=29.44, p<.01$. Newman-Keuls multiple comparisons indicated that subjects exposed to either the Apparatus or Shock treatment had significantly higher corticosterone levels in comparison to No Treatment subjects. The former two groups did not significantly differ. Interestingly, a main effect of the initial shock treatment was also demonstrated, $F(1, 30)=4.41, p<.05$, with shock subjects having higher corticosterone levels than those subjects within the No Treatment groups. Although no significant interaction between the two stressor experiences was determined, Newman-Keuls multiple comparisons were performed since a priori predictions had been made concerning this interaction. Indeed, multiple

comparisons indicated that subjects that were originally exposed to Shock and then placed in the Apparatus, had higher corticosterone levels than animals that were placed in the Apparatus but had not previously been shocked. Likewise, the effects of the second shock treatment were somewhat enhanced in animals previously exposed to Shock, but this difference did not reach statistical significance (see Table 3).

Insert Table 3 about here

Figure 7. Mean ($\pm$ S.E.M.) norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) concentrations (ng/ml protein) in animals that had originally been exposed to No Treatment (NT) or Shock (SH) and then one week later, reexposed to either NT, Apparatus (APP) or SH.

Figure 8a. Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) in animals that had originally been exposed to No Treatment (NT) or Shock (SH) and then one week later, reexposed to either NT, Apparatus (APP) or SH.

Figure 8b. Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) in animals that had originally been exposed to No Treatment (NT) or Shock (SH) and then one week later, reexposed to either NT, Apparatus (APP) or SH.

Figure 9 . Mean ($\pm$ S.E.M.) serotonin (5-HT) and 5-hydroxyindoloacetic acid (5-HIAA) concentrations (ng/ml protein) in animals that had originally been exposed to No Treatment (NT) or Shock (SH) and then one week later, reexposed to either NT, Apparatus (APP) or SH.

Table 3. Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml}$) in mice that initially had been given No Treatment (NT) or Shock (SH) and then one week later, were reexposed to either NT, Apparatus (APP) or SH.

		Initial Treatment	
		<u>No Treatment</u>	<u>Shock</u>
Subsequent Stressor Treatment	<u>No Treatment</u>	4.1 $\pm$ 0.9	4.8 $\pm$ 1.2
	<u>Apparatus</u>	12.4 $\pm$ 1.0	17.0 $\pm$ 2.3
	<u>Shock</u>	14.4 $\pm$ 0.9	17.5 $\pm$ 2.5

EXPERIMENT 4 Neurochemical Alterations Induced by the Combination of Interleukin-1 and Stressor Administration

It has been reported that the effects of stressors will enhance the behavioural and neurochemical changes induced by concurrently administered catecholamine stimulants (Hahn, Zacharko and Anisman, 1986; Antelman et al., 1980; Antelman & Eichler, 1979; Herman et al., 1984). Experiment 4 was undertaken to ascertain whether stressor exposure would likewise augment the neurochemical alterations induced by IL-1 treatment.

Method

Subjects and Procedures

A total of 60, naive, male, CD-1 mice as described in Experiment 1 served as subjects. Animals received either saline or IL-1 β (0.4 μ g). The IL-1 β was generously provided by Dr. Craig Reynolds of the Biological Modifiers Program, National Cancer Institute, Frederick, Maryland. The specific activity of the IL-1 β was 1.9×10^6 U/50 μ l and endotoxin content of 2.5 EU/mg protein. Aliquots were of 1.0 μ g in phosphate buffered saline were stored at -82°C. Fifteen minutes later mice were exposed to either No Treatment, Apparatus exposure for 1.1 hrs. or Footshock (360 shocks) as described in Experiment 3. Brains were taken immediately afterward and frozen for neurochemical determinations. Trunk blood was collected for analysis of plasma corticosterone levels. All HPLC and corticosterone procedures will follow those outlined in Experiment 1.

Results and Discussion

Norepinephrine

Figure 10 shows the NE and MHPG concentrations for each brain region as a function of the treatment mice received. As in earlier reports indicating that

stressors provoked increased NE turnover, analysis of variance revealed that MHPG levels within the hypothalamus varied as a function of the stressor treatment, $F(2, 48)=4.69$, $p<.01$. Newman-Keuls multiple comparisons ($\alpha=.05$) indicated that both apparatus exposure and administration of footshock increased MHPG levels in comparison to nonshocked subjects. The MHPG levels between the Apparatus and Shock subjects did not differ from one another. Unlike the results of Experiment 1, administration of IL-1 did not significantly affect NE or MHPG concentrations.

Paralleling the hypothalamic results, MHPG concentrations within the locus coeruleus were significantly increased as a function of Stressor treatment, $F(2, 48)=9.64$, $p<.01$. However, unlike the hypothalamic results, Newman-Keuls multiple comparisons indicated that Shock subjects differed significantly from both Apparatus and No Shock subjects, which did not differ from one another. Within the prefrontal cortex, MHPG concentrations varied as a function of the stressor treatment, $F(2, 48)=6.41$, $p<.01$. Newman-Keuls multiple comparisons revealed that shocked animals differed significantly from both Apparatus and No Shock subjects, which once again did not differ from one another. Finally, hippocampal MHPG was also influenced by the stressor treatment, $F(2, 52)=12.55$, $p<.01$. The multiple comparisons revealed that Subjects exposed to either shock or to the apparatus displayed higher MHPG concentrations relative to nonshocked subjects. The former two groups did not differ from one another.

 Insert Figure 10 about here

Dopamine

Analysis of variance revealed no significant variations of DA concentrations within any brain regions, however, several metabolite changes were observed (see

Figure 11). Within the prefrontal cortex, DOPAC was unaffected by the treatment, however, 3-MT varied as a function of the Stressor x Cytokine Treatment interaction, $F(2, 45)=6.89$, $p<.01$. Newman-Keuls multiple comparisons of the simple effects revealed that in the absence of shock treatment, IL-1 resulted in a significant increase of 3-MT concentrations. Curiously, stressor exposure appeared to counteract the effects of IL-1 treatment. In particular, in animals that received IL-1 followed by either Apparatus or Shock, 3-MT accumulation was lower than in nonstressed animals treated with this cytokine.

Consistent with earlier observations that stressors provoke moderate increases in DOPAC accumulation within the nucleus accumbens, analysis of variance indicated a significant main effect of both stressor exposure, $F(2, 54)=4.87$, $p<.01$ and IL-1 treatment, $F(1, 54)=5.91$, $p<.05$ within this brain region. Shock exposure resulted in increased DOPAC accumulation relative to nonshocked subjects and IL-1 treatment resulted in increased DOPAC levels relative to saline treated subjects. Although the interaction of shock and cytokine treatment did not reach significance, multiple comparisons were performed since a priori predictions had been made concerning this interaction. Indeed, Newman-Keuls multiple comparisons indicated that DOPAC accumulation in IL-1 treated animals that had been exposed to shock, exceeded that seen in either mice that had received IL-1 but had not been shocked or animals that had been shocked and injected with saline.

 Insert Figure 11 about here

Serotonin

Although Experiment 1 indicated a significant effect of IL-1 treatment upon 5-HIAA concentrations within the hypothalamus, a similar outcome was not

observed in Experiment 4 (see Figure 12). It was found that 5-HIAA varied as a function of the stressor treatment, $F(2, 46)=3.81, p<.05$. Newman-Keuls multiple comparisons revealed that subjects within the Shock groups had significantly higher levels of 5-HIAA than those within the No Shock groups. Subjects exposed to Apparatus had 5-HIAA levels moderately higher than those of the No Treatment group and somewhat lower than those of the Shock subjects. These latter groups were not found to be statistically different from one another. In contrast to these results, serotonin levels within the hypothalamus were significantly lower in IL-1 treated mice, $F(1, 40)=6.94, p<.01$.

Within the prefrontal cortex, 5-HIAA levels differed significantly as a result of stressor exposure, $F(2, 48)=5.69, p<.05$. Newman-Keuls multiple comparisons indicated that subjects exposed to Shock had significantly higher levels of 5-HIAA in comparison to No Shock groups. Once again, subjects within the Apparatus groups had 5-HIAA levels moderately higher than those of the No Treatment group and somewhat lower than those of the Shock subjects, but these differences did not reach statistical significance. Levels of serotonin within the prefrontal cortex did not vary as a function of the interleukin treatment, while the main effect of stressor exposure approached significance, $F(2, 47)=2.90, p=.065$. Subjects exposed to shock had somewhat higher levels of 5-HT than subjects within the No Treatment or Apparatus groups ($p<.10$).

Serotonin concentrations in the hippocampus were not significantly affected by either interleukin or stressor treatment. Unfortunately, 5-HIAA levels could not be analyzed due to several lost samples.

 Insert Figure 12 about here

Corticosterone

Plasma corticosterone concentrations for each group are shown in Table 4. Analysis of variance revealed a significant Treatment x Stresor interaction, $F(2,39)=4.74$, $p<.05$. Newman-Keuls multiple comparisons of the simple effects comprising the interaction revealed that subjects treated with IL-1 and exposed to either Apparatus or Shock, had significantly higher corticosterone levels than those injected with saline and exposed to No Shock or Apparatus. Shock had a comparable effect of increasing corticosterone levels regardless of IL-1 or saline injection.

Insert Table 4 about here

Figure 10 . Mean ($\pm$ S.E.M.) norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) concentrations (ng/ml protein) in mice injected with either IL-1, .4 μ g or saline, .4 ml, followed fifteen minutes later by 1.1 hours of NT, APP or SH.

Figure 11 . Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) in mice injected with either IL-1, .4 μ g or saline, .4 ml, followed fifteen minutes later by 1.1 hours of NT, APP or SH.

Figure 12 . Mean ($\pm$ S.E.M.) serotonin (5-HT) and 5-hydroxyindoloacetic acid (5-HIAA) concentrations (ng/ml protein) in mice injected with either IL-1, .4 μ g or saline, .4 ml, followed fifteen minutes later by 1.1 hours of NT, APP or SH.

Table 4. Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml}$) in mice injected with either $4\text{ }\mu\text{g}$ of IL- 1β or .4 ml of sterile saline, followed fifteen minutes later by 1.1 hrs. of NT, APP or SH.

		Treatment	
		<u>Saline</u>	<u>IL-1β</u>
Stressor Treatment	<u>No Treatment</u>	$4.4 \pm .9$	23.2 ± 1.6
	<u>Apparatus</u>	15.0 ± 1.8	31.6 ± 3.0
	<u>Shock</u>	27.8 ± 2.8	31.9 ± 3.8

EXPERIMENT 5 Neurochemical Sensitization of Stressor Exposure to Subsequent IL-1 Administration

If IL-1 challenge acts like a stressor, then it might be expected that sensitization or cross-sensitization with a stressor such as footshock might occur. Accordingly, Experiment 5 assessed whether stressor exposure would proactively influence the amine alterations ordinarily induced by IL-1. To this end, mice were exposed to a footshock stressor and then 1 week later, at a time when neurochemical activity and levels had returned to basal values, were challenged with this cytokine.

Method

Subjects and Procedure

Sixty, naive, male, CD-1 mice, as described in Experiment 4, served as subjects. Mice were assigned randomly to one of two conditions; No Treatment or Shock and then individually housed. Shock animals were then exposed to 360 shocks (300 μ A intensity, 2 second duration, at 9 sec intervals over a 1.1 hr period), while nonshocked animals were returned to the vivarium. One week later, mice of each group received either sterile saline injections (0.4 ml, ip.), IL-1 β (0.2 μ g in 0.2 ml of saline, ip.) or IL-1 β (0.4 μ g in 0.4 ml of saline, ip.). One hour later, mice were sacrificed and brains removed, sectioned and frozen for subsequent amine determinations. Trunk blood was collected for analysis of plasma corticosterone levels.

Results and Discussion

Norepinephrine

As in Experiment 1, treatment with IL-1 influenced MHPG accumulation within the hypothalamus, $F(2, 53)=4.60$, $p<.05$. As seen in Figure 13, and confirmed by Newman-Keuls multiple comparisons ($\alpha=.05$) of the main effect

indicated subjects treated with either 0.2 μg or 0.4 μg of IL-1 showed increased MHPG accumulation when compared to saline controls. Unlike Experiment 1, however, IL-1 did not influence hypothalamic NE levels. The possibility exists that protein differences in tissue samples between the experiments could account for the lower amine concentrations found in the saline controls in this experiment. In particular, in Experiment 5 the protein levels of hypothalamic tissue equalled 176.4 ± 12.13 , whereas in Experiment 1, the protein value was 245.44 ± 19.72 . As the latter value is closer to that more commonly attained, it is conceivable that the hypothalamic dissections in Experiment 5 were incomplete.

Within the hippocampus, MHPG levels varied as a function of the Stressor x Treatment interaction, $F(2, 47)=3.23$, $p<.05$. Newman-Keuls multiple comparisons of the simple effects comprising the interaction indicated that subjects originally exposed to a Shock stressor followed one week later by 0.2 μg of IL-1, showed higher MHPG accumulation when compared to either subjects who originally had not been stressed and then were injected with 0.2 μg of IL-1 or those subjects who originally were given Shock and then injected with either saline or 0.4 μg of IL-1.

Within the prefrontal cortex, no main effect of IL-1 treatment upon MHPG accumulation was evidenced. However, the Stressor x Treatment interaction approached significance, $F(2, 46)=3.08$, $p=.055$. Newman-Keuls multiple comparisons of the simple effects comprising the interaction indicated that subjects given No Treatment and subsequently injected with either 0.2 μg or 0.4 μg of IL-1 had significantly increased MHPG accumulation when compared to subjects given Shock stressor and subsequently injected with 0.4 μg of IL-1.

 Insert Figure 13 about here

Dopamine

As seen in Figure 16, within the prefrontal cortex, DA levels were unaffected by the treatments; however, DOPAC accumulation varied as a function of the cytokine Treatment, $F(2, 43)=3.48$, $p<.05$. Newman-Keuls multiple comparisons indicated that subjects injected with 0.2 μg of IL-1 had significantly higher DOPAC levels than those injected with 0.4 μg of IL-1, while DOPAC levels of saline treated mice were intermediate between those of mice that received the .2 μg and .4 μg doses of IL-1. Paralleling the DOPAC results within the prefrontal cortex, 3-MT accumulation varied as a function of the cytokine Treatment, $F(2, 38)=3.50$, $p<.05$ and Stressor exposure, $F(1, 38)=5.45$, $p<.05$. Subjects exposed to Shock a week prior to cytokine Treatment had lower 3-MT levels than subjects given No Treatment. Additionally, Newman-Keuls multiple comparisons of the cytokine effects indicated that subjects injected with .2 μg of IL-1 had significantly higher 3-MT accumulation than saline treated subjects. The mice injected with .4 μg of IL-1 had somewhat lower 3-MT levels than the .2 μg groups, but these did not differ from one another nor did they differ from those of the saline treated subjects.

Consistent with the previous Experiments involving IL-1, either administration of this cytokine or stressor application influenced DA and its metabolites within the nucleus accumbens. Specifically, DA levels varied as a function of the Cytokine treatment, $F(2, 49)=3.24$, $p<.05$. Newman-Keuls multiple comparisons indicated that there was no difference in DA levels between saline treated animals and those injected with either .2 μg or .4 μg of IL-1. Subjects injected with .2 μg of IL-1, however, had higher DA levels than those subjects injected with .4 μg of

IL-1. Although this was found to be a statistically significant result, the actual difference in DA levels between the .2 μ g and .4 μ g IL-1 groups was only 10%. Paralleling the results of Experiment 4, DOPAC accumulation within the nucleus accumbens varied as a function of Stressor application, $F(1, 52)=13.75$, $p<.01$ and cytokine administration, $F(2, 52)=5.10$, $p<.01$. Subjects exposed to the Shock stressor had increased DOPAC accumulation relative to nonshocked subjects. Moreover, the multiple comparisons revealed that mice injected with .4 μ g of IL-1 had significantly greater DOPAC accumulation than did subjects injected with saline.

Although no significant interaction existed between the Stressor and Cytokine treatment, Newman-Keuls multiple comparisons were performed since a priori predictions had been made concerning this interaction. Indeed, as determined in Experiment 4, DOPAC accumulation in animals treated with either dose of IL-1 and exposed to Shock, exceeded that seen in either mice that had received IL-1 and not been Shocked or in animals that had been Shocked and then injected with saline. Additionally, within the No Treatment groups, animals injected with .4 μ g of IL-1 had greater DOPAC accumulation than animals injected with either saline or .2 μ g of IL-1.

Like DOPAC, it appeared that 3-MT levels varied as a function of the Stressor x cytokine Treatment interaction, $F(2, 37)=5.58$, $p<.01$. Newman-Keuls multiple comparisons of the simple effects comprising this interaction revealed that within the No Stress groups, .2 μ g of IL-1 increased 3-MT accumulation relative to that seen in animals injected with either saline or .4 μ g of IL-1. Within saline treated subjects, those that were originally Shocked, had higher 3-MT levels than subjects originally given No Treatment. Within the Shock groups, no significant difference in 3-MT levels was observed, however, a continuing decline in accumulation was evident from saline through to the .4 μ g, IL-1 subjects. Finally, HVA levels within

the nucleus accumbens were found to vary as a function of the initial Stressor exposure, $F(1, 44)=4.26$, $p<.05$. Subjects exposed to Shock treatment had lower levels of HVA than did nonstressed subjects. Once again, although no significant interaction of Stressor x cytokine Treatment existed Newman-Keuls multiple comparisons of the simple effects were conducted. The multiple comparisons indicated that subjects exposed to the Shock stressor and then injected with either .2 or .4 μg of IL-1 had lower levels of HVA than subjects that had received either Shock and then injected with saline or subjects that had originally been given No Treatment and then injected with .4 μg of IL-1.

 Insert Figure 14 about here

Serotonin

The hypothalamic, hippocampal and prefrontal cortex concentrations of 5-HT and 5-HIAA for each group are shown in Figure 15. Although there was no significant effect of the Stressor or cytokine Treatment upon serotonin or its metabolites within the hypothalamus, increases in 5-HIAA levels within IL-1 treated subjects approached significance, $F(2, 48)=2.69$, $p=.07$. This pattern is consistent with the results obtained in Experiment 1 and with those of Lacosta et al., (1994).

Levels of 5-HT within the hippocampus varied with the cytokine Treatment, $F(2, 50)=11.57$, $p<.05$. Newman-Keuls multiple comparisons indicated that 5-HT levels were significantly increased within the 0.4 μg IL-1 group in comparison to subjects that received 0.2 μg of IL-1. The saline subjects had 5-HT levels slightly higher than those of the 0.2 μg IL-1 group but these were not statistically different from those subjects injected with 0.4 μg of IL-1.

Insert Figure 15 about here

Corticosterone

In contrast to the results obtained in Experiments 1 and 4, plasma corticosterone levels did not significantly vary as a function of IL-1 administration, $F(2, 34)=.86$, $p=0.43$. Given this peculiar result, a subsequent experiment was undertaken to compare the biological activity of the two different supplies used within these investigations. It was determined the both supplies of IL-1 were equally effective at provoking increases in plasma corticosterone levels (saline $\bar{x} = 3.73 \pm 1.71$; IL-1, R&D $\bar{x} = 13.94 \pm 1.1$; IL-1, NCI $\bar{x} = 14.06 \pm 1.11$). Thus, it seems that the results of corticosterone analyses within Experiment 5 were spurious.

Insert Table 5 about here

Figure 13 . Mean ($\pm$ S.E.M.) norepinephrine (NE) and 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG) concentrations (ng/ml protein) in mice that initially received either NT or SH followed one week later by systemic injection of either saline, .4 ml, IL-1, .2 μ g or IL-1, .4 μ g.

Figure 14 . Mean ($\pm$ S.E.M.) dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations (ng/ml protein) in mice that initially received either NT or SH followed one week later by systemic injection of either saline, .4 ml, IL-1, .2 μ g or IL-1, .4 μ g.

Figure 15 . Mean ($\pm$ S.E.M.) serotonin (5-HT) and 5-hydroxyindoloacetic acid (5-HIAA) concentrations (ng/ml protein) in mice that initially received either NT or SH followed one week later by systemic injection of either saline, .4 ml, IL-1, .2 μ g or IL-1, .4 μ g.

Table 5. Mean ($\pm$ S.E.M.) plasma corticosterone concentrations ($\mu\text{g}/100\text{ ml}$) in mice given either NT or SH followed one week later by injection with either .4 ml of sterile saline, .2 μg of IL-1 β or .4 μg of IL-1 β .

		Stressor Treatment	
		<u>No Treatment</u>	<u>Shock</u>
Subsequent Treatment	<u>Saline</u>	7.6 $\pm$ 2.2	5.2 $\pm$.9
	<u>.2μg IL-1β</u>	9.3 $\pm$ 2.2	9.4 $\pm$ 2.4
	<u>.4μg IL-1β</u>	7.7 $\pm$ 1.6	8.6 $\pm$ 2.4

2 of/de 2

PM-1 3½"x4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGETS

General Discussion

As indicated earlier, multidirectional communication has been shown to exist between the immune and endocrine systems (Blalock, 1989; Besedovsky et al., 1983; Anisman et al., 1988), as well as between the CNS and immune system. Further, it has been proposed that cytokines such as IL-1 or IL-2 may be acting as a messenger between the immune system and the CNS. In particular, it is supposed that IL-1 secreted from macrophages may gain access to the brain, initiating increased neuronal activity, particularly in the region of the hypothalamus (Dunn, 1990; Kabiersch et al., 1988; Zalcman et al., 1994). Among other things, IL-1 may provoke CRF release from the parvocellular aspect of the PVN, leading to pituitary ACTH and hence glucocorticoid release from the adrenal gland (Berkenbosch et al., 1987; Dunn, 1990; Rivier, 1991).

In the present investigation systemic administration of IL-1 increased plasma corticosterone concentrations. However, whereas such an effect was marked in Experiment 1 and Experiment 4, the results of Experiment 5 indicated there was no difference between mice injected with saline and those treated with IL-1. The possibility existed that there may have been a difference in activity level or viability of the cytokine used in the different experiments, since the cytokine was obtained from two different suppliers (R&D Systems vs. National Cancer Institute, Bethesda, Maryland). Accordingly, an additional study was conducted using IL-1 obtained from both suppliers in order to compare their effects with respect to neurochemical activity and plasma corticosterone levels. This experiment revealed that IL-1 from both sources were equally effective in raising corticosterone levels relative to that of saline controls (saline $\bar{x} = 3.73 \pm 1.71$; IL-1, R&D $\bar{x} = 13.94 \pm 1.1$; IL-1, NCI $\bar{x} = 14.06 \pm 1.11$). Thus, it is likely that the results of Experiment 5, where IL-1 did not affect corticosterone levels, was a spurious finding.

In contrast to the large corticosterone changes observed following IL-1 injection, IL-2 administration did not increase corticosterone concentrations. These results parallel those of Zalcman et al., (1994) and Berkenbosch et al., (1986) where IL-1 was shown to significantly increase corticosterone levels, while IL-2 and IL-6 did not. These data are consistent with the view that while IL-1 may be exerting its effects upon the CNS via activation of the HPA axis, any central effects of IL-2 and IL-6 operate via an alternate mechanism.

The documentation of neurochemical alterations following IL-2 administration are fewer in number than those of IL-1. Several areas of the rodent brain are known to contain IL-2 receptors, with the highest concentration being found within the hippocampus (Araujo et al., 1989). Receptors have also been found within the caudate, frontal cortex, median-eminence, arcuate nucleus, lateral septum, striatum, cerebellum and locus coeruleus (Lapchak et al., 1991). Indeed, recent reports have demonstrated several central neurochemical changes in response to the administration of IL-2. Both the present investigation and that of Zalcman et al., (1994) found that systemic IL-2 administration resulted in increased MHPG accumulation within the hypothalamus. Additionally, it seemed that MHPG within the locus coeruleus was influenced by IL-2 treatment.

Other researchers have suggested a role for IL-2 regulation of DA function within the striatum as well as modulation of DA release within the mesencephalon (Lapchak, 1992; Alonso, Chaudieu, Diorio, Krishnamurthy, Quirion & Boksa, 1993). It was determined that IL-2 application to striatal tissue slices in vitro could increase K^+ evoked (3H) dopamine release and that this effect was mediated by extracellular Ca^{++} concentrations. The possible role of IL-2 in regulation of striatal dopaminergic function could explain the neuropsychiatric side-effects seen in IL-2 treated cancer patients. The ability of haloperidol to block these IL-2-

induced effects supports the contention that IL-2 plays a role in increasing dopaminergic neuronal activity (Denicoff, Rubinow & Papa, 1987).

Within the present investigation, the only effect of IL-2 administration on DA was that of altered accumulation of the metabolite HVA within the caudate. It was observed that IL-2 injection resulted in decreased HVA levels within this brain region one hour following injection. However, by two hours these levels had risen significantly and did not differ from those of saline treated animals. Obviously, before firm conclusions can be drawn concerning the central effects of IL-2, additional studies are necessary to examine the dose-response relationship and time course for any IL-2 effects.

There are currently several reports documenting central neurochemical changes associated with cytokine treatment. In the main, they have been concerned with the effects of IL-1 on hypothalamic CRF or NE. In general, it has been observed that systemic or icv administration of the cytokine influences CRF release and the turnover of NE (Dunn, 1988; Kabiersch et al., 1988; Zalcman et al., 1994; Mohankumar & Quadri, 1993; Terao, Oikawa & Saito, 1993). Moreover, NE variations induced by IL-1 may be responsible for the CRF changes. In a similar fashion, in the present investigations it was observed that systemic IL-1 administration influenced hypothalamic NE levels as well as the accumulation of MHPG, indicating increased utilization of the amine. Unfortunately, however, this finding was not consistent across experiments. In Experiment 1 IL-1 administration resulted in a decrease of NE levels within the hypothalamus and an increase of MHPG accumulation. In contrast, in Experiment 4, no effect upon NE was observed and MHPG accumulation varied only as a function of stressor treatment. Finally, the results of Experiment 5, like those of Experiment 1 showed increased MHPG accumulation as a result of IL-1 injection, while NE levels were unaffected by the cytokine. In effect, it seems that in two of three experiments

MHPG accumulation was affected by IL-1, while NE levels was affected in one of the three studies. In earlier investigations conducted by Zalcman et al. (1994) both NE and MHPG varied as a function of the cytokine, a finding consistent with that observed by Dunn 1988 and Kabiersch et al., 1988. Thus, the present data are generally in support of previous reports, but suggest that the amine changes are not robust and may be influenced by subtle aspects of the experimental procedures. Unfortunately, the specific variables that lead to the differential outcomes are not clear. As indicated earlier, NE levels in Experiment 5 may have been unduely affected by incomplete dissection of hypothalamic tissues that were collected. Further, as previously discussed, the cytokine was obtained from different sources, but it seemed that both were biologically active. Finally, there were no remarkable differences in the subjects themselves, as all were obtained from the same supplier and were tested at about the same age.

Presently, the mechanism by which cytokines exert their actions upon central neurochemical functioning remains elusive. Controversy exists regarding whether IL-1 directly affects CRF containing neurons within the PVN or whether IL-1 affects NE neurons which in turn cause CRF release from the hypothalamus (Mohankumar et al., 1993; Kabiersch et al., 1988). It seems improbable that a large molecule such as IL-1 could cross the blood brain barrier directly; however, it was postulated that it may gain access through the circumventricular organs where the blood brain barrier is less restrictive. Several recent studies have indicated that IL-1 may be transported through the blood brain barrier by a saturable mechanism (Banks, Ortiz, Plotkin & Kastin, 1991). To date, identification of this mechanism has been elusive, but it has been postulated that interleukins may use a previously characterized endocytotic mechanism for macromolecules (Broadwell, 1989). A second proposition that has been advanced

is that IL-1 may exert its effects at the level of the locus coeruleus or other brain stem structures with NE projections to the hypothalamus.

It has been proposed that the hypothalamic NE changes induced by IL-1 may be due to interleukin effects upon CRF and an endogenous eicosanoid-cyclooxygenase product (Terao et al., 1993). This conclusion was based upon findings that anti-CRF antibody and indomethacin could effectively block IL-1's ability to increase NE turnover within the hypothalamus. It could be argued that since indomethacin has antipyrogenic effects, the NE changes were due to fever provoked by IL-1. However, other cytokines mediating the acute phase immune response, such as IL-6 and tumor necrosis factor (TNF α), do not influence hypothalamic NE turnover suggesting that IL-1's effect upon hypothalamic NE turnover may be independent of its role in the acute phase response (Terao et al., 1993). Further, in the present investigation, variations of NE/MHPG were detected with IL-1 doses as low as .2 μ g, which is well below that required to induce a pyrogenic effect (Dunn, 1990).

In addition to the IL-1 induced NE changes within the hypothalamus, other amine and metabolite alterations have been documented; however, these results have been inconsistent across studies. For instance, Kabiersch et al., (1988) detected no significant changes in DA or its metabolite, DOPAC, within the hypothalamus; a result consistent with the findings of the present investigation, but contrary to the findings of Mohankumar, Thyagarajan and Quadri (1990) who reported large increases in hypothalamic DA and DOPAC following IL-1 administration. Additionally, another investigation by Mohankumar, Thyagarajan and Quadri (1993) indicated increased 5-HIAA accumulation within the hypothalamus following IL-1 administration; once again, a result consistent with that of Experiment 1 of the present investigation as well as that of Dunn (1988), but contrary to the findings of Zalcman et al., (1994).

There are few reports concerning the effects of IL-1 on neurotransmitter activity in regions other than the hypothalamus. Moreover, it seems that even among the limited published studies, inconsistent results have been observed with respect to these changes. For instance, post mortem analyses revealed that DOPAC levels in prefrontal cortex of mice were altered by systemic IL-1 treatment (Zalcman et al., 1994). In contrast, analyses in freely moving rats, using microdialysis, revealed that IL-1 did not affect DA and induced only a modest DOPAC change (Iacosta et al., 1994). The source for these differences are not discernible given the great number of procedural differences between these studies (e.g., rats vs. mice, dose of IL-1, time of sampling after cytokine treatment). However, the results of the present investigation, like that of Zalcman et al. (1994), indicated that systemic administration of IL-1 resulted in increased accumulation of DOPAC within the prefrontal cortex without any corresponding changes in the amine itself.

To date, no investigations have reported IL-1 effects upon the extracellular DA metabolite, 3-MT. Throughout the present investigations involving IL-1, an interesting pattern of DOPAC and 3-MT accumulation was detected within the prefrontal cortex and nucleus accumbens. Specifically, in most of the investigations, both brain regions showed significant increases in DOPAC accumulation following IL-1 administration. Although the DOPAC levels within the prefrontal cortex in Experiment 4 did not increase to the point of being statistically significant, IL-1 treated animals had a 50% increase over those of saline subjects. Additionally, within Experiments 4 and 5, there was a significant increase in DOPAC accumulation within the nucleus accumbens as a result of IL-1 treatment. Paralleling the DOPAC results, IL-1 administration in Experiments 4 and 5 resulted in increased 3-MT accumulation within the prefrontal cortex.

Moreover, in Experiment 5, a corresponding increase in 3-MT levels was also observed within the nucleus accumbens.

Again, inconsistencies have been observed in the evaluation of serotonergic effects of IL-1 treatment. Whereas Kabiersch et al., (1988) and Zalcman et al., (1994) reported significant increases in 5-HIAA accumulation within the hippocampus following IL-1 administration, Dunn (1988) reported increases in the 5-HT/5-HIAA ratio that were restricted to the prefrontal cortex. Zalcman et al., (1994) also reported a significant increase in the 5-HIAA/5-HT ratio in prefrontal cortex following IL-1 administration, although variations in this ratio were also noted in the hippocampus.

Even within the present investigation the effects of IL-1 on central neurotransmitters were inconsistent across experiments. As in the aforementioned studies, it was observed in Experiment 1 that 5-HT and 5-HIAA accumulation increased within the prefrontal cortex following IL-1 administration. However, once again, Experiment 4 revealed only a stressor effect upon 5-HIAA accumulation within this region, while Experiment 5 indicated that IL-1 neither affected 5-HT nor its metabolite.

As already indicated, it is difficult to discern the factors that might be contributing to the inconsistent outcomes observed both in the present investigation and across laboratories. Certainly it can be argued that IL-1 does not affect central neurotransmitters, and that the results of the present investigation were largely spurious. Yet, the number of occasions where IL-1 was found to affect central amines was well beyond that expected by chance. Unfortunately, to date, there have been no published reports concerning the dose-response relationship between cytokines and variations of central amines or endocrines. This likely stems from the fact that attention to IL-1 as an immunotransmitter is only a recent phenomena, coupled with the exorbitant costs of the cytokine

(\$66/ug, U.S.) Interestingly, in Experiment 5, where two doses of IL-1 were employed, the lower dose was more effective than the higher dose in affecting amine levels/turnover. It is not clear what factors contribute to this, but when it is considered that cytokines may have an array of effects (e.g., behavioural, endocrine, anorexic, somnolescent, pyrogenic, inflammatory) it is conceivable that the higher dose results in recruitment of processes that minimize amine variations (e.g., changes in autoreceptor sensitivity, alterations of metabolite dispersion or uptake). Further to the same issue, as mentioned earlier, IL-1 does not readily pass into the brain, and it has been calculated that only .06-.08% of systemically injected IL-1 reaches the brain (Banks et al., 1991). Indeed, studies involving icv administration indicated pronounced effects using doses as low as 1-20 ng. It should be considered, however, that disruption of blood brain barrier mechanisms can be induced by events such as seizure or head trauma (Yan, Banos, Herregodts, Hooghe & Hooghe-Peters, 1992) and it would not be surprising to find that stressors also provoke such an outcome. In effect, it is conceivable that factors which influence circulatory processes, including stress, may come to affect accessibility of IL-1 into brain tissues. In view of the small percentage of systemically administered IL-1 that ordinarily reaches the brain, even small perturbations of the blood brain barrier may have profound effects. One can imagine that transporting animals from the vivarium to the laboratory may under some conditions be stressful (e.g., if rat smells are present in the hallway). Likewise, within the vivarium itself, factors such as cleaning of cages could potentially influence the effects of cytokine treatment. Indeed, in their highly influential papers, Riley, Fitzmaurice & Spachman (1981) reported that the effects of the laboratory environment could drastically effect antigenic and tumorigenic processes. Likewise, Zalcman et al., (1994) observed that the behavioural effects of cytokines can be greatly influenced by slight changes of laboratory stimuli.

Accordingly, it would not be unreasonable to suppose that similar factors might be operative in affecting the central consequences of cytokine treatment.

As alluded to earlier, it has been suggested that the behavioural and central neurochemical consequences of IL-1 treatment are in many ways similar to those induced by stressors (Dunn, 1988; Anisman et al., 1993). Thus, the possibility has been considered that these treatments might have synergistic effects, and perhaps stressors might also lead to sensitization of neurochemical mechanisms such that the effects of subsequently administered IL-1 would be enhanced.

It has recently been reported that stressors may act synergistically with IL-1 treatment (Lacosta, Merali, Anisman & Bielajew, 1994). Specifically, using microdialysis it was demonstrated that rats injected with IL-1 and exposed to an air puff forty minutes later, show greater 5-HIAA accumulation within the hippocampus and prefrontal cortex than rats given the air puff alone. To a considerable degree, the results of the present investigation also suggested combined actions of cytokine and stressor manipulations. Accumulation of DOPAC within the nucleus accumbens was affected by both IL-1 administration and stressor exposure. Moreover, it seemed that stressor exposure more profoundly influenced DOPAC accumulation in mice pretreated with IL-1 than in those treated with saline.

Unlike the aforementioned DA variations, the NE changes associated with the combination of IL-1 and stressor treatment were no greater than that of the stressor alone. However, it will be recalled that in Experiment 4 where the synergistic effects were evaluated, IL-1 itself did not modify NE levels or MHPG accumulation. Inasmuch as this finding was contrary to that of Experiment 1 and 5, as well as a subsequently conducted experiment, it seems that the failure to observe NE changes in Experiment 4 was an incongruent result. Accordingly, it is premature to conclude that IL-1 and stress do not yield synergistic effects.

An entirely different pattern, however, was evident in prefrontal cortex. In this region, where DOPAC was moderately increased as a result of IL-1 treatment, DA utilization was not further modified by stressors. Further, levels of the extracellular metabolite, 3-MT, were also enhanced by the cytokine, but in animals exposed to footshock the effects of IL-1 were eliminated. Although an explanation for this outcome cannot be given with great certainty, it is interesting that the optimal effects of IL-1 were actually obtained with a .2 μ g rather than .4 μ g dose of this cytokine. It is possible that the dose response relationship concerning IL-1 effects on brain neurotransmitters is an inverted U shape function. As such, it is conceivable that the combined effects of IL-1 and stressor exposure would result in a rightward shift along this function, leading to reduced amine/metabolite levels. Such an effect is, in fact, not unusual and has been observed with respect to the actions of opiate peptides on brain neurotransmitters and on immune functions (Carr, 1991).

Finally, turning to the potential sensitization effects of stressor exposure on responses to later IL-1 treatment, it was observed that the lower dose of IL-1 provoked an appreciably greater increase of hippocampal MHPG in previously stressed animals than in mice that received only one of the treatments. It is tempting to suggest that such an outcome reflects sensitization processes, particularly since this region was previously demonstrated to be exceptionally sensitive to sensitization effects (Irwin et al., 1986). Yet the fact that a similar sensitization was not seen with the .4 μ g dose of IL-1 makes it necessary to hold this conclusion in abeyance pending more complete dose response analyses.

In the case of dopaminergic changes, it was observed that within the nucleus accumbens, both stressor application and cytokine treatment enhanced accumulation of DOPAC. These effects appear to be cumulative. Thus, it seems that a previous stressor experience may augment the action of a subsequent

cytokine challenge. It is curious, however, that a stressor treatment would influence DA utilization measured one week later since amine changes induced by stressors typically persist for only a few hours (Anisman et al., 1991). It has been demonstrated previously that cues associated with a stressor may have effects on mesocorticolimbic DA activity (Anisman et al., 1991). Thus, it is possible that reexposure to cues associated with initial stressor treatment (e.g., handling, transport of mice from the vivarium to the testing area) were responsible for the enhanced DOPAC accumulation. Therefore, the proactive effects of the initial stressor treatment on the later response to IL-1 may reflect the conjoint actions of cytokines plus the effects of stressor-related cues, rather than a genuine sensitization effect.

In contrast to the DOPAC changes observed in the nucleus accumbens, 3-MT alterations were not indicative of a sensitization effect. To the contrary, IL-1 administration tended to eliminate the increased metabolite accumulation associated with a previously administered stressor. If, as indicated earlier, the dose response curve is one where the .2 µg dose yields an optimal effect on mesolimbic DA activity, then the combined action of the IL-1 and stressor treatment may again represent a rightward shift on this function. While such an explanation may be appealing with respect to the analysis of the 3-MT changes, it still remains that the changes of this metabolite did not parallel that of DOPAC accumulation. It has been argued that 3-MT as an extracellular metabolite may be a better index of DA utilization (Puglisi-Allergra, Imperato, Angelucci & Cabib, 1991) particularly since DOPAC accumulation may be subject to fluctuations associated with factors unrelated to utilization rate (e.g., differential rates of uptake into tissues as a function of experimental manipulations). Obviously the inconsistency between the effects of stressors and cytokines on 3-MT and DOPAC accumulation, coupled with the dearth of information regarding the previously discussed dose response

relationship, makes it difficult to provide firm conclusions regarding potential sensitization effects of the treatments. It should be underscored, however, that several recent reports have indicated that subtle manipulations (e.g., presentation of novel stimuli) may influence behavioural response to cytokines (Zalcman, 1994) and likewise cytokine treatment may proactively influence the response to rewarding stimuli (e.g., responding for rewarding stimuli from the lateral hypothalamus), and that such effects may be dependent on conditioning/sensitization processes (Anisman, Zalcman, Merali & Kokkinidis, 1994).

Summarizing, the results of the present investigation suggest that IL-1 and IL-2 may have CNS consequences including variations of NE and DA in several brain regions. These effects are clearly not exceptionally robust and appear to vary across experiments. In the main, however, it did appear that IL-1 affected hypothalamic NE utilization and DA turnover in prefrontal cortex, as well as plasma corticosterone concentrations. The response to interleukins was modifiable by prior or concurrent stressor exposure, and to a great extent both types of stressor treatments yielded comparable results. Unfortunately, the limited information concerning the conditions wherein cytokines would influence central neurotransmitter turnover precludes definitive conclusions being drawn concerning cytokine/stress interactions. Particular note must be made of the fact that the present series of studies was conducted under fairly well controlled conditions and were consistent across experiments in terms of subject characteristics, dissection and assay procedures. These data if nothing else, point to the capricious nature of potential effects of cytokines on central neurotransmitter function.

REFERENCES

- Abercrombie, E.D. & Jacobs, B.L. (1988). Systemic naloxone administration potentiates locus coeruleus noradrenergic neuronal activity under stressful but not non-stressful conditions. Brain Research, 441, 362-366.
- Abercrombie, E.D., Keefe, K.A., DiFrischia, D.S. & Zigmond, M.J. (1989). Differential effects of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J. Neurochem, 52, 1655-1658.
- Abercrombie, E.D., Wilkinson, L.O., & Jacobs, B.L. (1986) Environmental stress and activity of locus coeruleus noradrenergic neurons in freely moving cats. Soc. Neurosci. Abstr., 123, 1134.
- Alonso, R., Chaudieu, I., Diorio, J., Krishnamurthy, A., Quirion, R. & Boksa, P. (1993). Interleukin-2 modulates evoked release of [³H] dopamine in rat cultured mesencephalic cells. Journal of Neurochemistry, 61, 1284-1290.
- Anisman, H. & Sklar, L.S. (1979). Catecholamine depletion upon reexposure to stress: Mediation of the escape deficits produced by inescapable shock. J. Comp. Physiol. Psychol., 93, 610-625.
- Anisman, H., Zalcman, S. & Zacharko, R.M. (1993). The impact of stressors on immune and central neurotransmitter activity: Bidirectional communication. Reviews in the Neurosciences, 4, 147-180.
- Anisman, H., Irwin, J., Bowers, W., Ahluwalia, P., & Zacharko, R.M. (1986). Variations of norepinephrine concentrations following chronic stressor application. Pharmacol. Biochem. Behav., 26, 653-659.
- Anisman, H., Pizzino, A., & Sklar, L.S. (1980). Coping with stress, norepinephrine depletion and escape performance. Brain Research, 191, 583-588.
- Anisman, H., Zalcman, S., Shanks, N., & Zacharko, R.M. (1991). Multisystem regulation of performance deficits induced by stressors: An animal model of depression. In: Boulton A, Baker G, Martin-Iverson M. eds. Neuromethods, vol. 19: Animal Models of Psychiatry, II. New Jersey: Humana Press, 1-59.
- Anisman, H., Zalcman, S., Merali, Z. & Kokkinidis, L. (1994). Responding for rewarding brain stimulation following systemic interleukin-2 treatment. Neuroscience Abstracts.

- Antelman, S.M., Eichler, A.J., Black, C.A., & Kocan, D. (1980). Interchangeability of stress and amphetamine in sensitization. Science, 207, 329-331.
- Antelman, S.M., Kocan, D., Edwards, D.J., & Knopf, S. (1987). A single injection of diazepam induces long-lasting sensitization. Psychopharm. Bull., 23, 430-434.
- Antelman, S.M. (1988). Time-dependent sensitization as the cornerstone for a new approach to pharmacotherapy: drugs as foreign/stressful stimuli. Drug. Dev. Res. 14, 1-30.
- Antelman, S. & Eichler, A.J. (1979). Persistent effects of stress on dopamine-related behaviors; clinical implications. In Catecholamines: Basic and Clinical Frontiers, Eds. E. Usdin, I.J. Kopin and J. Barchas, Pergamon Press, New York.
- Antelman, S. & Chiodo, L.A. (1983). Amphetamine as a Stressor. In Stimulants: Neurochemical, Behavioral and Clinical Perspectives, Ed. I. Creese, Raven Press, New York.
- Antelman, S. & Chiodo, L.A. (1984). Amphetamine as a stressor. In L.L. Iversen, S.D. Iversen & S.H. Snyder, Eds. Handbook of Psychopharmacology, Plenum, New York.
- Antelman, S.M., Cunnick, J.E., Lysle, D.T., Caggiula, A.R., Knopf, S., & Kocan, D.J. (1990). Immobilization 12 days (but not one hour) earlier enhanced 2-deoxy-d-glucose-induced immunosuppression: Evidence for stressor-induced time dependent sensitization of the immune system. Prog. Neuro-Psychopharmacol. & Biol. Psychiat., 14, 579-590.
- Antelman, S.M., Kocan, D., Edwards, D.J., Knopf, S., Perel, J.M., & Stiller, R. (1986). Behavioral effects of a single neuroleptic treatment grow with the passage of time. Brain Res., 385, 58-67.
- Araujo, B.M., Lapchak, P.A., Collier, B. & Quirion, R. (1989). Localization of interleukin-2 immunoreactivity and interleukin-2 receptors in the rat brain: interaction with the cholinergic system. Brain Res., 498, 257-266.
- Archibald, H.C., & Tuddenham, R.O. (1965). Persistent stress reaction after combat: A twenty year follow-up. Arch. Gen. Psychiatry, 12, 475-481.
- Banks, W.A., Ortiz, L., Plotkin, R. & Kastin, J. (1991). Human interleukin (IL) 1, murine IL-1 and murine IL-1 β are transported from blood to brain in the

- mouse by a shared saturable mechanism. Journal of Pharmacology and Experimental Therapeutics., 259, 988-996.
- Berkenbosch, F., de Rijk, R., Del Rey, A., & Besedovsky, H. (1990). Neuroendocrinology of Interleukin-1. In: Porter J.C, Jezova, D. Eds. Circulating Regulatory Factors and Neuroendocrine Function. New York: Plenum Press, 303-314.
- Berkenbosch, F., van Oers, J., Del Rey, A., Tolders, F., & Besedovsky, H. (1987). Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. Science, 238, 524-526.
- Besedovsky, H., Del Rey, A., Sorkin, E., Da Prada, M., Burri, R., & Honegger, C. (1983). The immune response evokes changes in noradrenergic neurons. Science, 221, 564-566.
- Besedovsky, H.O., Sorkin, E., Felix, D., & Haas, H. (1977). Hypothalamic changes during the immune response. Eur. J. Immunol., 7, 323-325.
- Besedovsky, H., Sorkin, E., Keller, M., & Muller, J. (1975). Changes in blood hormone levels during the immune response. Proc. Soc. Exp. Biol. Med., 150, 466-470.
- Black, P.H. (1994,a). Central nervous system-immune system interactions: Psychoneuroendocrinology of stress and its immune consequences. Antimicrobial Agents and Chemotherapy, 38, 1-6.
- Black, P.H. (1994,b) Immune system-central nervous system interactions: Effect and immunomodulatory consequences of immune system mediators on the brain. Antimicrobial Agents and Chemotherapy, 38, 7-12.
- Blalock, J.E. (1984). The immune system as a sensory organ. J. Immunol., 132, 1067-1070.
- Blalock, J.E. & Smith, E.M. (1985). A complete regulatory loop between the immune and neuroendocrine systems. Fed. Proc., 44, 108-111.
- Breder, C.D, Cinarello, C.A, & Saper, C.B. (1988). Interleukin-1 immunoreactive innervation of the human-thalamus. Science, 240, 321-323.
- Broadwell, R.D. (1989). Transcytosis of macromolecules through the blood-brain barrier: a cell biological perspective and critical appraisal. Acta Neuropathol., 79, 117-128.

- Carlson, S.L., Felten, D.L., Livnat, S., & Felten, S.Y. (1987). Alterations of monamines in specific central autonomic nuclei following immunization in mice. Brain Behav. Immunity, 1, 52-63.
- Carr, D.J. & Blalock, J.E. (1991). Neuropeptide hormones and receptors common to the immune and neuroendocrine systems: Bidirectional pathway of intersystem communication. In: Ader, R., Felten, D.L., & Cohen, N. Eds. Psychoneuroimmunology, Academic Press, San Diego, 573-584.
- Carr, D.J. (1992). Neuroendocrine peptide receptors on cells of the immune system. In: Ishizaka, K., Lachmann, P.J., & Waksman, B.H., Eds. Chemical Immunology. Basel: Karger, 84-105.
- Cassens, G., Roffman, M., Kuruc, A., Orsulak, P.J., & Schildkraut, J.J. (1980). Alterations in brain norepinephrine metabolism induced by environmental stimuli previously paired with inescapable shock. Science, 209, 1138 - 1140.
- Cooper, E.L. (1980). Cell-mediated memory in invertebrates. In: Manning, M.J., ed. Phylogeny of Immunological Memory. Elsevier/North Holland, Amsterdam, 35-46.
- Cross, R.J., Markesbery, W.R., Brooks, W.H., & Roszman, T.L. (1980). The acute effects of anterior hypothalamic lesions on the immune response. Brain Res., 196, 79-87.
- Cross, R.J., Brooks, W.H., Roszman, T.L., & Markesbery, W.R. (1982). Hypothalamic-immune interactions. J. Neuro. Sci., 53, 557-566.
- Cross, R.J., Jackson, J.C., Brooks, W.H., Sparks, D.L., Markesbery, W.R., & Roszman, T.L. (1986). Neuroimmunomodulation: impairment of humoral immune responsiveness by 6-hydroxydopamine treatment. Immunology, 57, 145-152.
- Denicoff, K.D., Rubinow, D.R., Papa, M.Z., Simpson, C., Seipp, C.A., Lotze, M.T., Chang, A.E., Rostenstein, D. & Rosenberg, S.A. (1987). The neuropsychiatric effects of treatment with interleukin-2 and lymphokine activated killer cells. Ann. Intern. Med., 107, 293-300.
- Deutch, A.Y, Tam, S.Y, & Roth, R.H. (1985). Footshock and conditioned stress increase 3,4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. Brain Research, 333, 143-146.

- Doherty, M.D. & Gratton, A. (1992). High-speed chronoamperometric measurements of mesolimbic and nigostriatal dopamine release associated with repeated daily stress. Brain Research, in press.
- Dunn, A.J. (1990). Interleukin-1 as a stimulator of hormone secretion. Prog. NeuroEndocrinImmunol., 3, 26-34.
- Dunn, A.J. (1988). Changes in plasma and brain tryptophan and brain serotonin and 5-hydroxyindoleacetic acid after footshock stress. Life Sci., 42, 1847-1853.
- Dunn, A.J. (1988). Systemic interleukin-1 administration stimulates hypothalamic norepinephrine metabolism paralleling the increased plasma corticosterone. Life Sci., 43, 429-435.
- Dunn, A.J. & File, S.A. (1983). Cold restraint alters dopamine metabolism in frontal cortex, nucleus accumbens and striatum. Physiol Behav , 31, 511-513.
- Dunn, A.J., Antoon, M., & Chapman, Y. (1991). Reduction of exploratory behavior by intraperitoneal injection of interleukin-1 involves brain corticotropin-releasing factor. Brain. Res. Bull. , 26, 539-542.
- Dunn, A.J., Powell, M.L., Meitin, C., & Small, P.A. (1989). Virus infection as a stressor: Influenza virus elevates plasma concentrations of corticosterone, and brain concentrations of MHPG and tryptophan. Physiol. Behav., 45, 591-594.
- Farrar, W.L., Kilian, P.L, Ruff, M.R., Hill, J.M., & Pert, C.B. (1987). Visualization and characterization of interleukin-1 receptors in brain. J. Immunol., 139, 459-463.
- Hahn, B., Zacharko, R.M., & Anisman, H. (1986). Alterations of amphetamine elicited perseveration and locomotor excitation following acute and repeated stressor application. Pharmacol. Biochem. Behav., 1, 29-33.
- Hellhammer, D.H., Hingtgen, J.N., Wade, S.E., Shea, P.A. & Aprison, M. (1983). Serotonergic changes in specific areas of rat brain associated activity-stress gastric lesions. Psychosom. Med., 45, 115-122.
- Herman, J.P., Guillonueau, D., Dantzer, R., Scatton, B., Semerdjian-Rouquier, L., & LeMoal, M. (1982). Differential effects of inescapable footshock and stimuli previously paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat. Life Sci., 30, 2207-2214.

- Herman, J.P., Stinus, L., & Le Moal, M. (1984). Repeated stress increases locomotor response to amphetamine. Psychopharmacology, 84, 431-435.
- Irwin, J., Ahluwalia, P., & Anisman, H. (1986). Sensitization of norepinephrine activity following acute and chronic footshock. Brain Research, 379, 98-103.
- Kabiersch, A., Del Rey, A., Honegger, C.G., & Besedovsky, H.O. (1988). Interleukin-1 induces changes in norepinephrine metabolism in the rat brain. Brain Behav. Immun., 2, 267-274.
- Kandel, E.R. (1983). From metapsychology to molecular biology: Explorations into the nature of anxiety. Am J. Psychiatry., 140, 1277-1293.
- Karol, M.H., Stadler, J., & Magreni, C. (1985). Immunotoxicologic evaluation of the respiratory system: Animal models for immediate- and delayed-onset pulmonary hypersensitivity. Fundamental and Applied Toxicology, 5, 459-472.
- Koenig, J.I. (1991). Presence of cytokines in the hypothalamic-pituitary axis. Prog. NeuroEndocrinImmunol., 4, 143-153.
- Lacosta, S., Merali, Z., Zalcman S. & Anisman, H. (1994). Time-dependent in vivo mesolimbic dopamine variations following antigenic challenge. Brain Research., 664, 225-230.
- Lacosta, S., Merali, Z., & Anisman, H. (1994). Central neurochemical changes induced by IL-1. Neuroscience Abstracts.
- Lacosta, S., Merali, Z., Anisman, H. & Bielajew, C. (1994). Systemic interleukin-1 β and mild stress rapidly and additively increase interstitial levels of 5-HIAA and HVA at the nucleus accumbens. Neuroscience Abstracts.
- Lapchak, P.A. (1992). A role for interleukin-2 in the regulation of striatal dopaminergic function. NeuroReport, 3, 165-168.
- Lapchak, P.A., Araujo, D.M., Quirion, R., & Beaudet, A. (1991). Immunoautoradiographic localization of interleukin 2-like immunoreactivity and interleukin 2 receptors (Tac antigen-like immunoreactivity) in the rat brain. Neuroscience, 44, 173-184.
- Mefford, I.N. & Heyes, M.P. (1990). Increased biogenic amine release in mouse hypothalamus following immunological challenge: antagonism by indomethacin. J. Neuroimmunol., 27, 55-61.

- Miller, A.H., Spencer, R.L., McEwen, B.S., & Stein, M. (1993). Depression, adrenal steroids, and the immune system. Ann. Med., 25, 481-487.
- Mohankumar, P.S. & Quadri, S.K. (1993). Systemic administration of interleukin-1 stimulates norepinephrine release in the paraventricular nucleus. Life Sciences, 52, 1961-1967.
- Mohankumar, P.S., Thyagarajan, S. & Quadri, S.K. (1993). Interleukin-1 β increases 5-hydroxyindoleacetic acid release in the hypothalamus in vivo. Brain Research Bulletin, 31, 745-748.
- Mohankumar, P.S., Thyagarajan, jS. & Quadri, S.K. (1990). Interleukin-1 stimulates the release of dopamine and dihydroxyphenylacetic acid from the hypothalamus in vivo. Life Sci., 48, 925-930.
- Molina, V.A., Volosin, M., Cancela, L., Keller, E., Murua, V.S., & Basso, A.M. (1990). Effect of chronic variable stress on monoamine receptors: Influence of imipramine administration. Pharmacol. Biochem. Behav., 35, 335-340.
- Nance, D.M., Rayson, D. & Carr, R.I. (1987). The effects of lateral septal and hippocampal areas on the humoral immune response of adult female rats. Behav. Immun., 1, 292-305.
- Nisenbaum, L.K., Zigmond, M.J., Sved, A.F., & Abercrombie, E.D. (1991). Prior exposure to cold stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. J. Neurosci., 11, 1478-1484.
- Petty, F. & Sherman, A.D. (1982). A neurochemical differentiation between exposure to stress and the development of learned helplessness. Drug Devel. Res., 2, 43-45.
- Post, R.M. (1980). Intermittent versus continuous stimulation: Effect of the interval on the development of sensitization or tolerance. Life Sci., 26, 1275-1282.
- Puglisi-Allegra, S.P., Imperato, A., Angelucci, L. & Cabib, S. (1991). Acute stress induces time-dependent responses in dopamine mesolimbic system. Brain Res., 554, 217-222.
- Riley, V., Fitzmaurice, M.A., & Spachman, D.H. (1981). Psychoneuroimmunologic factors in neoplasia: Studies in animals. In: Ader, R. Psychoneuroimmunology. New York Academic.

- Ritter, S., & Pelzer, N.L. (1978). Magnitude of stress-induced norepinephrine depletion varies with age. Brain Res., 152, 170-175.
- Rivier, C. (1991). Role of interleukins in the stress response. In: Jasmin, G., Cantin, M. Eds. Stress revisited. Neuroendocrinology of Stress. Basel: Karger, 63-79.
- Robinson, T.E., Angus, A.L. & Becker, J.B. (1987) Sensitization to stress: The enduring effects of prior stress on amphetamine-induced rotational behavior. Neuropharmacology, 26, 679-691.
- Roszman, T.L. & Carlson, S.L. (1991). Neurotransmitters and molecular signalling in the immune response. In: Ader, R., Fleten, D.L., & Cohen, N., Eds. Psychoneuroimmunology. San Diego: Academic, 311-335.
- Roth, R.H., Tam, S-Y., Ida, Y., Yang, J-X, & Deutch, Y. (1988). Stress and mesocorticolimbic dopamine system. Ann. NY Acad. Sci., 537, 138-147.
- Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P., & Vale, W. (1987). Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor, Science, 238, 522-524.
- Segal, R.F., Bosh, K.O., & Bush, B. (1986). High performance liquid chromatography of biogenic amines and metabolites in brain, cerebrospinal fluid, urine and plasma. J. Chromatog., 377, 131-137.
- Shanks, N., Zalcman, S. & Anisman, H. (1988) Strain-specific catecholamine variations induced by stressors: Relation to behavioral change. Soc. Neurosci. Abst. 14, 969.
- Shanks, N., Zalcman, S., Zacharko, R.M., & Anisman, H. (1991). Alterations of central norepinephrine, dopamine and serotonin in several strains of mice following acute stressor exposure. Pharmacol. Biochem. Behav., 38, 69-75.
- Sherman, A.D., & Petty, F. (1980). Neurochemical basis of the action of anti-depressants on learned helplessness. Behav. Neur. Biol., 30, 119-134.
- Silverman, J.J. (1986). Post-traumatic stress disorder. Adv. Psychosom. Med., 16, 115-140.
- Stone, E.A. (1987). Central cyclic-AMP-linked noradrenergic receptors: New findings on properties as related to the actions of stress. Neurosci. Biobehav. Rev., 11, 391-398.

- Tache, Y., Du Rousseau, P., Tache, J., Selye, H., & Collu, R. (1976). Shift in adenohipophyseal activity during chronic intermittent immobilization of rats. Neuroendocrinology, 22, 325-336.
- Tanaka, M., Kohno, Y., Nakagawa, R., Ida, Y., Takeda, S., & Nagasaki, N. (1982). Time-related differences in noradrenaline turnover in rat brain regions by stress. Pharmacol. Biochem. Behav., 16, 315-319.
- Terao, A., Oikawa, M. & Saito, M. (1993). Tissue-specific increase in norepinephrine turnover by central interleukin-1, but not by interleukin-6, in rats. Amer. Psych. Soc. R400-R404.
- Terao, A., Oikawa, M. & Saito, M. (1993). Cytokine-induced change in hypothalamic norepinephrine turnover: involvement of corticotropin-releasing hormone and prostaglandins. Brain Research, 622, 257-261.
- Thierry, A.M., Tassin, J.P., Blanc, G., & Glowinski, J. (1976). Selective activation of the mesocortical DA system by stress. Nature., 263, 242-244.
- Weiss, J.M., Glazer, H.I., & Pohorecky, L.A. (1976). Coping behavior and neurochemical changes: An alternative explanation for the original "learned helplessness" experiments. In: Serban G, Kling A. Eds. Animal Models in Human Psychobiology, New York: Plenum Press, 141-173.
- Weiss, J.M., Goodman, P.A., Losito, B.G., Corrigan, S., Charry, J.M., & Bailey, W.H. (1981). Behavioral depression produced by an uncontrollable stressor: Relationship to norepinephrine, dopamine and serotonin levels in various regions of rat brain. Brain Res. Rev., 3, 167-205.
- Weiss, J.M., Sundar, S.K., Becker, K.J., & Cierpial, M.A. (1989). Behavioral and neural influences on cellular immune responses: Effects of stress and interleukin-1. J. Clin. Psychiat., 50, 43-55.
- Yan, H.Q., Banos, M.A., Herregodts, P., Hooghe, R. & Hooghe-Peters, E.L. (1992). Expression of interleukin (IL)-1 β , IL-6 and their respective receptors in the normal rat brain and after injury. Europ. Journ. of Imm., 22, 2963-2971.
- Zalcman, S., Shanks, N., & Anisman, H. (1991). Time-dependent variations of central norepinephrine and dopamine following antigen administration. Brain Research, 557, 69-76.
- Zalcman, S., Green-Johnson, J.M., Murray, L., Nance, D.M., Dyck, D., Anisman, H., & Greenberg, A.H. (1994). Cytokine-specific central monoamine alterations induced by interleukin. Brain Research: in press.

END

0 1 - 0 6 - 9 5

FIN