1

PM-1 3 1/2" x 4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGET

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.

**Stressor Induced Alterations in Locomotor Activity and Brain Stimulation
Performance From The Mesencephalon: Modification By Intraventricular
D-Ala²-Met⁵-Enkephalinamide, D-Ala², N-Me-Phe⁴, Gly-OI⁵-Enkephalin,
and D-Pen², D-Pen⁵-Enkephalin Administration**

by

Cindy Maddeaux, M.Sc.

A thesis presented to the faculty of Graduate Studies and Research of Carleton University
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
Department of Psychology

Carleton University

January 31, 1996

© copyright

1996, Cindy Maddeaux

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 accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

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

ISBN 0-612-08866-9

Canada

Name Caroly Meacham

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

PSYCHE BIOLOGY

0349 U·M·I

SUBJECT TERM

SUBJECT CODE

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
Special 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
Literature 0401
General 0294
Classical 0295
Comparative 0297
Medieval 0298
Modern 0316
African 0591
American 0305
Asian 0352
Canadian (English) 0355
Canadian (French) 0593
English 0311
Germanic 0312
Latin American 0315
Middle Eastern 0313
Romance 0314
Slavic and East European

PHILOSOPHY, RELIGION AND THEOLOGY

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

SOCIAL SCIENCES

American Studies 0323
Anthropology 0324
Archaeology 0326
Cultural 0327
Physical 0310
Business Administration 0272
General 0770
Accounting 0454
Banking 0338
Management 0385
Marketing 0501
Canadian Studies 0503
Economics 0505
General 0508
Agricultural 0509
Commerce-Business 0510
Finance 0511
History 0358
Labor 0366
Theory 0351
Folklore 0578
Geography 0366
Gerontology 0351
History 0578
General

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 0620
Sociology 0627
General 0631
Criminology and Penology 0628
Demography 0629
Ethnic and Racial Studies 0630
Individual and Family Studies 0700
Industrial and Labor Relations 0344
Public and Social Welfare 0709
Social Structure and Development 0999
Theory and Methods 0453
Transportation 0999
Urban and Regional Planning 0453
Women's Studies

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 0817
Plant Physiology 0777
Range Management 0746
Wood Technology
Biology 0306
General 0287
Anatomy 0308
Biostatistics 0309
Botany 0379
Cell 0329
Ecology 0353
Entomology 0369
Genetics 0793
Limnology 0410
Microbiology 0307
Molecular 0317
Neuroscience 0416
Oceanography 0433
Physiology 0821
Radiation 0778
Veterinary Science 0472
Zoology
Biophysics 0786
General 0760
Medical
Earth Sciences 0425
Biogeochemistry 0996
Geochemistry

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

Speech Pathology 0460
Toxicology 0383
Home Economics 0386

PHYSICAL SCIENCES

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

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 System Science 0793
Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
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,

Stressor Induced Alterations in Locomotor Activity and Brain Stimulation Performance
From The Mesencephalon: Modification By Intraventricular D-Ala2-Met5-
Enkephalinamide, D-Ala2, N-Me-Phe4, Gly-Or5-Enkephalin, and D-Pen2, D-Pen5-
Enkephalin Administration

Submitted by Cindy Maddeaux
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

Dr. W. Jones
Chairman, Department of Psychology

Dr. R. Zacharko
Thesis Supervisor

Dr. J. Telner
External Examiner

Carleton University
January, 1996

Abstract

Uncontrollable stressors provoke a diverse range of behavioral disturbances in infrahuman subjects which may simulate at least some of the symptoms associated with clinical depression. Evaluating responding for electrical stimulation from brain regions associated with central reward processing following stressor exposure provides a means of identifying the patterns of motivational change associated with aversive experiences, as well as the neural substrates which may be involved in the expression of anhedonia. The present series of experiments assessed responding for brain stimulation from the dorsal A10 area, the ventral A10 area, and the interpeduncular nucleus in male, CD-1 mice following uncontrollable footshock, as well as variations in activity and rearing. In addition, the modulatory role of intraventricular administration of the mixed μ/δ opioid receptor agonist D-Ala²-Met⁵-enkephalinamide (DALA), the selective μ receptor agonist, D-Ala², N-Me-Phe⁴, Gly-OI⁵-enkephalin (DAGO), and the selective δ receptor ligand, D-Pen², D-Pen⁵-enkephalin (DPDPE) in modifying stressor provoked behavioral pathology was determined. It was revealed that (1) the dorsal A10 region was particularly vulnerable to stressor provoked brain stimulation performance deficits while the ventral A10 region was resistant to stressor imposition, (2) transient brain stimulation performance deficits were evident from the interpeduncular region following footshock, indicating stressor provoked reward variations are not restricted to the mesocorticolimbic system, (3) transient decreases in locomotor activity and rearing were provoked by footshock but these motoric variations could not account for the expression of brain stimulation performance deficits, (4) administration of a low dose of DAGO prior to footshock antagonized the immediate and long term brain stimulation performance deficits from the dorsal A10 region, provoked self-stimulation deficits from the ventral A10 region, and had no ameliorative influence on self-stimulation performance from the interpeduncular nucleus, (5) administration of DPDPE prior to footshock failed to attenuate brain stimulation performance deficits from the dorsal A10 region, but antagonized the emergence of brain stimulation deficits 24-hours

and 168-hours post-stressor, (6) administration of DPDPE prior to stressor exposure reduced self-stimulation performance from the ventral A10 region on the immediate test day and interfered with the recovery of responding for brain stimulation from the interpeduncular nucleus 24-hours post-stressor, (7) administration of a low dose of DAGO or DPDPE following footshock exposure reversed brain stimulation deficits from the dorsal A10 region immediately post-stressor, (8) administration of DPDPE following footshock abolished long-term brain stimulation performance deficits from the dorsal A10 region, while DAGO administration was associated with moderate self-stimulation deficits 168-hours post-stressor, (9) DAGO but not DPDPE administration prior to footshock prevented the emergence of locomotor hypoactivity, while both DAGO and DPDPE had no influence on stressor provoked reductions in rearing. Taken together, these data indicate that different sub-areas of the mesencephalon are vulnerable to stressor provoked neurochemical variations associated with the emergence of anhedonia, and such changes are not limited to the mesocorticolimbic dopamine system. The release of endogenous μ receptor ligands during stressor exposure may enhance the ability to cope with aversive stimuli and protect the organism from the development of prolonged motivational changes. The release of endogenous δ receptor ligands may antagonize proactive or cascading neurochemical changes which contribute to long term behavioral pathology. In addition, δ receptor ligands may provoke therapeutic effects on behavioral pathology reminiscent of anhedonia, potentially owing to a link between dopamine, δ opioid receptors, and serotonin activity within the A10 region. Accordingly, the development of anhedonia may involve, at least in part, a failure of endogenous opioid peptide activity to initiate adaptive neurochemical variations in response to aversive life circumstances.

Acknowledgements

I would like to express my gratitude to Bob Zacharko for his many years of guidance and support. His dedicated efforts to produce excellent work were of tremendous value in the preparation of this thesis. I also thank Dan McIntyre and Hymie Anisman for serving as committee members numerous times throughout my graduate training. I would also like to acknowledge Rachel and Mike, Glenda and Alex, and Mary Ellen, who were always helpful in providing comic relief during eight and a half long years of graduate studies.

I would also like to express my gratitude to my family, especially my parents, Al and Anne Wolfe, whom I'm sure in their wildest dreams never expected me to be in university for 13 years! Thank you for your endless support and encouragement. I would also like to thank my mother-in-law, Doreen Maddeaux, for always being there when things get too hectic and for her endless prayers. Most of all, I thank my dearest husband, John, and my darling little Alexandra, who always make everything worthwhile.

Table of Contents

Abstract.....	i
Acknowledgements.....	iii
Table of Contents.....	iv
List of Figures.....	vi
 Introduction.....	 1
 Depression and Central Dopamine Activity.....	 8
 The Mesocorticolimbic Dopamine System:	
Anatomy of the Mesocorticolimbic System.....	12
The Mesocorticolimbic System and Reward Processing.....	14
Stressor provoked Alterations in Mesocorticolimbic Dopamine Activity.....	17
Stressor Induced Behavioral Pathology Associated with Dopamine Alterations....	23
 Regulation of Mesocorticolimbic Activity:	
Autoreceptor Regulation of Mesocorticolimbic Activity.....	28
Neurotransmitter Regulation of A10 Neural Activity.....	31
Neuropeptide Regulation of A10 Neural Activity.....	36
 Opioid-Dopamine Interactions Within the A10 Region:	
Opioid Receptor Types in the A10 Region.....	41
Functional Interaction Between Endogenous Opioids and Dopamine Activity....	45
Reward Alterations Associated with Opioid-Dopamine Interactions.....	49
 Stressor Provoked Opioid Activity: Implications for Reward Processing...	52
 Summary.....	 56
 General Methods.....	 58
 Preliminary Analyses.....	 60

Experiment 1:

Introduction.....	61
Procedure.....	63
Results.....	64
Discussion.....	81

Experiments 2a and 2b:

Introduction.....	88
Experiment 2a: Procedure.....	90
Experiment 2a: Results.....	91
Experiment 2b: Procedure.....	103
Experiment 2b: Results.....	103
Discussion.....	113

Experiment 3:

Introduction.....	131
Procedure.....	133
Results.....	134
Discussion.....	148

Experiment 4:

Introduction.....	155
Procedure.....	157
Results.....	157
Discussion.....	169

General Discussion.....174**References.....**182

Appendix A.....	236
Appendix B.....	237
Appendix C.....	238
Appendix D.....	239
Appendix E.....	241
Appendix F.....	242

List of Figures

- Figure 1a.** Mean ($\pm$ S.E.M) Baseline locomotor activity in CD-1 mice. Measures of locomotor activity were obtained at three minute intervals over a 45 minute period.66
- Figure 1b.** Mean ($\pm$ S.E.M) activity scores in CD-1 mice administered saline, DALA (1.0 ug), DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) intraventricularly and exposed to an acute session of uncontrollable footshock or the No Shock condition.69
- Figure 1c.** Mean ($\pm$ S.E.M) activity scores per three minute interval in CD-1 mice administered saline, DALA (1.0 ug), DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) intraventricularly and exposed to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the 15 time intervals to depict the Day x (Shock x Drug) interaction.72
- Figure 1d.** Mean ($\pm$ S.E.M) Baseline rearing in CD-1 mice. Rearing scores were obtained at three minute intervals over a 45 minute period.75
- Figure 1e.** Mean ($\pm$ S.E.M) rearing scores among CD-1 mice administered saline, DALA (1.0 ug), DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) intraventricularly and exposed to an acute session of uncontrollable footshock or the No Shock condition.77
- Figure 1f.** Mean ($\pm$ S.E.M) rearing per three minute interval in CD-1 mice administered saline, DALA (1.0 ug), DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) intraventricularly and exposed to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the 15 time intervals to depict the Day x Shock interaction.80
- Figure 2a.** Schematic representation of electrode placements within the dorsal VTA in CD-1 mice used in Experiment 2a.93
- Figure 2b.** Mean ($\pm$ S.E.M) Baseline self-stimulation rates among CD-1 mice responding for brain stimulation from the dorsal A10 region in Experiment 2a. Rates of responding for brain stimulation were assessed at three minute intervals over a 45 minute period.96

Figure 2c. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the dorsal A10 region in Experiment 2a following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition. ...98

Figure 2d. Mean ($\pm$ S.E.M) self-stimulation performance per three minute interval from the dorsal A10 region in mice administered saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) intraventricularly and exposed to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the 15 time intervals to depict the Day x (Shock x Drug) interaction.102

Figure 2e. Schematic representation of electrode placements within the dorsal A10 region in CD-1 mice used in Experiment 2b.106

Figure 2f. Mean ($\pm$ S.E.M) Baseline self-stimulation rates among CD-1 mice responding for brain stimulation from the dorsal A10 region in Experiment 2b. Rates of responding for brain stimulation were assessed at three minute intervals over a 45 minute period.108

Figure 2g. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the dorsal A10 region in Experiment 2b following exposure to an acute session of uncontrollable footshock or the No Shock condition. Rates of responding for brain stimulation were assessed at three minute intervals over a 15 minute period. Animals were then administered saline, DPDPE (1.0 ug), or DAGO (.01 ug) intraventricularly. The time of drug administration is represented by the broken line. ...111

Figure 2h. Mean ($\pm$ S.E.M) self-stimulation performance per three minute interval from the dorsal A10 region in mice administered saline, DPDPE (1.0 ug), or DAGO (.01) intraventricularly. The data shown are from the 24-hour and 168-hour test days to represent the long-term influence of opioid administration on footshock induced brain stimulation variations. The data are collapsed across the Shock conditions and the 24-hour and 168-hour test intervals to depict the Time x Drug interaction.115

Figure A: Schematic representation of the potential modulatory role of footshock provoked release of enkephalin on A10 DA activity.121

Figure B: Schematic representation of the potential modulatory role of δ receptor mediated release of 5-HT on A10 DA activity.125

Figure C: Schematic representation of the potential modulatory role of footshock provoked release of dynorphin on A10 DA activity.129

Figure 3a. Schematic representation of electrode placements within the ventral A10 region in CD-1 mice used in Experiment 3.135

Figure 3b. Mean ($\pm$ S.E.M) Baseline self-stimulation rates among CD-1 mice responding for brain stimulation from the ventral A10 region in Experiment 3. Rates of responding for brain stimulation were assessed at three minute intervals over a 45 minute period.139

Figure 3c. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the ventral A10 region following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition.141

Figure 3d. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the ventral A10 region following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the 15 time intervals to depict the Day x Drug interaction.144

Figure 3e. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the ventral A10 region following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the Immediate, 24-hour and 168-hour test days to depict the Time x (Shock x Drug) interaction.147

Figure 4a. Schematic representation of electrode placements within the interpeduncular nucleus in CD-1 mice used in Experiment 4.160

Figure 4b. Mean ($\pm$ S.E.M) Baseline self-stimulation rates among CD-1 mice responding for brain stimulation from the interpeduncular nucleus in Experiment 4. Rates of responding for brain stimulation were assessed at three minute intervals over a 45 minute period.162

Figure 4c. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the interpeduncular nucleus following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition.165

Figure 4d. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the interpeduncular nucleus following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the 15 Time intervals to depict the Day x (Shock x Drug) interaction. ..168

Introduction

It has been well documented that stressors may precipitate or exacerbate numerous psychological disturbances, including schizophrenia (Hirsch *et al.*, 1992; Braff *et al.*, 1990), anxiety (Lief *et al.*, 1995; Charney *et al.*, 1993; Chrousos *et al.*, 1992), bipolar affective disorder (Post *et al.*, 1993; Hunt *et al.*, 1992) and major depression (Dalgard *et al.*, 1995; Brown, 1993; Heninger, 1993; Post *et al.*, 1992; Lora *et al.*, 1992). The onset of major depressive episodes, in particular, are often preceded by the occurrence of severe life events, particularly those events which involve a significant loss (e.g., death of a spouse, parent, or child) (Dalgard *et al.*, 1995; Brown, 1993). Trauma such as domestic violence, sexual assault, or incest, have also been associated with the occurrence of depression (Yellowlees *et al.*, 1994). In addition, depressed individuals tend to report a higher incidence of major difficulties (e.g., unemployment) (Lora *et al.*, 1992) or chronic daily hassles (Brown *et al.*, 1989) relative to healthy individuals.

In light of the observed association between stressful life events and depression, animal models of depression have been utilized to further evaluate the relationship between stressors and depression (reviewed in Anisman *et al.*, 1992a; Willner, 1991; Richardson, 1988). It has been demonstrated that in some instances, the behavioral, neurochemical and hormonal variations induced by stressor exposure in infrahuman subjects are reminiscent of the variations detected in clinical depression (reviewed in Anisman *et al.*, 1991; Zacharko *et al.*, 1991; Willner, 1991). Moreover, chronic administration of pharmaceutical agents which exhibit antidepressant properties have been observed to attenuate the behavioral repercussions (Imperato *et al.*, 1994; Wolfe *et al.*, 1991a; Shanks *et al.*, 1989; Zacharko *et al.*, 1984) and neurochemical perturbations (Rossetti *et al.*, 1993) induced by stressor exposure in infrahuman subjects. Accordingly, an evaluation of stressor induced behavioral and central nervous system alterations in infrahuman subjects may provide a means of determining the neurochemical systems involved in the manifestation of clinical depression.

It should be emphasized that the qualitative and quantitative characteristics of the stressors facing an organism influence the symptom profile of behavioral pathology which is expressed following stressor exposure as well as the specific neurochemical alterations which emerge. While exposure to uncontrollable stressors produces behavioral pathology and central neurochemical variations, controllable stressors are typically without effect (reviewed in Anisman *et al.*, 1990). In addition, the intensity of a stressor may determine which central systems are activated and the accompanying behavioral alterations which are manifested. Mild, uncontrollable stressors appear to selectively influence mesocorticolimbic dopamine (DA) activity (Inoue *et al.*, 1995; Willner *et al.*, 1992). Stressor provoked DA alterations within the mesocorticolimbic system have been associated with reductions in responding for previously rewarding brain stimulation (Maddeaux *et al.*, 1992; Moreau *et al.*, 1992; Zacharko *et al.*, 1991; 1990), and decreased consumption of palatable substances (Monleon *et al.*, 1995; Papp *et al.*, 1993; Willner *et al.*, 1991). It has been suggested that behavioral alterations associated with variations in DA activity may simulate the anhedonic aspects of depression (Willner, 1992; Zacharko *et al.*, 1991). Uncontrollable stressors of a mild to moderate intensity have been noted to provoke noradrenergic (NE) variations within the locus coeruleus and its terminal regions, including the hypothalamus, hippocampus and frontal cortex (reviewed in Anisman *et al.*, 1992a; Weiss, 1991). Central NE variations have been associated with performance deficits in shuttle or water escape tasks (Shanks *et al.*, 1988), immobility in forced swim paradigms (Prince *et al.*, 1984), and retarded acquisition in response-choice discrimination tasks (Jackson *et al.*, 1980). Behavioral alterations associated with NE changes may be reminiscent of the symptoms of psychomotor retardation, reduced concentration, and perseverative tendencies characteristic of depressive illnesses (Anisman *et al.*, 1992a; 1991). However, it should be emphasized that the aforementioned performance deficits are not associated exclusively with central NE changes as a correspondence between deficits in escape behavior and alterations in central

serotonin (5-HT) (Edwards *et al.*, 1992) and DA activity (Imperato *et al.*, 1994; Rossetti *et al.*, 1993) have also been reported.

Stressors have also been observed to provoke alterations in central 5-HT activity, although more severe stressors are required to provoke 5-HT turnover in contrast to the stressors which activate DA or NE (e.g., Inoue *et al.*, 1995; Malyszko *et al.*, 1995). Stressor provoked alterations in 5-HT activity have been observed within the hippocampus, nucleus accumbens, frontal cortex, amygdala, lateral hypothalamus and midbrain (Inoue *et al.*, 1995; Edwards *et al.*, 1992; Heinsbroek *et al.*, 1991; Dunn, 1988). Stressor related variations in central 5-HT activity have been associated with escape deficits (Edwards *et al.*, 1992), hypophagia (Curzon *et al.*, 1992), and behaviors which have been proposed to be reminiscent of anxiety (e.g., ultrasonic vocalization, decreased exploration of novel environments) (De Vry *et al.*, 1993; Handley *et al.*, 1993; Kennet, 1992).

In parallel to the correspondence between central neurochemical variations and specific behavioral deficits in infrahuman subjects, divergent symptom profiles in psychological disturbance among human subjects may be associated with stressor dependent amine dysfunction within distinct brain regions. Indeed, while the central feature of depression is a disturbed mood and/or anhedonia, a diverse range of symptomatology may accompany mood alterations, including loss of appetite, increases or decrease in weight, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue and loss of energy, feelings of worthlessness or inappropriate guilt, diminished ability to think, concentrate, or make decisions, and recurrent thoughts of death or suicide (American Psychiatric Association, 1994). Considering the heterogeneous nature of depressive symptomatology, an evaluation of the behavioral manifestations of stressor provoked brain region dependent neurochemical alterations may provide a potential model for investigating the neural substrates of subtypes of depressive disorders and/or symptoms.

In addition to the characteristics of the stressor employed, organismic variables appear to contribute to the expression of stressor induced neurochemical variations and

behavioral pathology. Sex differences have been noted in both stressor induced neurochemical and behavioral variations. For example, increases in NE, 5-HT and DA metabolites following inescapable footshock have been observed to be more pronounced in female rats (Heinsbroek *et al.*, 1991). Age also appears to contribute to the effects of stressors with older animals exhibiting greater increases in the neurochemical, endocrine and immunological responses to stressor exposure (Shanks *et al.*, 1994; Perego *et al.*, 1993; Lorens *et al.*, 1990). The observation that stressor induced neurochemical and neuroendocrine alterations vary as a function of age and sex parallels the age and gender differences observed in clinical populations. The lifetime risk of a major depressive episode is 1.7 to 2 times higher in women (Kessler *et al.*, 1993; Angst *et al.*, 1992), with estimates of the lifetime risk for major depression ranging from 10% to 25% for women and 5% to 12% for men (American Psychiatric Association, 1994). Prevalence rates of major depression also tend to vary as a function of age, with the highest rates of depression being observed in both males and females between the ages of 18 and 44, and more moderate rates being observed in individuals between 45 and 65 (Wittchen *et al.*, 1994). Major depression appears to be prevalent again after the age of 65, with estimates of depression amongst institutionalized geriatric populations ranging from 15% to 30% (Stokes *et al.*, 1993; Ames, 1993). While some investigators have reported depression is relatively uncommon after the age of 65 (e.g., Wittchen *et al.*, 1994), it has been argued that depression in the elderly often goes undiagnosed due to the presence of comorbid disorders such as dementia, a tendency for the elderly to attribute symptoms of depression to physical illnesses, and the inability of commonly used psychiatric assessment tools to distinguish between physiological symptoms and the neurovegetative signs of depression (Knauper *et al.*, 1994).

Genetic factors also appear to contribute to an enhanced susceptibility to the neurochemical and behavioral repercussions of stressor exposure. Alterations in corticosterone as well as NE, DA and 5-HT show wide variability across different strains

of mice (Shanks *et al.*, 1994; 1991). Considerable interstrain variation has also been observed in stressor provoked behavioral alterations, including changes in shuttle escape performance, forced swim tasks, Y-maze spontaneous alternation performance (Shanks *et al.*, 1988), consumption of a palatable diet (Griffiths *et al.*, 1992) and responding for ICSS (Zacharko *et al.*, 1990a; 1987). However, the occurrence of behavioral alterations in one task does not appear to predict alterations in other paradigms, nor is there a direct correspondence between central amine variations and behavioral pathology (Anisman *et al.*, 1992b). Nevertheless, some strains appear to be more susceptible to both behavioral perturbations and neurochemical variations. For example, BALB/cByJ mice are particularly vulnerable to stressor provoked behavioral and neurochemical perturbations while C57BL/6J mice are more resistant to such alterations (see Shanks *et al.*, 1994; 1991). Certain lines of selectively bred rats (e.g., the Flinders sensitive rats) have also been noted to exhibit a higher susceptibility to the behavioral repercussions of stressors (Overstreet, 1993). In parallel to the genetic differences observed in response to stressor exposure in infrahuman subjects, it has been observed that genetic factors may contribute to depression in clinical populations. For example, there appears to be a high concordance rate of depression in identical twins (Thapar *et al.*, 1994; Kendler *et al.*, 1993; 1992) and depressed individuals often report a family history of depression and/or other forms of psychopathology (Kendler *et al.*, 1994; McGuffin *et al.*, 1989). Accordingly, in both infrahuman and clinical populations, the behavioral and neurochemical sequelae provoked by stressor exposure may be dependent on, at least in part, the organismic variables onto which a given stressor is imposed.

It has been argued that the enhanced turnover of central amines induced by acute controllable stressors may have adaptive consequences by enabling an organism to cope more effectively at a behavioral level (Anisman *et al.*, 1990). For example, enhanced central NE activity has been associated with increased alertness and vigilance to salient environmental stimuli (Delagrange *et al.*, 1993; Foote *et al.*, 1991; Aston-Jones *et al.*,

1991) while enhanced DA activity may play a role in the initiation of goal directed behaviors and facilitate motor output required for adaptive responding to stressors (Salamone, 1994). If an organism can control the onset or duration of a stressor or perceives that control is possible, then amine synthesis tends to keep pace with utilization such that the concentration of the transmitter is not reduced, at least in the short run. However, when a stressor is uncontrollable and of sufficient severity, excessive demands may then be placed on central neurochemical activity. The utilization of central neurotransmitters may then exceed synthesis, resulting in a net depletion in amine concentrations and the emergence of behavioral pathology (Anisman *et al.*, 1992a; Weiss, 1991). Therefore, while the initial mobilization of endogenous resources may be instrumental in dealing with a stressor, the resultant depletion of transmitters may render the organism less well prepared to deal with environmental demands, leading to the manifestation of behavioral deficits.

In contrast to the effects of acute stressor application, behavioral pathology is typically not evident following chronic stressor application (Anisman *et al.*, 1992; Shanks *et al.*, 1988; Zacharko *et al.*, 1984). Behavioral adaptation to chronic stressor regimens appears to be accompanied by central neurochemical alterations. For example, following chronic stressor exposure or prolonged application of a single stressor, central NE (Weiss *et al.*, 1989), 5-HT (Adell *et al.*, 1988) and DA concentrations (Kalivas *et al.*, 1989; Herman *et al.*, 1984) are equivalent to or exceed baseline levels. Adaptation is also evidenced by enhanced transmitter synthesis, as reflected in enhanced tyrosine hydroxylase (Nisenbaum *et al.*, 1991; Melia *et al.*, 1991) and tryptophan hydroxylase activity (Boadle-Bider *et al.*, 1989). Alterations have also been observed in central α - and β -NE receptor density or sensitivity (see Areso *et al.*, 1991; Weiss, 1991; Stone, 1987; 1990), D2 receptor sensitivity (Willner *et al.*, 1992), and variations in the adenylate cyclase-cyclic adenosine monophosphate (cAMP) linked second messenger systems (Stone, 1990) following chronic stressor exposure. It has been proposed that in some cases, behavioral

disturbances associated with chronic uncontrollable stressors may stem from the failure of adaptive neurochemical mechanisms (Anisman *et al.*, 1992b; Zacharko *et al.*, 1989). Ordinarily, the latency to effect adaptive central neurochemical variations is delayed when the nature, severity, or predictability of an uncontrollable stressor is varied (Anisman *et al.*, 1991; Molina *et al.*, 1990).

As previously discussed, numerous neurochemical and neuroendocrine systems likely contribute to the manifestation of the diverse range of symptomatology evident in depression. However, the potential role of DA in depressive disorders is particularly intriguing considering that the mesocorticolimbic DA system appears to play a central role in motivational/reward processing, and a reduced capacity to experience pleasure (i.e., anhedonia) is one of the fundamental characteristics of depression. The A10 region, in which the mesocorticolimbic system originates, plays a critical role in the neural circuitry mediating the rewarding properties of drugs of abuse, including opioids and psychomotor stimulants (Self, 1992; Barr *et al.*, 1992a), as well as the reward derived from reinforcing behaviors such as feeding, drinking, and sexual activity (Zhang *et al.*, 1994; Amgo *et al.*, 1993). It has been consistently demonstrated that stressors facilitate DA turnover within the VTA and its terminal regions (Rouge-Pont *et al.*, 1993; Sorg *et al.*, 1993; Deutch *et al.*, 1991; Imperato *et al.*, 1990; Abercrombie *et al.*, 1988), and that stressor exposure may result in decreased mesocorticolimbic DA release (Puglisi-Allegra *et al.*, 1991; Heinsbroek *et al.*, 1991). Moreover, stressor induced DA variations have been linked with behavioral pathology reminiscent of anhedonia (Willner *et al.*, 1992; Zacharko *et al.*, 1991). Accordingly, stressor provoked mesocorticolimbic DA alterations may contribute to the anhedonic aspects of depressive symptomatology.

Several converging areas of research suggest that a parallel activation of central DA and endogenous opioid systems in response to stressor exposure may contribute to alterations in reward processing. Acute and chronic stressors have been observed to provoke changes in central and peripheral endogenous opioid activity and chronic stressors

induce alterations in central opioid receptor density and/or sensitivity (reviewed in Yamada *et al.*, 1997). Stressor provoked activation of endogenous opioid activity may be instrumental in eliciting stressor related mesocorticolimbic DA alterations as the administration of opioid antagonists prior to stressor exposure attenuates stressor induced DA variations (Cabib *et al.*, 1989; Kalivas *et al.*, 1987a; Miller *et al.*, 1984). In contrast, opioid agonists attenuate or reverse stressor induced behavioral disruptions associated with stressor provoked DA alterations (Tejedor-Real *et al.*, 1993; Baamonde *et al.*, 1992; Barr, 1992; Wolfe *et al.*, 1990). These findings are of considerable interest in light of the proposition that endogenous opioid activity may be altered in depressive illness. Alterations in cerebrospinal fluid levels of opioids and/or central opioid receptors have been observed in depressed individuals and suicide victims (Frosst, 1993; Darko *et al.*, 1992; Zis *et al.*, 1991; Caldecott-Hazard *et al.*, 1991b). Moreover, effective treatments for depression, including tricyclic antidepressants (DeFelipe *et al.*, 1985; Baraldi *et al.*, 1983) and electroconvulsive therapy (Nakata *et al.*, 1985; Antkiewicz-Michaluk *et al.*, 1984) have been observed to induce opioid receptor alterations and/or enhance endogenous opioid activity. It has been suggested that the enhancement of central opioid activity is one of the common mechanisms underlying the therapeutic efficacy of antidepressant treatments (Sandyk, 1987). Taken together, these data suggest an interaction between stressor induced DA alterations and central opioid activity may function in parallel to mediate the expression of the anhedonic aspects of depressive disorders.

Depression and Central Dopamine Activity

Clinical studies have led to an accumulation of data which support the proposition that DA plays an important role in the expression of depressive symptomatology. One method which has been utilized to determine whether central DA activity is altered in depressed individuals is to measure the neuroendocrine response to the administration of DA agonists. Discrete groups of DA cells in the arcuate and paraventricular nuclei of the hypothalamus project to the median eminence and neurohypophysis, forming the

tuberoinfundibular system. The release of DA into the hypophyseal portal circulation stimulates the secretion of growth hormone and inhibits the secretion of prolactin from the anterior pituitary (see Moore, 1987). Several investigators have reported a blunted growth hormone response to apomorphine or clonidine challenge occurs in depressed populations (Pitchot *et al.* 1992; Annseau *et al.*, 1988). The decrease in growth hormone production in response to DA agonist challenge may reflect a decrease in the sensitivity of postsynaptic hypothalamic D2 receptors, potentially as a compensatory response to DA hyperactivity (see Diehl, 1992). However, differences in circulating basal plasma levels of growth hormone or prolactin have not been noted between depressed individuals and healthy control subjects (reviewed in Kapur *et al.*, 1992; Caldecott-Hazard *et al.*, 1991a). Moreover, other investigators have failed to detect a blunted growth hormone response or enhanced release of prolactin in response to DA agonists in depressed subjects (reviewed in Caldecott-Hazard *et al.*, 1991a). Subsequent studies have revealed that the blunted growth hormone responses to apomorphine challenge appears to be limited to a subpopulation of depressed individuals who exhibit significant symptoms of anxiety (Pitchot *et al.*, 1990). In general, neuroendocrine studies have provided little evidence that depression is associated with tuberoinfundibular DA alterations.

Additional methods of assessing central DA activity have revealed that distinct subtypes of depression may be associated with alterations in central DA functioning. For example, it has been noted that plasma or cerebrospinal fluid levels of homovanillic acid (HVA), the major DA metabolite in humans, are lower in depressed individuals relative to healthy controls, particularly in patients exhibiting psychomotor retardation (reviewed in Mann *et al.*, 1995; Brown *et al.*, 1993; Kapur *et al.*, 1992; Caldecott-Hazard *et al.*, 1991b). Depressed individuals who have attempted suicide have also been noted to exhibit lower urinary and cerebrospinal fluid levels of HVA relative to depressed individuals who have not attempted suicide (Roy, 1995). Depressed patients who exhibit low cerebrospinal fluid levels of HVA have also been observed to exhibit reduced REM latency (Mouret *et al.*,

1987). Piribedil, which stimulates postsynaptic DA receptors at high doses, and L-tyrosine, which enhances DA transmission under conditions of DA deficiency, have been observed to have antidepressant effects in the subset of depressed patients which exhibit low cerebrospinal fluid HVA levels, abnormal REM patterns, and psychomotor retardation (Mouret *et al.*, 1988; 1987; Gelenberg *et al.*, 1982). The selective DA reuptake inhibitor, nomifensine, also appears to be highly effective in treating symptoms of psychomotor retardation in patients with low cerebrospinal fluid HVA (see Mann *et al.*, 1995; Kapur *et al.*, 1992). The relatively new DA reuptake blocker, α -amineptine, has also been found to be an effective antidepressant, particularly in patients exhibiting psychomotor retardation (Rampello *et al.*, 1991; Kemali, 1989; Paes de Souza *et al.*, 1989). Accordingly, disturbances in central DA activity may be involved in depressions in which neurovegetative signs, such as psychomotor retardation and sleep disturbance, are prominent.

It has also been observed that melancholic depression, characterized by severe anhedonia, is associated with diminished cerebrospinal fluid and urinary levels of HVA and 3,4-dihydroxyphenylacetic acid (DOPAC) relative to patients with non-melancholic depression (Roy *et al.*, 1986; Asberg *et al.*, 1984). Melancholic depression is characterized by the near absence, rather than just a diminution, of the capacity for pleasure, and is typically accompanied by early morning awakening and psychomotor retardation or agitation (American Psychiatric Association, 1994). Anhedonia appears to be distinguishable from mood alterations *per se*, as the inability to experience pleasure is not correlated with blunted affect, nor with the severity of the depressive disorder (Loas *et al.*, 1994). Anhedonic depressives tend to exhibit a profound loss of interest or pleasure in appetite, sex, social contacts and work, while depressed individuals with a normal pleasure capacity are more likely to exhibit symptoms of hypochondriasis, irritability, indecision, and pessimism (Loas *et al.*, 1992; Fawcett *et al.*, 1983). In addition, melancholic forms of depressions are more responsive to antidepressant medication than reactive or neurotic

depression (Parker *et al.*, 1993). Interestingly, melancholia is often associated with psychomotor retardation (Parker *et al.*, 1992) and reduced REM sleep latency (Giles *et al.*, 1986). Accordingly, central DA alterations may play an etiological role in a subtype of depression, characterized by anhedonic symptomatology, psychomotor retardation, and abnormal REM patterns. However, it should be emphasized that such a proposition remains speculative and other investigators have failed to distinguish melancholic depression from non-melancholic depressions according to urinary HVA levels (Lykouras *et al.*, 1994) or decreases in REM latency (Stoner *et al.*, 1994).

Further support for the proposition that DA alterations play a permissive role in the expression of depressive symptomatology is derived from the finding that pharmaceutical agents which act as DA antagonists, including antihypertensives (e.g., reserpine, α -methyldopa) and neuroleptics, have been noted to induce or exacerbate depressive symptomatology in humans (reviewed in Brown, 1993; Kapur *et al.*, 1992; Caldecott-Hazard *et al.*, 1991b). In contrast, numerous compounds which enhance central DA activity appear to be effective antidepressants. The selective DA reuptake blockers, nomifensine and bupropion, have been noted to be as effective as tricyclics (e.g., amitriptyline, imipramine), selective 5-HT reuptake inhibitors (e.g., trazadone and zimelidine), as well as atypical antidepressants (e.g., maprotiline, clomipramine, and mianserin), in treating depression (Weisler *et al.*, 1994; Ferris *et al.*, 1993; Golden *et al.*, 1988). Deprenyl, a selective monoamine oxidase B inhibitor, which acts preferentially on DA, also appears to reduce depressive symptomatology (Mann *et al.*, 1989). Additional DA agonists which have not been utilized primarily as therapeutic agents to treat depression, including the D₂ antagonists bromocriptine (Wells *et al.*, 1989) and L-sulpride (Maier *et al.*, 1994; Del Zompo *et al.*, 1990), have also been noted to have antidepressant properties. Therefore, pharmaceutical manipulations which reduce DA activity promote depressive symptomatology while compounds which enhance DA activity have antidepressant properties.

Considering the heterogeneous nature of depressive symptomatology, there are likely multiple neurochemical, neuropeptidergic, and neuroendocrine systems involved in the expression of depressive symptomatology. Altered DA activity may play an etiological role in the subgroup of depressed patients which exhibit psychomotor retardation, altered REM patterns, and anhedonia. Infrahuman investigations have provided evidence that central DA activity within the mesencephalon, and the mesocorticolimbic system in particular, may be involved in reward and/or motivational processing. Accordingly, an evaluation of the mesocorticolimbic pathways with respect to the influences of stressor exposure, psychotherapeutic agents, and the neuromodulatory effects of other central neurotransmitters and neuropeptides on mesocorticolimbic DA activity may provide insight into the central mechanisms of action involved in the expression of anhedonia.

The Mesocorticolimbic Dopamine System

1. Anatomy of the Mesocorticolimbic Dopamine System

The midbrain DA neurons account for 70% of brain dopamine and form three major nuclei; the A8 (retrobulbar field), the A9 (substantia nigra), and the A10 (VTA) cell groups. Approximately 47% of midbrain DA neurons are located in the A10 region in the rat, while in humans, 21% of midbrain DA neurons are located in the A10 nuclei (German *et al.*, 1993). The A10 region has been further subdivided into five distinct nuclei; the parabrachial pigmentosis, paranigral, interfascicular, rostral linear and caudal linear nuclei (Swanson *et al.*, 1982). The nucleus parabrachialis pigmentosis represents the dorsal and lateral aspects of the VTA while the nucleus paranigralis corresponds to the more ventral region of the VTA. The interfascicular, rostral linear and caudal linear nuclei fall along the midline of the A10 region (Fallon, 1988; Oades *et al.*, 1987). While the A10 subnuclei can be demarcated anatomically, it should be emphasized that considerable local integration occurs throughout the A10 nuclei, as approximately 50% of A10 neurons form dendrosomatic, axodendritic or dendrodendritic synapses amongst the different nuclei (Bunney *et al.*, 1991). In addition, the dendritic fields of the A10 nuclei enter surrounding

brain regions to some extent, with the dendrites of the paranigral nuclei extending into the interpeduncular nucleus and the medial substantia nigra pars compacta (Oades *et al.*, 1987; Phillipson, 1979), while the parabrachial neurons overlap with dorsal substantia nigra pars compacta neurons (Phillipson, 1979).

The A10 neurons project primarily to limbic and cortical brain regions, forming the mesolimbic and mesocortical pathways respectively, while projections from the A9 neurons to the striatum form the nigrostriatal system (Oades *et al.*, 1987). The mesolimbic system is comprised of A10 projections to the nucleus accumbens, amygdala, limbic (entorhinal) cortices, olfactory tubercle, lateral septum, and stria terminalis. The mesocortical system consists of A10 projections to the neocortices (prefrontal, insular, cingulate, and pyriform cortices) as well as to regions of the sensory, motor and temporal cortex (Bunney *et al.*, 1991). Mesocortical projections tend to originate dorsorostrally in the A10 area (i.e., the rostral linear and parabrachial pigmentosus nuclei) while mesolimbic projections tend to originate in the ventrocaudal A10 region (i.e., the paranigral nuclei) (Oades *et al.*, 1987). However, it should be emphasized that A10 efferents do not form completely distinct projections, as VTA neurons projecting to different terminal regions are intermixed, rather than being clustered into the subnuclei of the A10 area (Albanese *et al.*, 1983; Swanson *et al.*, 1982). Indeed, other investigators have identified prominent pathways from the paranigral nucleus to the lateral septum and cortical areas as well as projections from the parabrachial pigmentosis subnucleus to the nucleus accumbens, amygdala and other limbic regions (see Fallon, 1988; Halliday *et al.*, 1988). Still others have delineated VTA projections according to a medial/lateral differentiation, with medial regions projecting to the septum and frontal cortex and more lateral region innervating the nucleus accumbens and olfactory tubercle (Phillipson, 1978). Moreover, approximately 10% of ascending DA projections from lateral aspects of the A10 region have been noted to collateralize to innervate both cortical and limbic terminal regions (Albanese *et al.*, 1983). Therefore, while

A10 neurons show a general dorsal/ventral differentiation with respect to terminal regions, both cortical and limbic sites receive projections from all aspects of the VTA.

While A10 DA neurons primarily innervate cortical and limbic regions, efferent projections to other brain regions have also been identified. For example, the ventromedial aspects of the A10 region appear to innervate the anteromedial striatum (Fallon, 1988; Oades *et al.*, 1987). Neurons within the VTA also innervate the locus coeruleus, the medial and dorsal raphe, and the parabrachial areas, forming the mesorhombencephalic system, as well as thalamic and hypothalamic nuclei, forming the mesodiencephalic system (Oades *et al.*, 1987). Projections from the A10 region to the cerebellar cortex (Ikai *et al.*, 1992) and the hippocampus (Gasbarri *et al.*, 1994) have also been identified. Accordingly, in addition to forming heterogeneous subnuclei, the A10 DA neurons innervate a diverse range of brain regions, potentially representing distinct anatomical and functional subsystems related to reward/motivational processing.

2. The Mesocorticolimbic System and Reward Processing

It has been consistently demonstrated in infrahuman subjects that drugs which enhance central DA activity have reinforcing or rewarding properties (reviewed in Kosobud *et al.*, 1994; Miller *et al.*, 1990). For example, a conditioned place preference can be established following the systemic administration of morphine, heroin, amphetamine, or cocaine (reviewed in Bardo *et al.*, 1995). Intra-VTA injections of psychomotor stimulants or opioids also result in a place preference for the drug paired environment (reviewed in Wise *et al.*, 1989; Fibiger *et al.*, 1988). Rats and mice have been observed to self administer opioids and psychomotor stimulants intravenously (McGregor *et al.*, 1995; Carelli *et al.*, 1994) or directly into the VTA (David *et al.*, 1995). The self-administration of opioids and psychomotor stimulants is associated with an increased firing rate of neurons within the VTA (Chang *et al.*, 1994) and nucleus accumbens (Chen *et al.*, 1994; Carelli *et al.*, 1994). In addition, enhanced dopamine release within the nucleus accumbens appears to accompany self-administration of opioids or cocaine (Chang *et al.*, 1994; Di Ciarl *et al.*,

1990; Pulverenti *et al.*, 1990), as well as the intra-VTA application of opioids (Devine *et al.*, 1993a; Leone *et al.*, 1991). In contrast to the reward associated with DA agonists, a reduction in DA activity induced by 6-OHDA lesions of the mesocorticolimbic pathways or the administration of DA antagonists (e.g., haloperidol, pimozide, spiroperidol, SCH 23390) into the nucleus accumbens or prefrontal cortex abolishes self-administration of rewarding substances (McGregor *et al.*, 1995; 1993; Le Moal *et al.*, 1991; Fibiger *et al.*, 1988) and the establishment of drug induced conditioned place preference (Cagganetti *et al.*, 1995). Accordingly, the mesocorticolimbic may play a central role in the neural circuitry mediating the rewarding aspects of drugs of abuse.

Activation of the mesocorticolimbic system also appears to play a role in the mediation of the rewarding properties of natural reinforcers. For example, DA release from the nucleus accumbens occurs during feeding behavior (reviewed in Salamone, 1994; Ljungberg *et al.*, 1992; Phillips *et al.*, 1991) and water ingestion (Yoshida *et al.*, 1992; Hoebel *et al.*, 1989). The release of DA from the nucleus accumbens is particularly conspicuous during the preparatory phase prior to feeding behavior and can also be elicited by presentation of a conditioned stimulus associated with food delivery (Phillips *et al.*, 1993). Enhanced release of DA from the nucleus accumbens is also observed during sexual behavior (Mas *et al.*, 1990; Pfaus *et al.*, 1990), again particularly during the preparatory phase of sexual activity (Melis *et al.*, 1995). Somatodendritic DA release within the VTA and the firing activity of A10 DA neurons is also enhanced in rats by palatable food presentation (Zhang *et al.*, 1994) or sucrose consumption (Kosobud *et al.*, 1994). A decrease in central DA transmission appears to disrupt the hedonic aspects of natural reinforcers, as pretreatment with the nonselective DA receptor antagonist, pimozide, the selective D1 antagonist, SCH23390, or the selective D2 antagonist, raclopride, prevents the conditioned place preference which can be established following water intake (Amgo *et al.*, 1993). Based on the role of the mesocorticolimbic in mediating the reinforcing properties of drugs of abuse and appetitively motivated behaviors, it has been proposed that the

mesocorticolimbic system may play a general role in mediating the rewarding effects of stimuli that act as positive reinforcers (reviewed in Salamone, 1994; Le Moal *et al.*, 1991).

Direct electrical stimulation of the mesocorticolimbic system also appears to be rewarding as animals will respond for brain stimulation from numerous sites along the mesocorticolimbic system, including the prefrontal cortex, nucleus accumbens and A10 area (Maddeaux *et al.*, 1992; Zacharko *et al.*, 1991; 1987; 1983). Dopamine appears to be at least one of the neural substrates mediating self-stimulation behavior as DA antagonists, including apomorphine, SCH23390, and spiperone, increase the threshold for responding for brain stimulation to the VTA and medial forebrain bundle (Hunt *et al.*, 1994; Fouriez *et al.*, 1992; Phillips *et al.*, 1989). In contrast, the postsynaptic D2 receptor agonist, bromocriptine (Knapp *et al.*, 1995; Hunt *et al.*, 1994) or opioid analogues (Johnson *et al.*, 1994; Bauco *et al.*, 1993; Heidebreder *et al.*, 1992) enhance responding for brain stimulation to the lateral hypothalamus and VTA. In addition, electrical stimulation to the VTA and medial forebrain bundle is accompanied by increases in DOPAC, HVA and DA release within the nucleus accumbens, prefrontal cortex and olfactory tubercle (Fiorino *et al.*, 1993; Phillips *et al.*, 1992; Nakahara *et al.*, 1992). Therefore, electrical stimulation induced DA release within mesocorticolimbic terminal regions may represent direct activation of the neural substrates mediating the hedonic properties of appetitively reinforcing stimuli.

The complex anatomical organization observed in the mesencephalon suggests that differential aspects of motivational and/or reward processing may be mediated by distinct subsystems within the mesocorticolimbic system. Indeed, it has been observed that the firing pattern of nucleus accumbens neurons varies as a function of the type of reinforcer presented (e.g., cocaine or water) and that distinct regions of nucleus accumbens cells increase in firing rate while others decrease in response to reinforcer presentation (Carelli *et al.*, 1994). In addition, while pimozide blocks the conditioned place preference established following water intake (Amgo *et al.*, 1993), pimozide is ineffective in preventing the

establishment of place preference following sexual behavior (Ango *et al.*, 1990). Accordingly, while the mesocorticolimbic may serve a general function in reward processing, different types of reward may be associated with activation of distinct neural subsystems, stimulation of specific DA receptor subtypes, and/or interactions with different neuromodulators within the A10 nuclei or the terminal regions of the mesocorticolimbic system. Indeed, as will be discussed in the following section, stressors appear to provoke variations in DA activity within the mesocorticolimbic system which are highly region specific. In light of the relationship between stressors, DA alterations and depression, region specific variations in the mesocorticolimbic system may contribute to the expression of the anhedonic aspects of depressive symptomatology.

3. Stressor Provoked Alterations in Mesocorticolimbic Dopamine Activity

It has been consistently demonstrated that uncontrollable stressors induce neurochemical alterations within the mesocorticolimbic DA system of infrahuman subjects which vary as a function of the brain region examined, as well as the intensity and duration of the stressor regimen employed. Uncontrollable stressors, including mild footshock (Inoue *et al.*, 1994; Heinsbroek *et al.*, 1991), restraint (Imperato *et al.*, 1991; Giorgi *et al.*, 1991; Abercrombie *et al.*, 1989; Roth *et al.*, 1988), conditioned fear (Inoue *et al.*, 1994; Claustre *et al.*, 1986; Deutch *et al.*, 1985; Herman *et al.*, 1982) and food deprivation (Carlson *et al.*, 1988) enhance DA activity in the prefrontal cortex. Increases in the DA metabolites, DOPAC and HVA, and changes in DOPAC/DA ratios have been demonstrated following uncontrollable stressor exposure (Inoue *et al.*, 1994; Shanks *et al.*, 1991; Kalivas *et al.*, 1989; Robinson *et al.*, 1987; Claustre *et al.*, 1986; Deutch *et al.*, 1985; Herman *et al.*, 1984; Dunn *et al.*, 1983; Blanc *et al.*, 1980; Herve *et al.*, 1979; Lavielle *et al.*, 1978; Fadda *et al.*, 1978). Activation of tyrosine hydroxylase in prefrontal cortex DA terminals (Iuvone *et al.*, 1986), an enhanced discharge rate of A10 DA neurons innervating the prefrontal cortex (Mantz *et al.*, 1989), and an augmented release of DA within the prefrontal cortex (Finlay *et al.*, 1995; Sorg *et al.*, 1993; Abercrombie *et al.*, 1989), have

also been demonstrated following footshock, restraint or tailpinch. More intense or protracted footshock, restraint or tail pinch are required to elevate DA metabolism within the nucleus accumbens (Shanks *et al.*, 1991; Bertolucci-D'Angio *et al.*, 1990; Dunn, 1988; Roth *et al.*, 1988; Robinson *et al.*, 1987; Speciale *et al.*, 1986; Claustre *et al.*, 1986; Herman *et al.*, 1984; Dunn *et al.*, 1983; Thierry *et al.*, 1976). However, even with more intense stressors, the magnitude of change in DA metabolites or DA release seen in the nucleus accumbens is typically smaller than that observed from the prefrontal cortex (Abercrombie *et al.*, 1989; Antelman *et al.*, 1988; Deutch *et al.*, 1985; Lavielle *et al.*, 1978).

The majority of studies evaluating the effects of stressors on DA activity have reported that footshock, restraint and tailpinch do not alter measures of DA, DOPAC, or DOPAC/DA ratios in the striatum (Giorgi *et al.*, 1991; Shanks *et al.*, 1991; Bertolucci-D'Angio *et al.*, 1990; Deutch *et al.*, 1985; Lavielle *et al.*, 1978; Fadda *et al.*, 1978). It has also been noted that mild to moderate stressor application fails to activate DA metabolic activity (Deutch *et al.*, 1988) or *c-fos* expression (Deutch *et al.*, 1991) in the A9 DA cell group. However, with more severe stressors, particularly those which involve a distinct physical component, enhanced striatal DA activity has been detected. For example, 30 minutes of prone restraint (Deutch *et al.*, 1991), forced locomotion (Bertolucci-D'Angio *et al.*, 1990), severe tail shock (Abercrombie *et al.*, 1989) or cold exposure (Dunn *et al.*, 1983; Keller *et al.*, 1983) enhances DOPAC levels and DA release in the striatum. Accordingly, stressors which evoke changes in locomotor activity or provoke a thermoregulatory response may enhance striatal DA turnover.

In contrast to the lack of effect of mild stressors on A9 DA activity, acute mild footshock, restraint, and conditioned fear increase DOPAC concentrations (Herman *et al.*, 1988; Ida *et al.*, 1987), enhance tyrosine hydroxylase activity (Deutch *et al.*, 1985; Hirada *et al.*, 1989), and provoke expression of Fos protein in A10 DA cells (Deutch *et al.*, 1991). Presentation of stimuli previously paired with footshock also appears to provoke *c-fos*

expression within the VTA region (Beck *et al.*, 1995a). It has further been demonstrated that tail pinch increases the firing rate of A10 neurons (Mantz *et al.*, 1989), and enhances the somatodendritic release of DA within the VTA (Zhang *et al.*, 1994). Moreover, in parallel to the differential effects of stressors on the terminal regions of the mesocorticolimbic system, stressors appear to provoke region specific changes within the A10 region. For example, acute restraint elicited increases in Fos-tyrosine hydroxylase expression in A10 neurons is greater in the caudal linear and parabrachial nuclei relative to the interfascicular and paranigral nuclei, in which Fos-tyrosine hydroxylase expression is more moderate, and the rostral linear neurons, in which Fos expression is minimal (Deutch *et al.*, 1991). Using retrograde tracers, it has been observed that the A10 cells expressing Fos-tyrosine hydroxylase following stressor application primarily innervate the prefrontal cortex (Deutch *et al.*, 1991). Electrophysiological studies have further demonstrated that acute tail pinch selectively increases the firing rate of A10 neurons which innervate the prefrontal cortex but not those innervating the nucleus accumbens (Mantz *et al.*, 1989). Accordingly, region specific alterations in DA activity within the A10 region correspond to the finding that the terminal regions of mesocorticolimbic cells are differentially activated by stressors. However, it should be noted that there are distinct subpopulations of VTA-DA neurons which project to the prefrontal cortex that do not show Fos-tyrosine hydroxylase following restraint, and DA neurons projecting to the amygdala and entorhinal cortices have been observed to exhibit stressor provoked Fos-tyrosine hydroxylase activity (Deutch *et al.*, 1991). Therefore, acute stressor application appears to activate areas of the VTA innervating both limbic and cortical terminal regions.

While acute stressors appear to enhance mesocorticolimbic DA activity, stressor induced alterations in DA have been noted to show a biphasic pattern of change with more protracted stressor exposure. For example, while 30 minutes of uncontrollable footshock produces an increase in DOPAC and HVA in the prefrontal cortex, 90 minutes of footshock provokes a decline in DOPAC levels and DOPAC/DA ratios (Heinsbroek *et al.*, 1991).

Sixty minutes of restraint or footshock has also been noted to produce an increase in DOPAC or HVA in the nucleus accumbens of mice, while levels of DOPAC and HVA decrease following 130 minutes of restraint (Puglisi-Allegra *et al.*, 1991). Elevations in the DA metabolite, 3-methoxytyramin (3-MT), which may provide a more accurate index of extracellular DA release than DOPAC (e.g., Wood *et al.*, 1988), have also been observed in the nucleus accumbens in mice subjected to five minutes of attack by a conspecific (Puglisi-Allegra *et al.*, 1990), or three to ten minutes of restraint or footshock (Puglisi-Allegra *et al.*, 1991). In contrast, dramatic decreases in 3-MT in the nucleus accumbens have been observed following 30 to 120 minutes of restraint (Cabib *et al.*, 1989; 1988; Puglisi-Allegra *et al.*, 1991) or 60 minutes of footshock (Cabib *et al.*, 1994; Puglisi-Allegra *et al.*, 1991). In vivo microdialysis studies have demonstrated similar biphasic alterations in DA release. For example, acute footshock or restraint provokes a peak increase of DA release in the prefrontal cortex approximately 15 to 20 minutes after stressor onset, which gradually decreases thus that DA release reaches baseline levels 70 minutes following stressor onset (Sorg *et al.*, 1993; Imperato *et al.*, 1991; Abercrombie *et al.*, 1989). Other investigators have observed enhanced nucleus accumbens DA release with 40 minutes of restraint, followed by a return to basal levels with 60 to 100 minutes of stressor application, and a significant decrease in DA release with 130 minutes of restraint (Puglisi-Allegra *et al.*, 1991). Accordingly, as stressor induced alterations in extracellular DA release and concentrations of 3-MT, DOPAC and HVA show a biphasic pattern, DA activity appears to be initially increased in response to stressors, followed by a decline of DA release with more protracted stressor exposure. It has been suggested that considering DOPAC and HVA levels do not decrease to the extent of that observed in extracellular DA concentrations or 3-MT levels, there may be an increase in intracellular DA synthesis following stressor application (Puglisi-Allegra *et al.*, 1991). However, while enhanced synthesis may occur during protracted stressor exposure, utilization may exceed synthesis rates as demonstrated by the decline in extracellular DA activity.

In addition to the nature of the stressor employed, organismic variables, including genetic factors, gender and prior stressor history, appear to influence the pattern of DA activation. For example, acute footshock has been observed to increase DOPAC accumulation in the frontal cortex in BALB/cByJ mice but not C57BL/6ByJ mice (Shanks *et al.*, 1991). Strain differences have also been observed amongst recombinant inbred strains, with DOPAC accumulation varying between strains in both the prefrontal cortex and nucleus accumbens following stressor exposure (Shanks *et al.*, 1994). A comparison of Roman high-avoidance (RHA/Verh) and Roman low-avoidance (RLA/Verh) rats, of which the latter are believed to be more emotional or anxious, has revealed that immobilization, exposure to a novel environment, or exposure to high intensity loud noise increases cortical DOPAC in the RHA/Verh but not RLA/Verh rats (D'Angio *et al.*, 1988). Individual difference within strains may also be predictive of stressor induced DA alterations. For example, Sprague Dawley rats which are more predisposed to drug seeking behavior (i.e., psychostimulant self-administration) and show higher locomotor reactivity in novel environments or in response to psychostimulant challenge (see Hooks *et al.*, 1992; 1991) exhibit higher and more protracted elevations of extracellular nucleus accumbens DA and DOPAC in response to ten minutes of tail pinch (Rouge-Pont *et al.*, 1993). Sex differences may also influence the pattern of DA activity observed following stressor exposure, as female rats exposed to inescapable footshock show higher concentrations of HVA and DA/HVA ratios than male rats (Heinsbroek *et al.*, 1991). Interestingly, the densities of D₂ receptors in limbic regions (e.g., the olfactory tubercle and striatum) show considerable variation across strains, and are lower in female rats relative to male rats, potentially accounting for differential sensitivities to stressor exposure (Vadasz *et al.*, 1992). Finally, animals previously exposed to stressors exhibit more pronounced DA activation in response to stressors relative to naive animals, demonstrating that sensitization occurs within the mesocorticolimbic system (see Wise *et al.*, 1993; Kalivas *et al.*, 1991). It has been proposed that sensitization of DA activity occurs as a result of desensitization of

D1 autoreceptors located on inhibitory γ -aminobutyric acid (GABA) neurons within the A10 region, secondary to the somatodendritic release of DA (Kalivas *et al.*, 1993; Wise *et al.*, 1993). Accordingly, the DA alterations induced by stressors vary as a function of genetic makeup, sex, and prior stressor history of the organism onto which the stressor is imposed, potentially mediated by different sensitivities and/or densities of D1 and D2 receptors.

As previously discussed, it has been argued that initial stressor provoked increases in neurochemical activity may enable an organism to cope behaviorally with environmental stressors (Anisman *et al.*, 1992a). In this respect, it has been suggested that elevated mesocortical DA activity following stressor exposure may reflect heightened attention of the animal or activation of cognitive processes in an attempt to cope with the stressor (see Bertolucci-D'Angio *et al.*, 1990). Accordingly, elevated DA activity may reflect the preparatory cortical processes (i.e., avoidance or escape strategies) which lead to effective coping with a stressor, returning to baseline levels when control is established. Indeed, enhanced DA activity in the prefrontal cortex is not observed in animals who learn to avoid or terminate footshock (Driscoll *et al.*, 1989). It has further been proposed that considering DA release within the nucleus accumbens is increased by reinforcing stimuli as well as by stressors, enhanced mesolimbic DA activity may serve to initiate goal directed behavior whether appetitively or aversively motivated (Kosobud *et al.*, 1994; Salamone, 1994). Activation of mesocorticolimbic DA activity may facilitate the ability to effectively cope with a stressor by enhancing the ability to use sensory information and by regulating adaptive motor output (Salamone, 1994).

Taken together, studies which have assessed DA variations following stressor application suggest that when faced with an environmental challenge, attempts to cope may initially be manifested as behavioral activation associated with increased mesocorticolimbic DA activity. Increased mesocorticolimbic activity may facilitate attention to relevant stimuli and regulate behavioral interaction with the environment (i.e., sensorimotor integration),

resulting in appropriate goal directed behavior. When behavioral coping is not possible (i.e., the organism cannot exert control over the stressor or the stressor is severe and protracted), decreased mesocorticolimbic DA activity appears to ensue. As is the case with infrahuman subjects, stressors of varying intensity, nature and duration, superimposed on an individual's stressor history and/or genetic background, may promote differential degrees of pathology and diverse symptom profiles in human populations. Stressor related alterations influencing DA neurotransmission, in particular, may be associated with the emergence of the anhedonic aspects of depression.

4. Stressor Induced Pathology Associated With Mesocorticolimbic DA Alterations

Stressor provoked alterations in DA activity are paralleled by behaviors which may be reminiscent of depressive symptomatology. For example, stressor provoked DA reductions within the nucleus accumbens have been associated with shuttle escape deficits (Anisman *et al.*, 1979), and immobility in the Porsolt forced swim paradigm (Imperato *et al.*, 1994; Rossetti *et al.*, 1993). At least some strains of mice (e.g., DBA/2J) which exhibit a stressor provoked reduction in consumption of a palatable diet show altered DOPAC levels in the nucleus accumbens following acute footshock (Griffiths *et al.*, 1992). Reductions in post-synaptic D2 receptors within the nucleus accumbens have also been associated with a reduction of sucrose intake following chronic mild, unpredictable stressor application (Papp *et al.*, 1994; Wilner *et al.*, 1991). It has been suggested that reductions in the consumption of sucrose may potentially reflect a loss of the rewarding value associated with palatable substances (Monleon *et al.*, 1995; Willner, 1992). The rewarding properties of amphetamine or the D2 agonist, quinpirole, as determined by diminished ability to establish a conditioned place preference, have also been associated with stressor provoked reductions in nucleus accumbens D2 receptors (Papp *et al.*, 1993).

Additional evidence that stressor provoked mesocorticolimbic DA variations may be associated with reward alterations comes from the finding that uncontrollable stressors provoke decreases in responding for brain stimulation from mesocorticolimbic sites but not

from the substantia nigra (Zacharko *et al.*, 1990a; 1990b; Zacharko *et al.*, 1983). As previously discussed, stressor provoked DA variations are consistently observed within the A10 area and mesocorticolimbic terminal regions, but not the nigrostriatal system. Accordingly, stressor provoked alterations in brain stimulation performance directly parallel region specific stressor related DA alterations. Moreover, the time course of stressor induced self-stimulation variations also appears to parallel the time course of stressor induced DA variations. Exposure to relatively brief stressors, such as 30 seconds of tail pinch, have been observed to facilitate brain stimulation performance from the medial forebrain bundle (Katz *et al.*, 1979), and 20 minutes of escapable footshock has been found to facilitate the acquisition of responding for brain stimulation to the medial prefrontal cortex (Balleine, 1991). In contrast, more protracted uncontrollable stressors, such as 60 minutes of intermittent footshock, decrease self-stimulation performance from the medial forebrain bundle (Zacharko *et al.*, 1983), medial prefrontal cortex (Molfe *et al.*, 1990), and nucleus accumbens (Bowers *et al.*, 1987). Stressor exposure has also been found to induce a large attenuation in brain stimulation performance from the dorsal A10 region (Maddeaux *et al.*, 1992; Zacharko *et al.*, 1991), while no change in responding for brain stimulation is observed from the ventral regions of the A10 area (Zacharko *et al.*, 1990b). Interestingly, stressors preferentially activate Fos-tyrosine hydroxylase activity within the nucleus parabrachial pigmentosus, which corresponds to the dorsolateral aspects of the A10 region (Deutch *et al.*, 1991). Accordingly, stressor provoked reductions in brain stimulation directly correspond to the stressor provoked alterations observed in the mesocorticolimbic system and are consistent with the biphasic time course of stressor induced DA alterations. Considering an alteration in reward/motivational processing is a fundamental characteristic of depression, stressor provoked reductions in brain stimulation performance subserved by region specific midbrain DA variations may provide a model of the anhedonia evident in clinical depression (Zacharko *et al.*, 1991; 1983).

In further support of the proposition that behavioral pathology associated with central DA alterations may parallel depressive symptomatology, psychotherapeutic agents which are effective for treating clinical depression have been noted to reverse or attenuate behaviors associated with DA alterations. Chronic prophylactic administration of imipramine has been noted to attenuate shuttle escape deficits following acute footshock (Gambarana *et al.*, 1995). Forced swim induced immobility, and the accompanying reductions in extracellular DA within the ventral striatum, is prevented by chronic pretreatment with imipramine (Rossetti *et al.*, 1992; 1993). A subchronic regimen (i.e., 9 days) of imipramine has been observed to enhance in vivo dopamine release in the ventral striatum and to attenuate stressor induced immobility in a forced swim task (Imperato *et al.*, 1994). The chronic therapeutic administration of desipramine has also been observed to reverse forced swim induced immobility (Borsini *et al.*, 1990). Chronic administration of tricyclics (e.g., imipramine, desipramine, amitriptyline), the selective 5-HT reuptake inhibitor, fluoxetine, or the selective NE reuptake inhibitor, maprotiline, also reverses the stressor induced reduction of sucrose intake (Monleon *et al.*, 1995; Papp *et al.*, 1994; Willner *et al.*, 1992). The imipramine provoked reversal of diminished sucrose intake has been associated with an attenuation of the stressor induced decrease in post-synaptic D₂ receptors in the nucleus accumbens (Papp *et al.*, 1994). Stressor provoked decreases in sucrose consumption are also reversed by chronic administration of the DA agonists pramipexole (Willner *et al.*, 1995) or chronic intermittent administration of quinpirole or bromocriptine (Muscat *et al.*, 1992). Chronic administration of the tricyclics, desmethylinipramine and amitriptyline, have been noted to reverse the stressor induced disruption in brain stimulation performance observed from the prefrontal cortex (Wolfe *et al.*, 1991a) and nucleus accumbens (Baille *et al.*, 1988; Zacharko *et al.*, 1984). The chronic administration of imipramine has also been observed to prevent stressor provoked reductions in responding for brain stimulation from the VTA (Moreau *et al.*, 1992), while

chronic mianserin treatment reverses stressor related decreases in VTA self-stimulation (Moreau *et al.*, 1994).

While many of the aforementioned antidepressants, particularly tricyclics, do not acutely affect DA reuptake, antidepressant administration appears to indirectly enhance extracellular DA levels. Fluoxetine, clomipramine, imipramine and desipramine have been noted to enhance DA levels in the prefrontal cortex (Tanda *et al.*, 1994). Buspirone, tianeptine and amineptine have also been observed to enhance extracellular DA levels in the nucleus accumbens (Tanda *et al.*, 1994; Sacchetti *et al.*, 1993; Invernizzi *et al.*, 1992a; 199b). Antidepressant agents also appear to provoke changes in DA receptor density and/or sensitivity with prolonged administration. Chronic imipramine administration has been noted to reduce the density of D1 receptors in limbic regions, including the olfactory tubercle, nucleus accumbens, septum and amygdala, as revealed by [³H]SCH 23390 binding (Nowak *et al.*, 1991; DeMontis *et al.*, 1989, 1990; Klimek *et al.*, 1987). Chronic imipramine or desipramine also reduces the D1 stimulated production of adenylate cyclase in the nucleus accumbens, olfactory tubercle, and striatum (Okada *et al.*, 1994; DeMontis *et al.*, 1990). While chronic imipramine fails to alter D2 receptor density in limbic regions (Nowak *et al.*, 1991), chronic administration of imipramine has been noted to enhance the affinity for D2 receptor agonists (e.g., spiperone) in the nucleus accumbens (Klimek *et al.*, 1989). Chronic administration of iprindole or mianserin have also been observed to potentiate the apomorphine or *d*-amphetamine induced locomotor activity in animals (Serra *et al.*, 1990; Spryaki *et al.*, 1981; Klimek, 1985). A potentiation of DA mediated behaviors following chronic antidepressant administration may reflect enhanced D2 receptor sensitivity in the mesolimbic system and/or changes distal to the D2 site in second messenger pathways (see Serra *et al.*, 1992; DeMontis *et al.*, 1990). The prevention of behavioral pathology by chronic prophylactic antidepressant administration may be related to alterations in both D1 and D2 postsynaptic receptors as acute administration of either the D1 antagonist, SCH 23390, or the D2 antagonist, sulpride, reduces the anti-immobility

effect of chronic prophylactic desipramine or imipramine in forced swim paradigms (D'Aquila *et al.*, 1994; Cervo *et al.*, 1990). Moreover, chronic administration of SCH 23390 or sulpride prevents the reversal of stressor produced attenuation of sucrose consumption by desipramine or imipramine (see Willner, 1992). However, the DA receptor alterations which ensue may be dependent on the stressor regimen employed, considering chronic administration of SCH 23390, but not sulpride, prevents shuttle escape deficits following acute footshock (Gambarana *et al.*, 1995). Taken together, the finding that antidepressants provoke alterations in extracellular DA levels, DA receptor linked neurotransmission, and the behavioral pathology associated with stressor provoked DA alterations provides further support for the proposition that DA alterations may be associated with depressive symptomatology.

In summary, the mesocorticolimbic system appears to play a major role in central reward processing and stressor induced disruptions in mesocorticolimbic transmission may mediate at least some of the symptoms of depression, particularly anhedonia. Considering compensatory central alterations occur following chronic stressor exposure which potentially minimize the neurochemical and behavioral repercussions of stressors, anhedonia may occur when there is a disturbance in adaptive neural alterations influencing the mesocorticolimbic system. Antidepressants appear to facilitate mesolimbic DA transmission, potentially accounting for the prevention and reversal of stressor provoked reward alterations. However, the conceptualization of the mesocorticolimbic DA projections as forming a unitary system may be overly simplistic in consideration of the complex anatomical organization of the mesencephalon, as well as evidence that different aspects of the mesocorticolimbic system may be activated by different types of rewarding stimuli. Moreover, stressors do not uniformly effect DA activity within the A10 area or mesocorticolimbic terminal regions and stressor provoked effects on brain stimulation performance are region specific within the VTA. As will be discussed in the ensuing section, a complex interface occurs between central neurotransmitters and neuropeptides at

the level of the A10 region. Accordingly, it is important to consider the neural mechanisms that interact with and regulate mesocorticolimbic DA activity under conditions of stressor exposure which may potentially contribute to the expression of region specific alterations in reward processing.

Regulation of Mesocorticolimbic Activity

1. Autoreceptor Regulation of Mesocorticolimbic Activity

One of the mechanisms which may influence region specific alterations in mesocorticolimbic DA activity following stressor exposure is feedback regulation from DA autoreceptors. Presynaptic D2 receptors serve as inhibitory autoreceptors through inhibition of the adenylate cyclase-cAMP second messenger system (Andersen *et al.*, 1990). Somatodendritic D2 receptor stimulation hyperpolarizes DA cells, decreasing the rate of tyrosine hydroxylase induction (hence DA synthesis) and reducing impulse flow (reviewed in Bunney *et al.*, 1991). It is well established that somatodendritic release occurs in response to depolarization of DA cells within the A10 region (Rice *et al.*, 1994). Accordingly, it has been suggested that the primary function of DA release within the VTA is to provide autoinhibition of impulse generation, thereby regulating DA release in terminal regions (Kalivas, 1993). Somatodendritic DA release within the A10 region may inhibit DA firing through activation of D2 receptors on DA perikarya, as well as by modulating release of GABA and 5-HT from presynaptic terminals through stimulation of D1 receptors on non-DA cells in the A10 region (Cameron *et al.*, 1993). As will be discussed in more detail, GABA and 5-HT both appear to inhibit DA release from A10 DA cells.

A differential distribution of D2 autoreceptors has been detected between mesocortical and mesolimbic neurons. Mesocortical neurons originating in the medial A10 region appear to be devoid of D2 autoreceptors, while autoreceptors are present on neurons originating in the lateral A10 region, which collateralize to innervate cortical as well as limbic and striatal regions (Shepard *et al.*, 1984). Other investigators have also observed that somatodendritic autoreceptors are absent on subpopulations of DA neurons in the VTA which project to the

prefrontal and cingulate cortices (Hoffman *et al.*, 1988; Deutch *et al.*, 1985; Chiodo *et al.*, 1984; Bannon *et al.*, 1983). Mesocortical neurons exhibit a number of functional characteristics which differ from that of mesolimbic and/or nigrostriatal neurons which may be accounted for, at least in part, by the lack of synthesis and impulse regulating autoreceptors. Mesocortical neurons which lack D2 autoreceptors exhibit fast basal firing patterns, while DA neurons which contain autoreceptors and innervate limbic and striatal regions tend to have slower firing patterns (Shepard *et al.*, 1984). In addition, in vitro preparations have demonstrated that mesocortical DA neurons exhibit greater stimulation induced release of DA and sustain higher levels of DA release at rapid stimulation rates relative to mesolimbic and nigrostriatal neurons (Cubeddu *et al.*, 1990; Hoffmann *et al.*, 1988). Mesoprefrontal neurons, in particular, show a greater degree of DA release in response to DA agonists, higher basal firing rates, and a greater frequency of burst firing relative to mesolimbic neurons (Deutch *et al.*, 1990).

It has been suggested that electrophysiological and stressor related differences observed between subpopulations of midbrain DA cells may be associated with the differential distribution of autoreceptors in the mesocorticolimbic system (Bunney *et al.*, 1991). However, while the medial prefrontal, cingulate and entorhinal cortices all appear to be largely devoid of somatodendritic impulse and synthesis modulating autoreceptors, they do possess release regulating autoreceptors on their terminals (reviewed in Bunney *et al.*, 1991). Moreover, a small group of mesoprefrontal cortical DA neurons which possess impulse modulating D2 receptors have been identified, which appear to originate in the lateral aspects of the paranigral nucleus (Gariano *et al.*, 1989). Accordingly, autoreceptor regulation is not completely absent within the mesocortical A10 projections. In addition, while both the cingulate and prefrontal cortices lack synthesis and impulse modulating autoreceptors, only the prefrontal cortex exhibits heightened DA synthesis and metabolic activity in response to mild stressors (Hirada *et al.*, 1989). Accordingly, the more profound

effect of stressors on DA alterations within the mesocortical terminal regions cannot be attributed solely to the lack of autoreceptors.

It has recently been demonstrated utilizing electrical field stimulation in cell preparations that mesocortical neurons exhibit differences in DA release characteristics relative to striatal neurons which are both dependent on and independent of autoreceptor control (Agneter *et al.*, 1994). With repetitive stimulation, DA release from striatal neurons is reduced to a greater extent than DA release from neurons in the prefrontal cortex. When the D₂ receptor antagonist, sulpride, is added to the cell preparation, the frequency dependent per pulse release of DA is increased from striatal neurons but not neurons in the prefrontal cortex, indicating an absence of autoreceptor mediated release inhibition in prefrontal cortex cells. However, the DA release evoked from prefrontal cortex neurons by single pulse stimulation is lower than that observed from striatal DA neurons, an effect which is not modified by sulpride, suggesting midbrain DA neurons also exhibit autoreceptor independent differences in DA release characteristics (Agneter *et al.*, 1994). Adaptive changes in protein kinase C activity, as well as differential sensitivity to depolarizing agents and to extracellular calcium requirements have been proposed as potential mechanisms underlying differences in release characteristics between mesocortical and mesolimbic/nigrostriatal neurons (Cubeddu *et al.*, 1990). Accordingly, differences in firing pattern, DA metabolic activity, and DA release between mesocortical and nigrostriatal or mesolimbic systems in response to stressors, may be due, in part, to intrinsic differences in the release characteristics of midbrain DA systems.

It should be noted that recent studies have identified D₃, D₄ and D₅ receptor subtypes, all of which appear to be localized primarily to the limbic system (Kostrzewa *et al.*, 1995; Vadasz *et al.*, 1992). The D₃ receptor appears to have a higher affinity for DA than the D₂ receptor and may also serve an autoreceptor function in the A10 region (Schwartz *et al.*, 1993). However, little attention has focused on the differential physiological functions of DA receptor subtypes with respect to functional differences

amongst mesocortical and mesolimbic systems. Accordingly, the relative distribution of DA receptor subtypes between mesocortical and mesolimbic neurons and the physiological relevance of DA autoreceptor subtypes in terms of the response of mesocorticolimbic DA cells to stressor exposure remains to be determined.

2. Neurotransmitter Regulation of A10 Neural Activity

In addition to DA mediated modulation of A10 cell activity via somatodendritic D₂ receptor activation, A10 neurons are regulated by additional neurotransmitters, including GABA. Anatomical studies have demonstrated that GABA containing interneurons are located within the VTA and form synapses with DA neurons (Masuko *et al.*, 1992; Nagai *et al.*, 1983). In addition, GABAergic afferents to the VTA directly innervate A10 DA neurons as well as GABAergic interneurons (Sugita *et al.*, 1992; Bayer *et al.*, 1991). The GABAergic projections to the VTA originate in the medial shell regions of the nucleus accumbens (Kalivas *et al.*, 1993; Heimer *et al.*, 1991; Groenewegen *et al.*, 1984) and primarily innervate the paranigral nucleus (Kalivas *et al.*, 1993). In addition, GABAergic projections from the ventromedial aspects of the ventral pallidum innervate the VTA (Klitenick *et al.*, 1992b; Zahm, 1989). Inhibition of GABA release within the A10 region following application of GABA antagonists results in disinhibition of DA cells and increased extracellular DA content within the VTA and nucleus accumbens (Shaud-Chagny *et al.*, 1992; Klitenick *et al.*, 1992b; Johnson *et al.*, 1992a; Oakley *et al.*, 1991). Accordingly, GABAergic afferents from the nucleus accumbens and ventral pallidum and GABA containing interneurons may tonically inhibit A10 DA activity.

GABAergic mechanisms appear to interact with DA activity in response to stressor exposure. Infusions of the GABA_B agonist, baclofen, or the benzodiazepine, chlorodiazepoxide, into the VTA prevents stressor induced alterations in DA activity within the prefrontal cortex (Kalivas *et al.*, 1990; Deutch *et al.*, 1990). Systemic administration of benzodiazepines (e.g., diazepam), which enhance chloride flux through the GABA-activated ionophore (Sieghart, 1994), also prevents the stressor induced increase in DA

metabolism in both the prefrontal cortex and nucleus accumbens (Finlay *et al.*, 1995; Roth *et al.*, 1988; Scarton *et al.*, 1988). Pretreatment with diazepam also reduces the number of stressor provoked DA cells immunoreactive for Fos in the A10 caudal linear and parabrachial nuclei (Deutch *et al.*, 1991) and prevents the conditioned fear provoked expression of *c-fos* in the VTA (Beck *et al.*, 1995a). In contrast to the effects of diazepam, the benzodiazepine inverse agonist, methyl- β -carboline-3-carboxamide (FG 7142), enhances DA metabolic activity in the prefrontal cortex, nucleus accumbens and VTA (McCullough *et al.*, 1992; Bradberry *et al.*, 1991; Deutch *et al.*, 1991; Bertolucci-D'Angio *et al.*, 1990; McGregor *et al.*, 1990). Interestingly, FG 7142 increases the number of A10 DA neurons expressing Fos protein in a region specific manner, with enhanced Fos being observed primarily in the parabrachial nuclei, and to a lesser extent, the paranigral nuclei (Deutch *et al.*, 1991). Accordingly, enhancing GABAergic neurotransmission may attenuate the neurochemical repercussions of stressor exposure while GABA antagonists simulate the stressor induced alterations in mesocorticolimbic DA activity. It has recently been demonstrated that when rats can exert control over a stressor (e.g., terminate tail shock by turning a wheel), brain levels of endogenous benzodiazepine receptor agonists are increased relative to non-stressed or yoked control animals (Drugan *et al.*, 1994). These data suggest a facilitation of GABAergic transmission may occur during active behavioral coping which may interfere with the cascade of events which places excessive demands on central neurotransmitters, thereby preventing the expression of behavioral pathology.

Excitatory amino acids also appear to play an important role in the regulation of A10 DA activity. Three excitatory amino acid receptor types have been localized in the A10 region, including N-methyl-D-aspartate (NMDA), kainate, and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (see Wang *et al.*, 1994). It has been reported that stimulation of both NMDA and non-NMDA receptors in the A10 region depolarizes DA neurons (Johnson *et al.*, 1992a). For example, application of glutamate, quisqualate or kainate enhances the activity of DA cell firing and extracellular levels of DA

in the nucleus accumbens and prefrontal cortex (Shaud-Chagney *et al.*, 1992; Imperato *et al.*, 1990; Kalivas *et al.*, 1989; Grace *et al.*, 1986). However, only stimulation of NMDA receptors induces burst firing of A10 DA neurons (Wang *et al.*, 1994; Shaud-Chagney *et al.*, 1992), while stimulation of AMPA and kainate receptors promote pacemaker-like activity, potentially through modulation of non-DA neurons in the A10 region (Wang *et al.*, 1995). The stimulation of subtypes of excitatory amino acid receptors may be dependent on the margin of excitatory amino acid activity within the A10 area as glutamate has been observed to selectively activate NMDA receptors within the VTA at low doses and to activate both NMDA and non-NMDA receptors at higher doses (Wang *et al.*, 1993). The excitatory amino acid receptor subtypes may be differentially linked with A10 neurons innervating cortical and limbic terminal regions considering DA release in the nucleus accumbens appears to be modulated by non-NMDA receptors in the VTA while enhanced prefrontal cortex DA metabolism is associated with activation of NMDA receptors in the A10 region (see Deutch *et al.*, 1990; Kalivas *et al.*, 1989).

There is evidence that excitatory amino acids may modulate stressor related mesocorticolimbic activity as NMDA receptor antagonists (e.g., HA-966, CPP), prevent the stressor induced enhancement of medial prefrontal cortex DA utilization without affecting basal DA activity (Goldstein *et al.*, 1994; Kalivas *et al.*, 1989). Accordingly, activation of NMDA receptors within the A10 region by excitatory amino acid release during stressor exposure may be instrumental in increasing DA cell bursting activity. Excitatory amino acid containing neurons which terminate in the VTA are believed to originate in the medial prefrontal cortex, pedunculopontine and subthalamic nuclei (reviewed in Kalivas, 1993). However, the excitatory amino acid projections from the prefrontal cortex are believed to be primarily responsible for stimulation of NMDA receptors within the A10 region (Wang *et al.*, 1995; Gariano *et al.*, 1988). These data suggest that burst firing of A10 DA cells during stressor exposure may depend, in part, on excitatory amino acid feedback regulation from the prefrontal cortex.

Serotonin has also been observed to play a role in the regulation of mesocorticolimbic DA activity. The medial aspects of the A10 region contain a low density of 5-HT perikarya and 5-HT fibers ascending from the dorsal and medial raphe nuclei pass through the caudal border of the A10 area (Halliday *et al.*, 1989; Oades *et al.*, 1987). Serotonin axon terminals have been observed within all of the subnuclei of the A10 region, which form axodendritic synapses with both DA and non-DA neurons (Herve *et al.*, 1987). The majority of axon terminals within the paranigral and linear rostral nuclei appear to be serotonergic in nature (Halliday *et al.*, 1989). The 5-HT receptors in the VTA are primarily of the 5-HT_{1B} subtype, although 5-HT_{1C} and 5-HT₂ receptors are also present (reviewed in Prisco *et al.*, 1994). The intra-VTA application of 5-HT has been observed to increase DA metabolic activity in the nucleus accumbens (Guan *et al.*, 1989). Local application of selective 5-HT_{1B} agonists enhances A10 DA activity indirectly by inhibiting GABA release within the VTA (Johnson *et al.*, 1992). However, decreases in central 5-HT activity, induced by electrolytic lesions of the raphe nuclei, have also been observed to increase DA metabolic activity in the nucleus accumbens (Herve *et al.*, 1981). Suppression of dorsal and median raphe cell activity with systemic administration of 8-hydroxy-2(di-n-propylamino)tertralin (8-OH-DPAT), a 5-HT_{1A} autoreceptor agonist, has also been observed to increase burst firing of VTA DA neurons (Prisco *et al.*, 1994; Arborelius *et al.*, 1993) and to elevate medial prefrontal cortex DA utilization (Rasmusson *et al.*, 1994). In addition, systemic administration of the 5-HT₂ antagonist, ritanserin, increases burst firing of A10 DA cells (Ugedo *et al.*, 1989). Based on these paradoxical findings, it has been suggested that 5-HT may exert a biphasic effect on mesocorticolimbic DA neurons, with basal endogenous release of 5-HT acting on 5-HT₂ receptors to promote dendritic DA release, resulting in D₂ autoreceptor stimulation and hyperpolarization of DA cells. However, when 5-HT activity is increased, enhanced release of 5-HT may then stimulate 5-HT_{1B} receptors and activate mesocorticolimbic DA cells by presynaptically inhibiting descending GABAergic inputs (Kalivas, 1993; Johnson *et al.*, 1992c). It has also recently

been observed that the selective 5-HT_{1C} antagonist, mesulergine, increases basal A10 DA cell firing, suggesting 5-HT_{1C} receptors may also tonically inhibit A10 DA cell activity (Prisco *et al.*, 1994). Accordingly, basal 5-HT release may tonically inhibit A10 activity through stimulation of 5-HT₂ and 5-HT_{1C} receptors, while stressors which further augment 5-HT release may inhibit GABAergic inputs to the A10 region through activation of 5-HT_{1B} receptors, leading to disinhibition of A10 DA neurons (see Kalivas, 1993). Interestingly, systemic pretreatment with 8-OH-DPAT, which increases basal A10 and prefrontal cortex DA activity, partially antagonizes footshock induced increases in DA utilization in the medial prefrontal cortex (Rasmusson *et al.*, 1994). Based on the aforementioned findings, it has been suggested that 5-HT_{1A} agonists may potentially render VTA neurons more resistant to stressor activated excitatory inputs (Rasmusson *et al.*, 1994).

Considering GABA and EEA transmitters within the A10 region originate in regions innervated by the mesocorticolimbic system, feedback from A10 terminal fields may play a central role in modulating stressor provoked DA activity. As prefrontal cortex neurons are activated preferentially by stressors, excitatory amino acid containing inputs to A10 neurons from the prefrontal cortex may provide further excitation of DA cells or activate inhibitory GABAergic interneurons depending on the nature and intensity of the stressor. GABAergic feedback from the nucleus accumbens or ventral pallidum to the VTA may also contribute to the overall activity of DA cells during stressor application. In addition, particularly severe stressors may enhance 5-HT inputs from the raphe, inhibiting GABA transmission and provoking disinhibition of DA A10 cell activity. Accordingly, a complex interaction between excitatory amino acids, DA, GABA and 5-HT neurons within the VTA, nucleus accumbens, prefrontal cortex, ventral pallidum, and raphe nuclei may regulate the overall activity of the mesocorticolimbic system.

3. Neuropeptide Regulation of A10 Neural Activity

In addition to modulation by central neurotransmitters, neuropeptides appear to play a central role in modulating mesocorticolimbic DA activity. Neuropeptide containing nerve terminals, peptide containing perikarya, and a vast variety of neuropeptide receptors have been localized in the VTA. Neuropeptides are both colocalized with DA and non-DA perikarya in the A10 region, as well as contained in the terminals of neurons which innervate the A10 region. The most extensively studied A10 neuropeptides include neurotensin, the tachykinins, cholecystokinin and enkephalin (reviewed in Kalivas 1993; 1985).

A moderate to high density of neurotensin immunoreactive fibers have been observed within the A10 region, with the highest densities being localized to the parabrachial pigmented and linear nuclei (Jayaraman *et al.*, 1990). Neurotensin is colocalized within A10 DA neurons, as well as contained in axon terminals which originate in the nucleus accumbens and the amygdala (reviewed in Kalivas, 1993). Neurotensin receptors appear to be located directly on DA perikarya and dendrites within the VTA (Dilts *et al.*, 1989). The majority of A10 DA neurons containing neurotensin project to the medial prefrontal cortex (Febvre *et al.*, 1991; Seroogy *et al.*, 1988), although a percentage of neurotensin positive neurons also innervate the nucleus accumbens (Kalivas *et al.*, 1984). Neurotensin has been observed to increase the firing frequency of A10 DA cells and to augment DA levels in mesocorticolimbic terminal fields (reviewed in Kalivas, 1993). It has been demonstrated that neurotensin reduces the affinity of D₂ receptors (Fuxe *et al.*, 1995), thus neurotensin release in the A10 area may antagonize the autoreceptor induced inhibition of DA neurons induced by somatodendritic DA release.

Midbrain DA neurons have also been observed to contain cholecystokinin. Within the A10 region, cholecystokinin appears to colocalized with neurotensin within DA cell bodies (Seroogy *et al.*, 1988). However, in contrast to neurotensin, cholecystokinin appears to be solely colocalized within VTA DA cells as 6-OHDA lesions completely

eliminate the expression of mRNA for cholecystokinin in the A10 region (Seroogy *et al.*, 1989). Neurons containing cholecystokinin have been visualized throughout the VTA, but are somewhat more dense within the parabrachial pigmented nucleus (Seroogy *et al.*, 1988). Approximately 40% of the VTA neurons projecting to the caudal aspects of the nucleus accumbens contain cholecystokinin (Vaccarino, 1994). In addition, DA neurons containing cholecystokinin project to the striatum, prefrontal cortex, and amygdala (reviewed in Kalivas, 1993). The intra-VTA administration of cholecystokinin stimulates the firing frequency of DA cells, primarily through activation of cholecystokinin_A rather than cholecystokinin_B receptors (Kelland *et al.*, 1991). Indeed, cholecystokinin appears to be one of the most potent excitants of DA cell firing, and it has been suggested that cholecystokinin may function to switch DA neurons from single spike mode to bursting mode (Bunney *et al.*, 1991). Similar to neurotensin, cholecystokinin has been observed to desensitize D₂ autoreceptors (Fuxe *et al.*, 1995). However, it should be noted that cholecystokinin also augments the ability of D₂ autoreceptor agonists, including apomorphine, to inhibit DA cells, indicating subtypes of cholecystokinin receptors may be differentially coupled to DA and/or GABAergic cells (see Kalivas, 1993).

Moderate to high levels of the tachykinins (substance P, neurokinin A (substance K), and neurokinin B) have also been demonstrated in the VTA (reviewed in Kalivas, 1993). Intra-VTA infusions of either substance P or the neurokinins increases mesocorticolimbic activity (see Elliot *et al.*, 1992). However, substance P may preferentially activate non-DA cells within the A10 region while neurokinin A acts primarily on DA cells (Kalivas, 1993). In addition, substance P appears to preferentially activate mesocortical neurons, while neurokinins activate DA metabolic activity in the nucleus accumbens (Deutch *et al.*, 1985b). Substance P also appears to have opposing functions in the subnuclei of the VTA, promoting an inhibitory effect on DA cells in the paranigral nucleus while exerting an excitatory effect on DA cells in the parabrachial pigmented

nucleus (Halliday *et al.*, 1988). In contrast to other neuropeptides, the tachykinins are not involved in the regulation of bursting activity of A10 DA neurons (Kalivas, 1993).

Opioid containing fibers have also been localized to the A10 region, which appear to overlap with dopaminergic cell bodies and dendrites in the VTA (Sesack *et al.*, 1992; Fallon *et al.*, 1986; Zamir *et al.*, 1984). Dynorphin (Pickel *et al.*, 1991), leu-enkephalin (Liang *et al.*, 1992; Sesack *et al.*, 1992) and met-enkephalin (Fallon *et al.*, 1990; 1986; Gaspar *et al.*, 1983) containing fibers have all been observed to make synaptic contacts with A10 neurons. The nucleus accumbens and ventral pallidum appear to be the primary sources of VTA terminals which contain enkephalin (Kalivas *et al.*, 1993). However, enkephalin containing perikarya have also been localized in the rostral regions of the nucleus parabrachial pigmentosus and the adjacent substantia nigra (Vankova *et al.*, 1991). In addition, enkephalinergic projections may arise from the frontal cortex, hypothalamus, amygdala, and raphe nuclei (see Sesack *et al.*, 1992). Enkephalinergic axon terminals within the A10 region form somatodendritic and axoaxonic synapses with DA neurons. In addition, enkephalinergic terminals form axo-axonic connections with non-DA neurons, which in turn form synaptic connections with DA dendrites (Sesack *et al.*, 1992). Enkephalin also appears to be colocalized within non-DA interneurons in the A10 region (Liang *et al.*, 1993; Sesack *et al.*, 1992). Similar to other neuropeptides in the A10 region, enkephalin and opioid analogues have a stimulatory effect on A10 DA neurons and enhance DA turnover in mesocorticolimbic terminal fields (e.g., Grant *et al.*, 1994; Devine *et al.*, 1993a; Stinus *et al.*, 1992).

Taken together, the finding that various neuropeptides are localized within A10 cells suggests that a highly complex set of interactions between various peptides and neurotransmitters may modulate mesocorticolimbic DA activity. Neuropeptides are known to be colocalized with classic neurotransmitters in large, dense core vesicles and are released extrajunctionally, rather than into the synaptic cleft (reviewed in Hokfelt, 1991). Neuropeptides are able to diffuse long distances and influence neural transmission for a

longer duration than classical neurotransmitters (Fuxe *et al.*, 1991). Accordingly, one of the primary functions of mesencephalic neuropeptides may be to potentiate the time course and release of DA or other colocalized transmitters within the A10 region (discussed in Malek-Akmal, 1992). Considering neuropeptide release requires bursting or high frequency activity (Hokfelt, 1991), neuropeptides may play a key role in the modulation of A10 DA cell activity in response to stressors. Indeed, it has been demonstrated that substance P plays a role in the activation of DA neurons in response to stressors, as intra-VTA infusions of monoclonal antibody to substance P attenuates stressor induced increases in DA metabolism in the prefrontal cortex (Bannon *et al.*, 1983). A naloxone reversible increase in enkephalinergic activity has also been observed in the VTA, and intra-VTA naloxone prevents stressor provoked increases in DA metabolism within the prefrontal cortex and nucleus accumbens (Kalivas *et al.*, 1987a). As cholecystokinin and neurotensin desensitize D₂ autoreceptors (Fuxe *et al.*, 1995), cholecystokinin and neurotensin may prolong DA activity during stressor exposure by antagonizing the inhibitory action of somatodendritic DA release in the A10 area.

The environmental conditions under which different A10 neuropeptides are released are poorly understood at the present time but are likely dependent on the nature of the stressor regimen employed as demonstrated in other brain regions. For example, within the paraventricular nucleus of the hypothalamus, footshock or interperitoneal saline injection increases corticotropin releasing factor and enkephalin mRNA, while restraint or forced swim selectively enhances corticotropin releasing factor mRNA, and ether challenge selectively increases enkephalin mRNA (Lightman *et al.*, 1993b; Watts, 1991). Similarly, stressors of varying intensity or duration may influence differential peptide release in the VTA, potentially dependent on stressor related activation of afferents to the VTA which may differentially innervate the A10 subnuclei and interact selectively with certain peptides. Depending on which peptides are released in the A10 region, different terminal regions of the mesocorticolimbic system may be activated. For example, while intra-VTA enkephalin

appears to activate both the nucleus accumbens and prefrontal cortex, substance P more selectively enhances DA metabolism in the prefrontal cortex, while neurokinin or neurotensin preferentially activate DA neurons projecting to the nucleus accumbens (Cador *et al.*, 1989; Kalivas *et al.*, 1987b; Deutch *et al.*, 1985). Accordingly, depending on the combinations of transmitters and/or peptides released in the VTA in response to stressors, different ascending DA systems and therefore, different forebrain regions may be activated, ultimately producing variable effects on the behavioral responses to stressors.

Neuropeptide release within the A10 region appears to have a general activating effect on behavior, as the intra-VTA administration of substance P, neurokinin, neurotensin and enkephalin all elicit an increase in locomotor activity (Badiani *et al.*, 1995; Druhan *et al.*, 1993; Elliot *et al.*, 1992; Kelley *et al.*, 1989). However, considering the diverse network of neuropeptides in the A10 region, it has been suggested that differential peptidergic modulation may constitute the substrate of distinct behavioral processes (Kelley *et al.*, 1989). Indeed, as the mesocorticolimbic region is involved in reward/motivational processing, neuropeptide specific activation of different aspects of the mesocorticolimbic system may be involved in different rewarding behaviors. Enkephalin, in particular, appears to play a central role in reward processing associated with activation of the A10 region. For example, intra-VTA injections of enkephalin facilitate operant responding for food reinforcement, while neurotensin inhibits responding, and substance P elicits no change in operant responding (Kelley *et al.*, 1989; Cador *et al.*, 1986). In addition, a conditioned place preference can be established with intra-VTA injections of morphine or enkephalin analogues (Barr, 1992a; Herz *et al.*, 1992; Phillips *et al.*, 1982), while intra-VTA administration of substance P (Kelley *et al.*, 1989), neurotensin (Kelley *et al.*, 1991), and cholecystokinin (Pettit *et al.*, 1989) are ineffective in conditioned reinforcement paradigms. Activation of A10 neurons appears to be critical in mediating the reinforcing properties of opioids as administration of morphine or enkephalin analogues to the lateral hypothalamus, nucleus accumbens, prefrontal cortex, substantia nigra or striatum is

ineffective is establishing a place preference (Bals-Kubik *et al.*, 1993; Baumeister *et al.*, 1993). Moreover, the application of morphine immediately dorsal, caudal or rostral to the VTA is ineffective in establishing a place preference (Bozarth, 1987). Accordingly, enkephalin appears to selectively modulate reward processing at the level of the A10 region.

It should be noted that non-opioid neuropeptides may influence central reward processing at brain regions other than the A10 nuclei. For example, peripheral administration of substance P, as well as local injections of substance P into the medial forebrain bundle, septum, and nucleus basalis magnocellularis results in a place preference for the drug paired environment (Mattioli *et al.*, 1995; Boix *et al.*, 1995; Staubli *et al.*, 1985). Rats will also self-administer substance P to the ventromedial caudate-putamen (Krappman *et al.*, 1994). Systemic administration of the cholecystokinin antagonist, L-365,260, antagonizes intravenous morphine induced place preference, suggesting cholecystokinin also plays a role in reward/motivational processing (Higgins *et al.*, 1992). As intra-nucleus accumbens cholecystokinin increases cocaine self-administration and facilitates responding for brain stimulation from the VTA, activation of cholecystokinin receptors at the level of the nucleus accumbens may influence reward (reviewed in Crawley *et al.*, 1994; Vaccarino, 1994). Accordingly, while enkephalins modulate reward processing at the level of the A10 region, other neuropeptides may influence reward at different sites along the mesocorticolimbic system. The following section provides a more comprehensive discussion of the interactions between opioids and DA within the A10 region, and the implications that opioid-DA interactions have for stressor provoked alterations in central reward processing.

Opioid-Dopamine Interactions within the A10 Region

1. Opioid Receptor Subtypes in the A10 Region

The differential distribution and distinct physiological roles of opioid receptor subtypes within the central nervous system, particularly μ , δ and κ receptors, has been the

focus of a considerable amount of research (reviewed in Simon, 1991; Slater *et al.*, 1991). Exogenous opioid alkaloids, as well as the endogenous opioid peptides derived from the three peptide families (pro-opiomelanocortin, proenkephalin and prodynorphin) bind to μ , δ and κ receptors (reviewed in Mansour *et al.*, 1994). Met-enkephalin, leu-enkephalin, and β -endorphin appear to bind preferentially with μ and δ receptors, while dynorphin and neodyorphin interact with κ receptors (Lutz *et al.*, 1992). The three receptor subtypes appear to have overlapping functions in the regulation of pain (Pasternak, 1993), consummatory behavior, and cardiovascular functioning (reviewed in Olson *et al.*, 1994; 1993). Activation of μ and κ receptors appear to have opposing effects on fluid balance and thermoregulation (reviewed in Olsen *et al.*, 1994). In addition, as will subsequently be discussed, μ and δ receptor agonists appear to exert differential effects on DA activity, locomotor behavior, and reward processing relative to κ agonists.

A highly heterogeneous distribution of μ opioid receptors occurs throughout the central nervous system, as evidenced by binding of the selective μ receptor agonists 3[H]-(D-Ala²,N-MePhe⁴,Gly-ol⁵)-enkephalin (DAMGO) (Mansour *et al.*, 1994), 3[H]-Tyr-D-Ala²-Gly-(Me)Phe-Gly-ol⁵ (DAGO), (Gross-Isseroff *et al.*, 1992; Dilts *et al.*, 1989; Mansour *et al.*, 1988), and [125]-FK33-824 (Mokharti *et al.*, 1993). Binding for μ receptors in the mouse and rat brain is densest in the amygdala, nucleus accumbens, striatum, and thalamus, while more moderate binding is observed in the cortex, hypothalamus and hippocampus. A similar μ receptor distribution has been observed in humans, although μ receptor binding is the highest in the hypothalamus and thalamus, while more moderate binding is expressed in the amygdala and caudate, and binding is lower in the hippocampus and substantia nigra (Raynor *et al.*, 1995). In situ hybridization studies have localized μ receptor mRNA in the caudate putamen, nucleus accumbens, olfactory tubercle, the lateral and medial septal nuclei, frontoparietal cortex, hippocampus, thalamus, hypothalamus, locus coeruleus, superior olive and cerebellar cortex of the rat (Zastawny *et al.*, 1994). The distribution of μ receptors and μ receptor mRNA is relatively

low at the level of the mesencephalon (Mansour *et al.*, 1994; German *et al.*, 1993). Within the A10 region, μ receptors and μ receptor mRNA are higher in the caudal relative to the rostral aspects, with notable μ receptor binding being observed in the interfascicular nucleus and the region surrounding the fasciculus retroflexus (Mansour *et al.*, 1994; German *et al.*, 1993). However, μ receptor ligand binding is minimal in the parabrachial pigmented and paranigral nuclei, regions in which there are high densities of DA somata (German *et al.*, 1993). Relatively dense μ receptor binding occurs in the caudal VTA immediately dorso-lateral to the interpeduncular nucleus (German *et al.*, 1993). Very prominent μ receptor binding occurs throughout the interpeduncular nucleus, with higher levels of cellular μ receptor mRNA being observed in the rostral and intermediate nuclei relative to the paranigral and central subdivisions (Mansour *et al.*, 1994; German *et al.*, 1993). Accordingly, dense μ receptor binding occurs near the A10 region, but not overlapping with regions containing DA somata, suggesting μ receptors are located on non-DA neurons in the A10 region.

The distribution of δ receptors in the rat and mouse brain has been determined utilizing the selective δ ligands 3[H]-(D-Pen2, D-Pen5)-enkephalin (DPDPE) (Speciale *et al.*, 1993; Dilts *et al.*, 1990; Mansour *et al.*, 1988), 3[H]-D-Ser(otBu)²-Gly-Phe-Leu-Thr (DSTBULET) (Delay-Goyet *et al.*, 1990), [125]-D-Ala²-deltorphin-I (Mokharti *et al.*, 1993) and 3[H]-natriindole (Lever *et al.*, 1992). Immunohistochemistry and in situ hybridization studies have also been utilized to determine the distribution of δ receptors (Arvidsson *et al.*, 1995; Mansour *et al.*, 1993). As with μ receptors, δ receptors show a wide distribution throughout the central nervous system, although the distribution of δ receptors appears to be more restricted and binding density for δ receptors is ten fold lower relative to μ receptors (Mokharti *et al.*, 1993; Mansour *et al.*, 1993). The highest densities of δ receptors have been identified in the striatum, olfactory tubercle, amygdala and cortical regions. More moderate binding is observed in the hippocampus, thalamus and hypothalamus, while the lowest levels are observed in the cerebellum and brainstem (Lever

et al., 1992; Mansour *et al.*, 1988). Relatively low levels of binding for δ ligands has been observed throughout the midbrain DA nuclei, with δ binding being the highest in the caudal portion of the substantia nigra pars reticulata (Arvidson *et al.*, 1995). Binding for δ receptor ligands is relatively sparse in the VTA, localized primarily to the interfascicular and paranigral nuclei of the A10 region (Delay-Goyet *et al.*, 1990). In general, δ receptor binding is higher in the rostral aspects of the VTA and sparse in more caudal aspects where there are a high number of DA cell bodies (Speciale *et al.*, 1993). Relatively dense δ binding is observed in the rostral and lateral nuclei of the interpeduncular nucleus (Arvidsson *et al.*, 1995; Speciale *et al.*, 1993). However, fewer δ sites are present in the interpeduncular nucleus relative to μ receptors (Dilts *et al.*, 1990; Moskowitz *et al.*, 1984).

Kappa receptors are also widely distributed throughout the central nervous system, as determined by receptor binding for the selective κ ligand, 3[H]-U-69593 (Speciale *et al.*, 1993), as well as the detection of κ opioid receptor mRNA utilizing in situ hybridization (DePaoli *et al.*, 1994). Kappa receptors are particularly conspicuous in subcortical telencephalic structures, thalamic, and hypothalamic nuclei (DePaoli *et al.*, 1994; Mansour *et al.*, 1988). Within the midbrain region, κ binding is highest throughout the rostral-caudal extent of the substantia nigra pars compacta while relatively sparse κ binding is observed in the retrorubral field and the central linear nucleus raphe. There is a paucity of κ receptors at the level of the caudal VTA, with minimal binding being observed in the interfascicular nucleus and nucleus paranigralis, although κ receptors are somewhat more prominent in the rostral VTA (Speciale *et al.*, 1993).

In summary, opioid receptor binding appears to be higher in the terminal regions of the mesocorticolimbic system relative to the cell body regions of the mesencephalon. The A10 region contains a heterogeneous distribution of μ , δ and κ receptors, although μ receptors appear to predominate. Binding for all three receptor subtypes is higher in the rostral aspects of the A10 nuclei and relatively sparse within the parabrachial pigmented and paranigral nuclei.

2. Functional Interaction Between Endogenous Opioids and Dopamine Activity:

Both endogenous enkephalins and synthetic opioid analogues have been demonstrated to enhance electrophysiological and neurochemical activity in the VTA and mesocorticolimbic terminal regions. Systemic or intra-VTA administration of morphine leads to a naloxone reversible increase in the activity of A10 DA cells (Grant *et al.*, 1994; Kiyatkin, 1988; Matthews *et al.*, 1984; Gysling *et al.*, 1983), increased extracellular accumulation of DA in the VTA (Klitenick *et al.*, 1992b), and elevations in DOPAC and HVA in the nucleus accumbens, prefrontal cortex, and olfactory tubercle (Ivengar *et al.*, 1989; Kim *et al.*, 1986). Intra-VTA application of β -endorphin, Leu-enkephalin, or the mixed μ and δ receptor agonists, D-Ala²-Met⁵-enkephalinamide (DALA), DADLE and DAME, increases the firing rates of A10 cells (Trulson *et al.*, 1985; Gysling *et al.*, 1983), and produces an increase in DOPAC and DOPAC/DA ratios in the nucleus accumbens, prefrontal cortex and VTA (Stinus *et al.*, 1992; Leone *et al.*, 1991; Cador *et al.*, 1989; Latimer *et al.*, 1987; Kalivas *et al.*, 1987b). Inhibition of the peptidases which inactivate endogenous enkephalins also enhances mesocorticolimbic activity. For example, systemic administration of the neutral endopeptidase inhibitor, SCH 32615, enhances DA metabolic activity in the nucleus accumbens (Giorgi *et al.*, 1991). Intra-VTA injection of the neutral endopeptidase inhibitor, thiorphin, or the mixed inhibitor of neutral endopeptidase and aminopeptidase, kelatorphin, also produces a naloxone reversible elevation of DOPAC and HVA in the nucleus accumbens and prefrontal cortex and extracellular DA accumulation in the nucleus accumbens (Dauge *et al.*, 1992; Kalivas *et al.*, 1986). Accordingly, both exogenous opioid compounds and endogenous opioid peptides facilitate mesocorticolimbic DA neurotransmission.

It was initially proposed that enhanced mesocorticolimbic DA activity following opioid administration was mediated primarily by μ receptor activation (Kim *et al.*, 1987; Latimer *et al.*, 1987). However, intra-VTA injections of either selective μ agonists (e.g., DAGO, DAMGO), or selective δ receptor agonists (e.g., DPEN, DPDPE), appear to

increase DOPAC/DA ratios and extracellular DA release in the nucleus accumbens and prefrontal cortex (Devine *et al.*, 1993a; 1993b; Spanagel *et al.*, 1992; Kalivas *et al.*, 1990). Furthermore, pretreatment with the selective μ receptor antagonist D-Pen-Cys-Tyr-D-Trp-Om-Thr-Pen-Thr-NH₂ (CTOP) or the selective δ receptor antagonist, ICI 174,864, antagonizes the enhanced DA activity produced by intraventricular β -endorphin administration (Spanagel *et al.*, 1990). The selective δ receptor antagonist, natrindole, but not the selective μ receptor antagonist, CTOP, blocks the enhanced DA release and DA metabolic activity produced by DPDPE, demonstrating that specific δ ligands do not increase DA activity by cross reacting with μ receptors (Devine *et al.*, 1993a). However, the effective dose of DPDPE in increasing DA release from the nucleus accumbens is 100 to 1000 times higher than that of DAMGO (Devine *et al.*, 1993a). Accordingly, while both μ and δ receptor activation facilitates mesocorticolimbic DA activity, μ receptors agonists appear to have a greater facilitory effect on DA activity.

In contrast to the effects of μ and δ receptor ligands, intra-VTA injections of the κ agonists, U-50,488H or U-69593, or the κ receptor antagonist, norbinaltorphimine (nor-BNI) fails to alter DA release or DA metabolic activity within the nucleus accumbens (Devine *et al.*, 1993a, Spanagel *et al.*, 1992). In contrast, systemic or intra-nucleus accumbens injection of U-69593 decreases DA release from the nucleus accumbens (Devine *et al.*, 1993a; Di Chiara *et al.*, 1988), while intra-nucleus accumbens administration of nor-BNI increases nucleus accumbens DA release (Spanagel *et al.*, 1992). Moreover, while intra-VTA administration of DAGO enhances DA release in the nucleus accumbens and intra-VTA injection of CTOP decreases basal release of nucleus accumbens DA, injections of DAGO or CTOP into the nucleus accumbens has no effect on nucleus accumbens DA activity (Spanagel *et al.*, 1992). Based on the differential effects of μ and κ agonists on DA release, two opposing endogenous opioid systems which modulate mesolimbic DA activity may exist. It has been suggested that there is a tonically active μ opioidergic system at the level of the VTA which increases basal DA release and an

inhibitory opioidergic system which is activated by κ receptors in the nucleus accumbens (Spanagel *et al.*, 1992). In view of the observation that intra-VTA administration of κ agonists does not alter mesolimbic DA activity, it has been suggested that κ receptors within the A10 region do not participate in the tonic modulation of mesolimbic activity (Spanagel *et al.*, 1992). However, other investigators have demonstrated that U69593 or dynorphin inhibits the spontaneous or potassium stimulated release of DA from VTA neurons in culture (Ronken *et al.*, 1993; Smith *et al.*, 1992). Accordingly, κ receptor activation at the level of the nucleus accumbens and the A10 region may inhibit mesolimbic DA activity.

There is considerable evidence which suggests that μ receptor stimulation activates A10 DA cells by inhibiting GABA activity within the midbrain. Intra-VTA administration of morphine or DAMGO has been noted to hyperpolarize GABA neurons and inhibit the release of GABA in the A10 region (Johnson *et al.*, 1992b; Klitenick *et al.*, 1992a; Gysling *et al.*, 1983). The intra-VTA injection of the GABA_B agonist, baclofen, antagonizes the intra-VTA DAMGO stimulated release of DA from the nucleus accumbens, as well as the morphine provoked somatodendritic release of DA in the A10 region (Klitenick *et al.*, 1992a; Kalivas *et al.*, 1990). Accordingly, it has been proposed that stimulation of μ receptors increases A10 DA cell activity by removing tonic inhibition from GABA interneurons (Kalivas *et al.*, 1990; 1988; Matthews *et al.*, 1984; Gysling *et al.*, 1983). In addition to GABA interneurons, μ receptors also appear to be located on enkephalin efferents to the VTA (Dilts *et al.*, 1989). Interestingly, enkephalin terminals within the A10 area have been noted to contain both small clear vesicles as well as large core vesicles, suggesting colocalization of enkephalin with GABA (Liang *et al.*, 1993). Indeed, GABA projections from the nucleus accumbens and ventral pallidum appear to contain a distinct enkephalinergic component (Kalivas *et al.*, 1993). Accordingly, the release of enkephalin from GABA/enkephalin containing terminals may also inhibit GABA release through an activation of presynaptic μ receptors located on terminals which form synapses with A10

DA cells (see Devine *et al.*, 1993b). Therefore, A10 DA activity may be increased by μ agonists as a result of decreased inhibition from GABA interneurons as well as presynaptic inhibition of GABA release from afferent neurons directly innervating A10 DA cells.

The mechanisms through which δ opioid receptors activate mesocorticolimbic DA activity are poorly understood. As 6-OHDA lesions do not reduce δ receptor binding within the VTA, δ receptors do not appear to be localized to DA cells (Dilts *et al.*, 1990). However, in contrast to μ receptors, δ receptors are not localized on GABAergic interneurons, as DPDPE fails to hyperpolarize non-DA neurons in slice preparations, and natrindole fails to antagonize the hyperpolarization of non-DA A10 cells induced by met-enkephalin application (Johnson *et al.*, 1992b). Recent data suggest that δ receptors in the mesencephalon (i.e., the substantia nigra and interpeduncular nucleus) may be localized on terminal regions of 5-HT containing neurons originating in the brainstem (Arvidson *et al.*, 1995). As 5-HT projections from the raphe are known to influence A10 cell activity, δ receptors may potentially modulate the release of 5-HT within the VTA. However, such a proposition is speculative at the present time and the exact mechanisms through which δ receptors modulate A10 DA activity remain to be determined.

In summary, exogenously administered as well as endogenously released opiates modulate A10 cell firing and DA turnover in the cortical and limbic terminal fields of the VTA. The stimulation of κ receptors within the nucleus accumbens appears to inhibit mesocorticolimbic DA activity. In contrast, activation of μ receptors located on GABAergic neurons facilitates the activity of the mesocorticolimbic system by inhibiting the release of GABA from both GABA interneurons and GABA afferents to the A10 region. The stimulation of δ receptors within the A10 region also enhances mesocorticolimbic transmission, although the neural mechanisms by which δ receptors increase DA activity remain relatively unknown.

3. Reward Alterations Associated with Opioid-Dopamine Interactions

Conditioned place preference, self-administration and self-stimulation paradigms have all demonstrated that the mesocorticolimbic system, and the A10 region in particular, plays a critical role in mediating the rewarding effects of opiates. Intra-VTA microinjections of morphine are effective in establishing a conditioned place preference (Barr, 1992a; Phillips *et al.*, 1980), an effect which is antagonized by systemic injections of haloperidol or 6-OHDA lesions of DA pathways (Phillips *et al.*, 1983). Intra-VTA or intraventricular administration of DALA, DAMGO, and DPDPE are also effective in establishing a place preference (Herz *et al.*, 1992; Bals-Kubik *et al.*, 1990a, 1990b; Shippenberg *et al.*, 1987; Phillips *et al.*, 1982; Katz *et al.*, 1979). Conditioned place preference has also been demonstrated following intra-VTA injections of the enkephalinase inhibitor thiorphin (Glimcher *et al.*, 1984). In contrast, intraventricular or intra-VTA administration of U-50, 488H produces conditioned aversion to the drug paired environment (Bals-Kubik *et al.*, 1990b; Shippenberg *et al.*, 1987; Mucha *et al.*, 1985). Accordingly, activation of μ or δ receptors at the level of the A10 region elicits appetitively reinforcing effects, while stimulation of κ receptors appears to be aversive.

It has been demonstrated that rats will self-administer morphine, heroin, DAGO or DPDPE into the A10 region, a behavior which is attenuated by systemic treatment with naloxone (David *et al.*, 1995; Devine *et al.*, 1994; Bozarth *et al.*, 1980; 1981). Self administration of opioids is abolished by 6-OHDA lesions of the mesocorticolimbic system, further demonstrating that activation of mesocorticolimbic DA activity mediates the hedonic properties derived from opioids (Bozarth *et al.*, 1987). Stimulation of the A10 region, in particular, appears to be mediate the reward associated with systemically administered opiates, as the opiate antagonist, diallyl-normorphinium bromide, induces a compensatory increase in intravenous heroin self administration when injected into the VTA, but not other brain regions (Britt *et al.*, 1983). Interestingly, intra-VTA administration of the opioid antagonists, naltrexone and buprenorphine, have also been

observed to prevent the establishment of a place preference following cocaine administration (see Calcagnetti *et al.*, 1995). Pretreatment with naloxone has also been observed to antagonize the conditioned place preference which can be established following sexual activity or water consumption (Amgo *et al.*, 1990; 1993), behaviors which are associated with increased mesocorticolimbic activity (see Melis *et al.*, 1995; Salamone, 1994). Accordingly, opioid-DA interactions within the A10 region may play a general role in mediating the hedonic aspects of reinforcing stimuli.

Opioids also increase self-stimulation performance from a number of brain regions, potentially reflecting an increase in the reward value associated with electrical brain stimulation. Systemic or intra-VTA application of morphine facilitates responding from a number of brain sites including the lateral hypothalamus, prefrontal cortex, and VTA (Bauco *et al.*, 1993; Wise, 1987; Kornetsky *et al.*, 1983; Broekkamp *et al.*, 1979; 1976). Both μ and δ receptors appear to be involved in the opioid induced facilitation of brain stimulation performance as intra-VTA or intra-nucleus accumbens administration of DALA, DAGO, or the selective δ receptor agonists DPDPE, DSTBULET, and (D-Thr²)-leucine enkephalin (DTLET) have been noted to enhance self-stimulation responding from the lateral hypothalamic medial forebrain bundle (Johnson *et al.*, 1994; Heidbreder *et al.*, 1992; West *et al.*, 1989; De Witte *et al.*, 1989; Jenck *et al.*, 1987). The administration of DAMGO or DPDPE to caudal regions of the ventral pallidum also enhances self-stimulation performance to the lateral hypothalamus (Johnson *et al.*, 1994; 1993). Intra-VTA injections of DAGO and D-Ala², D-Ala⁵-enkephalin (DADME) have also been observed to facilitate responding for brain stimulation from the VTA (Singh *et al.*, 1994). The facilitative effects of opioids on brain stimulation performance appear to be DA dependent as pretreatment with α -methyl-para-tyrosine or 6-OHDA lesions of the mesocorticolimbic system has been found to prevent the systemic morphine and intra-nucleus accumbens DALA mediated enhancement of self-stimulation to the lateral hypothalamus (Johnson *et al.*, 1994; Hand *et al.*, 1985; Pert *et al.*, 1975). Taken together, the aforementioned studies suggest that the

facilitative effects of opiates on brain stimulation performance are mediated by activation of mesocorticolimbic DA activity induced by central μ and δ receptor stimulation.

In addition to increasing the reinforcing properties of brain stimulation, opioids appear to facilitate responding for naturally reinforcing stimuli. The intra-VTA administration of DAMGO, but not U50,488H, decreases the latency to feed in non-food deprived rats and increases behaviors directed towards incentive stimuli, including water and gnawable objects (Badiani *et al.*, 1995). Intra-VTA DAMGO or DPDPE also increases the speed of eating in food-deprived rats, although DAMGO has a shorter latency to enhance feeding and a more potent effect (Noel *et al.*, 1995). The intraventricular administration of DAMGO and DTLET also increase operant responding (i.e., lever pressing) for saccharin, while the κ ligand, DAKLI, is without effect (Gosnell *et al.*, 1993). Intra-nucleus accumbens administration of DALA, DPDPE or DAMGO also enhances conditioned lever pressing acquired through the contingent availability of sucrose or food reinforcement (Phillips *et al.*, 1994; Cunningham *et al.*, 1992). Accordingly, activation of μ and δ but not κ opioid receptors appears to facilitate the reward associated with appetitively motivated behaviors, potentially through activation of the mesocorticolimbic DA system.

In summary, behavioral studies suggest that the reward associated with opiates may involve activation of the mesocorticolimbic DA system. The intra-VTA application of opioids results in a conditioned place preference, enhanced responding for brain stimulation, and a facilitation of responding for naturally rewarding stimuli. The administration of opioid antagonists attenuates the reward associated with opioid agonists, as well as the rewarding properties associated with cocaine, brain stimulation, sex, food and water consumption. Accordingly, a common DA-opioid link may mediate the hedonic properties of drugs of abuse and brain stimulation, as well as natural reinforcers.

Stressor Provoked Opioid Activity: Implications for Reward Processing

Several areas of research suggest that stressors facilitate central endogenous opioid activity. For example, footshock, forced swim and restraint paradigms have been demonstrated to result in analgesia (Pasternak, 1993). Stressor induced analgesia appears to be dependent on the release of endogenous opioids as pretreatment with naloxone or more specific μ , δ or κ receptor antagonists prevents stressor related analgesia (reviewed in Olson *et al.*, 1994; 1993). Acute stressors also appear to modify central opiate receptors, with changes in opioid binding being related to the severity of the stressor. For example, handling provokes decreases in both μ and δ receptor binding in the frontal cortices and olfactory tubercle, while 20 minutes of acute footshock decreases μ but not δ receptor binding in the septum (Stein *et al.*, 1992). More prolonged stressors, including 90 hours of water deprivation increases δ receptors in the caudate and nucleus accumbens and increases μ receptors in the septum and VTA (Stein *et al.*, 1992; Blake *et al.*, 1987). Increased μ and δ receptor binding has also been observed in the midbrain, striatum and cortex following 72 hours of food deprivation (Tsuji *et al.*, 1986). Acute stressor induced alterations in opioid receptor binding may reflect mechanisms of receptor inactivation (e.g., receptor phosphorylation, uncoupling from G proteins), or stressor induced endogenous opioid release which competes with exogenous ligands. In contrast, upregulation of opioid receptors induced by chronic stressors may reflect compensatory receptor alterations in response to a depletion of endogenous opioids (discussed in Stein *et al.*, 1992).

Interestingly, behavioral alterations induced by stressors appear to be accompanied by alterations in endogenous opioid activity. For example, conditioned suppression of motility in response to footshock and forced swim induced immobility are accompanied by decreased concentrations of met-enkephalin in the striatum and hypothalamus, decreased leu-enkephalin in the hypothalamus, and increased dynorphin in the hypothalamus and pituitary (Nabeshima *et al.*, 1992). Accordingly, a decrease in endogenous opioids acting at μ and δ receptors and an increase in opioids acting at κ receptors may be associated with

stressor related behavioral pathology. In contrast, the central administration of μ or δ receptor ligands, but not κ receptor ligands, appears to attenuate stressor provoked behavioral pathology. For example, the intraventricular administration of D¹LA or (D-Ala², D-Leu⁵)-enkephalin (DADLE) following footshock prevents the conditioned suppression of motility, as well as forced swim induced immobility, while dynorphin exacerbates stressor provoked behavioral pathology (Kato *et al.*, 1991; 1990). The intraventricular administration of DPDPE and DAMGO or intra-nucleus accumbens morphine application prior to isolation stress reduces vocalizations in rat pups (Barr *et al.*, 1992b, 1992c), while U-50, 488H increases vocalization (Carden *et al.*, 1992). Augmentation of endogenous opioid activity with the enkephalinase inhibitor, RB 101, prior to forced swim reduces the subsequent duration of immobility (Baamonde *et al.*, 1992). The therapeutic administration of the mixed enkephalinase and endopeptidase inhibitor, RB 38A, and the selective neutral endopeptidase inhibitor, EC 3.4.24.11, attenuates the number of escape failure in a shuttle escape task induced by uncontrollable footshock (Tejedor *et al.*, 1993). The enkephalinase inhibitors, bestatin and thiorphin, have also been observed to enhance the efficacy of subtherapeutic doses of imipramine and iprindole in decreasing immobility in the Porsolt swim test (de Felipe *et al.*, 1989). Accordingly, administration of μ or δ receptor ligands or augmentation of endogenous opioids prior to or following stressor exposure appears to ameliorate subsequent behavioral pathology.

It should be noted that other investigators have reported that pretreatment with opioid antagonists, including naloxone and naltrexone, prevents the shuttle escape deficits induced by inescapable shock (Blustein *et al.*, 1992; Hunziker *et al.*, 1992; McCubbin *et al.*, 1984), as well as footshock provoked decreases in responding for brain stimulation from the VTA (Kamata *et al.*, 1986). The central mechanisms underlying the paradoxical finding that both opioid antagonists and opioid agonists attenuate stressor induced behavioral pathology are not readily apparent. However, one potential explanation may

involve the biphasic nature of stressor related neurochemical alterations. As previously discussed, stressors initially provoke enhanced mesocorticolimbic activity, with DA depletion becoming evident with more protracted stressor exposure (e.g., Sorg *et al.*, 1993; Puglisi-Allegra *et al.*, 1992; Imperato *et al.*, 1991). Opiate antagonists have been found to block both the initial increases in DA metabolism as well as subsequent decreases in DA activity induced by stressors. For example, systemic administration of naloxone has been observed to attenuate the footshock induced increase in DOPAC and HVA in the prefrontal cortex (Miller *et al.*, 1984), while intra-VTA administration of naltrexone prevents footshock provoked increases in DOPAC in the prefrontal cortex and nucleus accumbens (Kalivas *et al.*, 1987a). Other investigators have reported that naloxone does not antagonize the increase in DOPAC and HVA in the nucleus accumbens of mice restrained for two hours, but it does attenuate subsequent decreases in nucleus accumbens 3-MT levels (Cabib *et al.*, 1989). Accordingly, endogenous opioid release may contribute to the adaptive phase of increased DA activity which promotes behavioral coping, as well as to the phase of decreased DA release observed with more protracted stressors which is associated with behavioral pathology. Indeed, it has been suggested that the release of endogenous opiate peptides during the initial phases of stressor exposure may interfere with the development of neurochemical alterations associated with the expression of behavioral pathology (Tanaka *et al.*, 1989). Accordingly, the augmentation of opioids during acute stressor exposure may promote adaptive increases in mesocorticolimbic DA activity which permit behavioral coping. However, with protracted, uncontrollable stressor exposure, excessive release and utilization of central DA may result in behavioral pathology.

Stressor related interactions between endogenous opioids and mesocorticolimbic DA activity may have important implications for reward processing, as reflected by region specific stressor provoked alterations in brain stimulation performance. The stressor provoked release of met-enkephalin within the A10 region appears to be limited to the ventromedial aspects of the VTA (Kalivas *et al.*, 1987). Anatomical data have further

demonstrated that enkephalin containing fibers may be more conspicuous in the ventral aspects of the A10 area (Fallon *et al.*, 1990; Pollard *et al.*, 1989). In addition, the most dense distribution of μ receptors has been noted immediately dorsolateral to the interpeduncular nucleus, corresponding to the ventral-caudal region of the A10 area (German *et al.*, 1993). Accordingly, region specific enkephalin activity may contribute to the regional variations in DA activity within the nuclei of the A10 region observed following stressor exposure (Deutch *et al.*, 1991), as well as regional VTA differences with respect to stressor provoked alterations in responding for brain stimulation (Zacharko *et al.*, 1990b). Considering stressor exposure does not decrease self-stimulation performance from the ventral VTA, it is possible that a stressor provoked augmentation of enkephalin release prevents neurochemical perturbations which permit the expression of behavioral pathology. In support of this proposition, the administration of DALA prior to stressor exposure has been found to attenuate disruptions in brain stimulation from the dorsal A10 region (Wolfe *et al.*, 1990), an area in which endogenous enkephalin activity is relatively sparse. By augmenting endogenous opiate levels in the dorsal A10 region, the initial effects of the stressor appear to be blunted and the more long term behavioral repercussions are largely attenuated (i.e., self-stimulation decrements are absent 24-hours and 168-hrs post-stressor). Accordingly, enkephalin may interfere with the cascade of neurochemical events promoting reward alterations at the level of the dorsal A10 area. In addition, DALA has also been found to reverse stressor induced alterations in brain stimulation when administered following footshock exposure, possibly by enhancing DA neural activity which is reduced following protracted stressor exposure (Maddeaux *et al.*, 1992).

While the dorsal A10 region appears to be more vulnerable to the detrimental effects of stressors, pathology can also be induced in the ventral A10 area with a combination of footshock and exogenous opioid administration. The intraventricular administration of DALA prior to stressor exposure has been found to result in substantial decreases in brain stimulation performance from the ventral A10 region (Wolfe *et al.*, 1991b). In view of the

conspicuous distribution of enkephalin within the ventral A10 region, the superimposition of exogenous opioid receptor stimulation on the endogenous release of enkephalin may diminish midbrain DA activity and reduce brain stimulation performance. Accordingly, resistance to behavioral pathology appears to be related to the margin of activation of endogenous opioid-DA activity. The extent to which the modulatory role of opioids on stressor provoked alterations in reward processing at the level of the A10 region is attributable to the activation of μ or δ receptors is relatively unknown.

In summary, stressors promote variations in endogenous opioid activity in several brain regions, including the A10 nuclei. Stressor provoked decreases in enkephalin activity have been associated with behavioral pathology while opioid augmentation prior to or following stressor exposure appears to antagonize stressor related performance deficits. However, pretreatment with opioid antagonists also prevents behavioral deficits, as well as stressor related DA variations. Accordingly, stressor induced opioid release may contribute to adaptive increases in DA activity as well as the DA reductions associated with pathology. Region specific enkephalin activation at the level of the A10 area may mediate, at least in part, region specific brain stimulation deficits induced by uncontrollable stressors.

Summary

Stressor induced behavioral deficits in infrahuman subjects are accompanied by region specific alterations in DA activity within the mesocorticolimbic system. The mesocorticolimbic system plays a critical role in mediating the rewarding properties of appetitively motivating stimuli, and accordingly, stressor related variations in central DA activity may mediate the anhedonic aspects of depressive illnesses. An interaction between endogenous opioids and DA at the level of the A10 area may mediate stressor provoked region specific alterations in responding for previously rewarding brain stimulation from the VTA. Stressor related decreases in self-stimulation performance are observed from dorsal regions of the A10 area in which there is minimal enkephalin activity while brain

stimulation deficits are not observed from the ventral A10 region in which there is a more conspicuous distribution of enkephalin. The exogenous administration of the mixed μ and δ receptor agonist DALA has previously been shown to attenuate reductions in responding for brain stimulation from the dorsal A10 region associated with stressor exposure. However, the extent to which activation of μ and δ receptors at the level of the VTA contributes to the attenuation of stressor provoked disruptions in brain stimulation is unclear. The following experiments evaluated whether selective μ and δ receptor agonists differentially modulate stressor induced alterations in reward processing from the dorsal and ventral aspects of the A10 region. The relatively unknown role of the adjacent interpeduncular nucleus in reward processing, in which there is a dense distribution of both μ and δ receptors, was also examined. In addition, the extent to which μ and δ receptor activation modulates stressor related variations in subcomponents of motoric behavior (e.g., activity and rearing) was assessed to examine a broader spectrum of behaviors associated with stressor exposure and intraventricular opioid administration.

GENERAL METHODS

Subjects

Two hundred and fifty two, naive, male, CD-1 mice, obtained at five weeks of age from Charles Rivers (St. Constant, Quebec) were used as subjects. The mice were initially housed in groups of five in polypropylene cages and allowed food and water ad libitum.

Surgery

After approximately two months of acclimatization to the laboratory, mice were anesthetized with sodium pentobarbital (Somnotol; 65 mg/kg) and implanted with a 0.15 mm bipolar stimulating electrode (Plastic Products, Roanoke, VA.) using a David Kopf Micromanipulator. Electrodes were placed in (a) the dorsal VTA (A.P. -2.9 to 3.0 mm from Bregma, L. +0.5 mm from the sagittal suture and V. -4.70 mm from a flat skull surface), (b) the ventral VTA (A.P. -2.9 to 3.0 mm from Bregma, L. +0.4 mm from the sagittal suture and V. -4.85 mm from a flat skull surface), or (c) the interpeduncular nucleus (A.P. -3.0 mm from Bregma, L. +0.1 mm from the sagittal suture and V. -4.9 mm from a flat skull surface). Animals were also implanted with a cannula in the lateral ventricle, ipsilateral to the electrode site (A.P. +0.8 mm from Bregma, L. +0.7 mm from the sagittal suture and V. -2.8 mm from a flat skull surface). The cannulae were constructed from 23 gauge hypodermic needles (Becton-Dickson; Rutherford, NJ) and cut to a length of 1.1 cm. To maintain the patency of the cannulae, stilletes fashioned from 30 gauge hypodermic needles (Becton-Dickson) cut to a length of 1.5 cm were employed.

Apparatus

Self stimulation testing was conducted in nine white polyethylene tubs (30 cm diameter x 24 cm). The floor of each tub contains a 2 cm diameter well positioned in the center of the self-stimulation chamber. Head dipping through a distance of 1 cm interrupts an infrared photobeam, resulting in the delivery of biphasic square wave stimulation. Brain

stimulation was delivered by a constant current stimulator through a mercury swivel commutator.

Locomotor activity was assessed in three black circular tubs (28 cm diameter x 32 cm high) with grey granulated polyvinylchloride floors. Locomotor activity was monitored by an array of photobeams mounted 0.5 cm above the tub floor and recorded via a Macintosh Plus microcomputer. Rearing was recorded by a second set of photobeams positioned 7 cm. above the floor of the locomotor tubs.

Uncontrollable footshock was delivered in black Plexiglas boxes measuring 30 x 40 x 15 cm. The floor of each box is comprised of 0.32 cm stainless steel rods spaced 1.0 cm apart, connected in series by neon bulbs. The end walls of each chamber are lined with stainless steel plates connected in series with the grid floor. Footshock (150 μ A, 60 x 6 sec) was delivered by a microcomputer controlled 3000-V source (Carleton University Science Technology Center) providing relatively constant current.

Drugs were administered intraventricularly, employing a 30 gauge injector (Becton-Dickson) inserted to a depth of 0.5 mm below the guide cannula tip. The microinjector was connected via polyethylene tubing to a Hamilton 1 μ l syringe, attached to a bar over the top of each self stimulation tub. Drug or vehicle was injected in a 1 μ l volume over a one minute period. The microinjector was left in place for an additional 1 minute period to ensure adequate intracerebral drug diffusion. Intraventricular administration of both μ and δ ligands has been noted to result in homogeneous drug diffusion throughout the brain (Meucci *et al.*, 1989).

Histology

Following completion of behavioural testing, animals were sacrificed with an overdose of sodium pentobarbital and perfused intracardially with physiological saline followed by a 10% formalin solution. The entire assembly was kept in formalin for an additional 3 day interval prior to electrode removal. The brains were subsequently removed

from the formalin solution, blocked in the appropriate plane and frozen on a microtome. Every fifth section (40 μ) was mounted and stained with cresyl violet to aid in visualization of electrode placements.

Preliminary Analyses

Ten animals with electrodes in the dorsal A10 region were administered an intraventricular injection of DAGO (1.0 μ g) or DPDPE (1.0) prior to being placed in the No Shock apparatus for a 66 minute period. The dose of 1.0 μ g was the same dose which was previously utilized in examining the influence of DALA on stressor provoked brain stimulation performance deficits from the dorsal A10 area (see Maddeaux *et al.*, 1991; Wolfe *et al.*, 1990). Responding for brain stimulation was assessed for a 45 minute test session immediately following exposure to the No Shock condition. The aim of the preliminary analyses was to establish whether a 1.0 μ g dose of DAGO or DPDPE influenced brain stimulation in the absence of shock. Only DAGO was observed to provoke a marked decrease in self-stimulation performance. The lower doses of .5 (n=4), .10 (n=4) and .01 μ g of DAGO (n=5) were then tested in a similar procedure. The doses of .5 and .10 μ g of DAGO were also noted to decrease ICSS in the absence of shock while the dose of .01 μ g had minimal effects. The dose of 1.0 μ g of both DAGO and DPDPE were employed in the following experiments in order to make comparisons with previous experiments utilizing a 1.0 μ g dose of DALA (Maddeaux *et al.*, 1992; Wolfe *et al.*, 1990). In addition, the dose of .01 μ g of DAGO was utilized to determine the modulatory effect of a dose of DAGO which had minimal effect on brain stimulation performance from the dorsal VTA in the absence of shock, as previously noted with a 1.0 μ g dose of DALA

EXPERIMENT 1:

Locomotor Alterations Associated with Uncontrollable Footshock and Intraventricular Enkephalin Administration

It has been consistently demonstrated that central administration of opioid agonists provokes alterations in locomotor activity. Intra-VTA or intraventricular injections of mixed μ/δ receptor ligands (e.g., morphine), selective μ receptor agonists (e.g., DAMGO), or selective δ receptor agonists (e.g., DPDPE, DSLET or BUBU) produce an increase in locomotor activity (Badiani *et al.*, 1995; Meyer *et al.*, 1995; Druhan *et al.*, 1993; Michael-Titus *et al.*, 1991; Calenco-Choukroun *et al.*, 1991a; Mickley *et al.*, 1990). Intra-VTA administration of kelatorphin or thiorphin also produces a naloxone or naltrexone reversible increase in locomotor activity (Dauge *et al.*, 1992; Calenco-Choukroun *et al.*, 1991a; Kalivas *et al.*, 1986). Enhanced locomotor activity following opioid administration appears to coincide with the release of DA in the nucleus accumbens (Devine *et al.*, 1993; Spanagel *et al.*, 1992). In addition, the locomotor stimulating effects of opioid analogues and enkephalinase inhibitors are attenuated by 6-OHDA lesions of the nucleus accumbens (Calenco-Choukroun *et al.*, 1991b) or systemic administration of D1 or D2 receptor antagonists (Zarrindast *et al.*, 1992). Accordingly, the locomotor enhancing influence of exogenously administered opioids and endogenously released enkephalins appears to involve modulation of DA transmission within the mesocorticolimbic system.

The locomotor stimulating effects of selective μ and δ receptor agonists appear to differ in several respects. Intra-VTA or intra-nucleus accumbens administration of low doses of μ agonists, including morphine, DAGO, and DAMGO, provokes bursts of locomotion, grooming and rearing. In contrast, moderate doses of μ receptor agonists promote biphasic behavioral change, characterized by a period of behavioral inhibition followed by locomotor stimulation. Higher doses produce catalepsy, characterized by freezing behaviors and muscular rigidity (Bechara *et al.*, 1992; Cunningham *et al.*, 1992; Dauge *et al.*, 1989; 1988; Michael-Titus *et al.*, 1989; Gaetano *et al.*, 1987). In contrast to μ

agonists, intra-VTA or intra-nucleus accumbens administration of the selective δ receptor agonists, DPDPE and DTLET, stimulates locomotor activity across a wide range of doses (Michael-Titus *et al.*, 1989; Dauge *et al.*, 1989; 1988). The locomotor alterations induced by δ receptor agonists also appear to be qualitatively different from that observed with μ agonist or mixed δ and μ receptor ligands. For example, intra-VTA or intra-nucleus accumbens application of DPDPE enhances horizontal and vertical activity (i.e., rearing) while DAGO appears to enhance horizontal activity but suppress rearing (Meyer *et al.*, 1995; Churchill *et al.*, 1992; Cunningham *et al.*, 1992; Calenco-Choukroun *et al.*, 1991a; Mickley *et al.*, 1990; Michael-Titus *et al.*, 1989). Accordingly, locomotor alterations are induced by both μ and δ receptor stimulation, although different components of locomotor behavior are modulated depending upon the dose employed as well as the specific opioid receptor subtype which is activated.

In view of the observations that augmentation of endogenous enkephalin levels induced by central enkephalinase administration or stressor exposure elicits a naloxone reversible enhancement in mesocorticolimbic DA release and locomotor activity (see Dauge *et al.*, 1992; Kalivas *et al.*, 1987a; 1986), an interaction between mesencephalic DA activity and endogenous enkephalins may contribute to the expression of stressor related behavioral alterations. Relatively few studies have examined variations in the different subcomponents of locomotor activity following exposure to uncontrollable stressors which may be dependent upon alterations in mesolimbic DA and/or enkephalin release. In addition, the potential influence of exogenous opioid administration in modulating locomotor variations provoked by stressor exposure remains largely unexamined. As the modulatory influence of opioids on stressor related reward variations will be evaluated in Experiments 2-4, it is of interest to assess a broader spectrum of behavioral alterations associated with uncontrollable footshock, as well as the influence of intraventricular opioid administration on stressor related behavioral change. It is conceivable that the motoric variations and reward/motivational alterations associated with uncontrollable footshock may be

differentially amenable to opioid administration. Accordingly, the purpose of Experiment 1 was to assess (a) the extent to which uncontrollable footshock influences horizontal and vertical locomotion (i.e., activity and rearing) in CD-1 mice, (b) the effects of intraventricular administration of the mixed μ/δ receptor ligand, DALA, and the selective μ and δ receptor agonists, DAGO and DPDPE respectively, on activity and rearing, and (c) whether μ and/or δ receptor activation prior to stressor administration modulates footshock induced variations in different components of motor activity.

Procedure

Sixty animals which had been implanted with a cannula in the lateral ventricle were used as the subjects in Experiment 1. Following a 10 day postoperative recovery period, mice were placed in the locomotor tubs for 45 minutes per day and levels of activity and rearing were recorded at three minute intervals. Animals were tested for three to six days, until stable levels of activity were attained (at least three consecutive days with less than 10% variation). Mean activity and rearing scores were calculated using the data from the three days in which stable activity rates were obtained, forming the baseline for each of the animals. The animals were then matched according to baseline locomotor rates and subdivided into five groups ($n=12/\text{group}$). The mice were administered DALA (1.0 μg), DAGO (.01, 1.0 μg), DPDPE (1.0 μg) or Vehicle into the lateral ventricle. The animals were then further subdivided ($n=6/\text{group}$) such that half were exposed to 60 inescapable footshocks while the remaining mice were placed in an identical shock apparatus for an equivalent period of time but shock was withheld. Immediately following the stressor session, mice were returned to the locomotor tubs and tested for a 45 minute session in which activity and rearing rates were recorded at three minute intervals. Activity and rearing were again assessed for a 45 minute period 24-hours and 168-hours following the initial stressor exposure.

Results

Cannula placements for each of the mice used in Experiment 1 were histologically verified. Data for any animals exhibiting severe tissue displacement or necrosis were excluded from the data analyses.

Baseline Activity: An analysis of variance of Baseline locomotor activity revealed that the ten groups of mice ($n=6/\text{group}$) subsequently assigned to the Drug and Shock conditions did not differ in activity levels (see Appendix A). A significant main effect for Time was observed, $F(14,700) = 77.02$, $p < .001$. Newman Keuls multiple comparisons revealed that baseline activity rates declined progressively across the first 15 minutes of testing then remained at a stable level across the final 30 minutes (see Figure 1a).

 Insert Figure 1a about here

Post-Shock and Drug Activity: An analysis of variance of locomotor activity following Shock and Drug administration revealed a Day $\times$ Shock, $F(2,100) = 10.25$, $p < .001$, a Day $\times$ (Shock $\times$ Drug), $F(8,100) = 2.30$, $p < .05$, a Day $\times$ Time, $F(28, 1400) = 1.84$, $p < .01$, and a (Day $\times$ Time) $\times$ Shock interaction, $F(28,1400) = 1.51$, $p < .05$ (see Appendix A). Newman Keuls multiple comparisons of the simple effects associated with the Day $\times$ (Shock $\times$ Drug) interaction revealed that on the Immediate test day, Shocked animals pretreated with Vehicle exhibited a decrease in activity relative to the Non Shocked Vehicle treated animals. Mice administered DPDPE prior to footshock exhibited lower rates of activity relative to non-stressed mice treated with DPDPE or Vehicle. Shocked animals which were administered DALA showed a moderate, but non-significant decrease in activity relative to Non Shocked mice treated with DALA or Vehicle. Non-stressed subjects pretreated with 1.0 ug of DAGO exhibited a significant increase in locomotor activity on the

Figure 1a. Mean ($\pm$ S.E.M) Baseline locomotor activity in CD-1 mice. Measures of locomotor activity were obtained at three minute intervals over a 45 minute period.

Immediate test day relative to the remaining drug conditions, while activity in Shocked animals administered 1.0 ug of DAGO was comparable to Non Shocked Vehicle treated subjects. Shocked animals treated with the .01 ug dose of DAGO exhibited comparable activity scores to Non Shocked animals treated with the .01 ug dose of DAGO or Vehicle. No difference was observed in activity levels between Shocked and Non Shocked animals at the 24-hour and 168-hour test intervals (see Figure 1b). Activity scores collapsed across the 15 time intervals are presented in Figure 1c to depict the Day x (Shock x Drug) interaction.

 Insert Figures 1b-1c about here

Newman Keuls multiple comparisons of the simple main effects associated with the (Day x Time) x Shock interaction revealed that at the Immediate test day, activity scores were comparable between the Shocked and Non Shocked animals only during the initial three minutes of testing. Over the remaining 42 minutes of the test session, activity scores were lower in mice exposed to uncontrollable footshock relative to non-stressed animals. It should be noted that while the (Day x Time) x (Shock x Drug) interaction failed to reach an appropriate level of statistical significance ($p = .067$), the (Day x Time) x Shock interaction was primarily attributable to the increase in activity observed in non-stressed animals treated with 1.0 ug of DAGO. At the 24-hour and 168-hour test intervals, no differences were observed in locomotor activity as a function of time between stressed and non-stressed mice (see Figure 1b).

Baseline Rearing: An analysis of variance of Baseline rearing revealed that the 10 groups of mice ($n = 5/\text{group}$) did not differ in rearing scores prior to the Shock or Drug manipulations (see Appendix B). It should be noted that the experimental groups were reduced from six to

Figure 1b (pages 69-70). Mean ($\pm$ S.E.M) activity scores in CD-1 mice administered saline, DALA (1.0 ug), DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) intraventricularly and exposed to an acute session of uncontrollable footshock or the No Shock condition. Measures of locomotor activity were obtained at three minute intervals over a 45 minute period immediately following footshock and again 24-hours and 168-hours following stressor exposure.

IMMEDIATE

24 HOURS

168 HOURS

SHOCK
NO SHOCK

VEHICLE 69

ACTIVITY / 3 MIN.

DALA (1.0 UG)

ACTIVITY / 3 MIN.

DPDPE (1.0 UG)

ACTIVITY / 3 MIN.

DAGO (.01 UG)

ACTIVITY / 3 MIN.

DAGO (1.0 UG)

ACTIVITY / 3 MIN.

Figure 1c. Mean ($\pm$ S.E.M) activity scores per three minute interval in CD-1 mice administered saline, DALA (1.0 ug), DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) intraventricularly and exposed to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the 15 time intervals to depict the Day x (Shock x Drug) interaction.

five animals per cell in order to obtain equivalent rates of rearing across the groups, considering it was not always possible to adequately match animals according to both baseline activity and rearing scores. A significant main effect for Time was detected, $F(14, 560) = 3.22$, $p < .001$, although no consistent pattern of change across the 15 three minute intervals was apparent. While an increase in rearing was noted over the initial six minutes of testing, only minor behavioral fluctuations were detected during the remaining test intervals (see Figure 1d).

 Insert Figure 1d about here

Post-Shock and Drug Rearing: An analysis of variance of rearing scores following Shock and Drug administration revealed a main effect for Time, $F(14, 560) = 3.24$, $p < .001$, and a Day x Shock interaction, $F(2, 80) = 8.35$, $p < .001$ (see Appendix A). Newman Keuls multiple comparisons associated with the Time effect revealed that rearing declined over the first twelve minutes of testing, followed by lower but stable rates of rearing during the ensuing portion of the behavioral test session. Multiple comparisons of the simple effects associated with the Day x Shock interaction revealed that at the Immediate test day, Shocked animals exhibited lower rates of rearing relative to Non Shocked animals irrespective of Drug condition. At the 24-hour and 168-hour test intervals, no significant difference was observed between the rearing scores among Shocked and Non Shocked mice (see Figure 1e). Rearing scores collapsed across the 15 time intervals are presented in Figure 1f to depict the Day x Shock interaction.

 Insert Figures 1e to 1f about here

Figure 1d. Mean ($\pm$ S.E.M) Baseline rearing in CD-1 mice. Rearing scores were obtained at three minute intervals over a 45 minute period.

Figure 1e (pages 77-78). Mean ($\pm$ S.E.M) rearing scores among CD-1 mice administered saline, DALA (1.0 ug), DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) intraventricularly and exposed to an acute session of uncontrollable footshock or the No Shock condition. Rearing scores were obtained at three minute intervals over a 45 minute period immediately following footshock and again 24-hours and 168-hours following stressor exposure.

163 HOURS

SHOCK
NO SHOCK

REARING / 3 MIN.

REARING / 3 MIN.

REARING / 3 MIN.

DAGO (.01 UG)

REARING / 3 MIN.

DAGO (1.0 UG)

REARING / 3 MIN.

Figure 1f. Mean ($\pm$ S.E.M) rearing per three minute interval in CD-1 mice administered saline, DALA (1.0 ug), DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) intraventricularly and exposed to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the 15 time intervals to depict the Day x Shock interaction.

In summary, mice pretreated with Vehicle or 1.0 ug of DPDPE and exposed to uncontrollable footshock exhibited a decrease in activity and rearing immediately following stressor exposure. Stressed animals treated with 1.0 ug of DALA exhibited a decrease in rearing but more moderate reductions in activity. Mice treated with either 1.0 ug or .01 ug of DAGO prior to footshock showed a decrease in rearing but not activity. Intraventricular opioid administration did not influence activity or rearing scores in non-stressed animals with the exception of the 1.0 ug dose of DAGO which induced a transient period of hyperactivity.

Discussion

Consistent with recent investigations in this laboratory (e.g., Mendella, 1994), uncontrollable footshock was noted to provoke reductions in both locomotor activity and rearing in vehicle treated CD-1 mice immediately following stressor exposure. It should be noted that in at least some instances, failure to detect changes in locomotor activity following uncontrollable footshock have been reported (Dickson, 1993; Zacharko *et al.*, 1983). Apparent discrepancies in the elicitation of locomotor alterations may be accounted for, at least in part, by procedural variations. For example, in the investigation of Dickson (1993) drug administration and handling occurred following footshock exposure, while Zacharko *et al* (1983) assessed locomotor activity during the period corresponding to self-stimulation responding from the medial forebrain bundle. Accordingly, the interactive influence of handling and the time at which intraventricular drugs are administered relative to stressor imposition, as well as the impact of external stimuli, may influence the expression of stressor related changes in locomotor activity.

Mesolimbic DA activity has been implicated as playing a key role in the expression of locomotor activity. Pharmacological manipulations which enhance mesolimbic DA turnover, including intraventricular, intra-nucleus accumbens, or intra-VTA administration of direct DA receptor agonists (e.g., apomorphine), psychomotor stimulants, and opioids appear to facilitate locomotor activity (see Badiani *et al.*, 1995; Meyer *et al.*, 1995; Phillips

et al., 1994; Geoffroy *et al.*, 1993; Le Moal *et al.*, 1991). A depletion or reduction in central DA functioning following administration of 6-OHDA, α -methyl-para-tyrosine, or DA receptor antagonists (e.g., SCH 23390) blocks the locomotor enhancing influence of opioids and psychomotor stimulants (Miller *et al.*, 1990). Accordingly, assessment of locomotor activity has been employed as a behavioral assay of increased or decreased central DA activity (discussed in Phillips *et al.*, 1994). In this respect, the decrease in locomotor activity and rearing noted in the present investigation immediately following exposure to uncontrollable footshock may reflect a stressor induced decline in central DA release and/or turnover. Consistent with such a proposition, a number of performance deficits observed following stressor exposure, including shuttle escape deficits (see Anisman *et al.*, 1990), and forced swim induced immobility (Imperato *et al.*, 1994; Rosetti *et al.*, 1993), have also been associated with reductions in mesolimbic DA activity.

The extent to which stressor associated reductions in locomotor activity and rearing reflect changes in the motivational aspects of behavior rather than motoric deficits is difficult to determine. Locomotor activity is a fundamental component of both appetitively and aversively motivated behaviors (see Vaccarino *et al.*, 1994; Katz, 1988, Mogenson *et al.*, 1980). Accordingly, locomotor activity may well reflect the emotive aspects of behavior (i.e., the motivation to engage in a behavior) as well as the motoric capabilities of the organism. The emotive and motoric components of locomotor activity are interrelated and both are influenced by manipulations of central DA activity, particularly at the level of the nucleus accumbens. It has been suggested that the nucleus accumbens serves as a forebrain-midbrain interface between processes associated with the initiation of goal directed behavior and the extrapyramidal motor system which provides specific motor responses to environmental stimuli (see Mogenson *et al.*, 1993; 1980, for reviews). The nucleus accumbens receives direct and indirect innervation from central sites associated with the initiation of motivated behavior (e.g., food procurement, attack, sexual activity), including the prefrontal cortex, thalamus, limbic forebrain structures (e.g., hippocampus,

amygdala, septum), and the A10 region. The nucleus accumbens also provides direct and indirect innervation to structures involved in the execution of motor output (e.g., cerebral cortex, substantia nigra, globus pallidus, striatum), by virtue of reciprocal interconnections with the ventral pallidum (Churchill *et al.*, 1994; Klitenick *et al.*, 1992b; Mogenson *et al.*, 1980). In light of the role of mesolimbic DA functioning in both motivation and motor output, it is difficult to differentiate the extent to which the behavioral disturbances associated with stressor related reductions in mesolimbic DA activity (e.g., shuttle escape deficits) represent a disturbance in response initiation and/or the motoric capabilities of the organism to maintain vigorous responding (see Imperato *et al.*, 1994; Rosetti *et al.*, 1993; Anisman *et al.*, 1990). Similarly, it is not readily apparent whether the decrease in activity and rearing observed following uncontrollable footshock exposure represents a disturbance in the motivational state of the animals, the motoric components of motivated behavior, or both.

While not dismissing the role of multiple neurotransmitters and neuropeptides in modifying locomotor behavior, an interaction between mesolimbic DA and GABA containing neurons may play a prominent role in the stressor related expression of locomotor variations. Reciprocal GABAergic interconnections are apparent between the nucleus accumbens and the ventral pallidum (Churchill *et al.*, 1994; Klitenick *et al.*, 1992b), and descending GABAergic projections from the ventral pallidum and nucleus accumbens innervate the VTA (Kalivas *et al.*, 1992; Zahm *et al.*, 1989). Administration of the GABA_B agonists, 3-aminopropane sulphonic acid and baclofen, into the nucleus accumbens decreases locomotor activity (Wong *et al.*, 1991). Ventral pallidum injections of GABA_A receptor agonists antagonize the locomotor stimulating properties of intra-nucleus accumbens administration of DA (see Churchill *et al.*, 1994, for review). Injection of baclofen into the VTA, which reduces DA release in the nucleus accumbens, also prevents novelty induced locomotor activation (Hooks *et al.*, 1995). In contrast to GABA agonists, administration of the GABA antagonist, picrotoxin, to the nucleus accumbens or ventral

2

PM-1 3 1/2" x 4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGET

pallidum has been observed to enhance locomotor activity (see Klitenick *et al.*, 1992a; Wong *et al.*, 1991; Austin *et al.*, 1991). Taken together, inhibition of a tonically active GABA projection from the nucleus accumbens to the ventral pallidum enhances mesolimbic DA activity and promotes locomotor activation (e.g., Bourdelais *et al.*, 1992). Conversely, a deficiency of mesolimbic DA release may result in disinhibition of GABA neurons projecting to the ventral pallidum, resulting in reduced locomotor behavior (see Mogenson *et al.*, 1980). Indeed, electrolytic lesions of the VTA which deplete mesolimbic DA levels are associated with enhanced GABA activity within the nucleus accumbens (Retaux *et al.*, 1994) and intra-nucleus accumbens application of D2 receptor antagonists increases Fos expression in accumbal neurons which innervate the ventral pallidum (Robertson *et al.*, 1995). Therefore, it is conceivable that a stressor provoked decrease in mesolimbic DA release may result in enhanced GABAergic release from nucleus accumbens efferents, contributing to the footshock related decline in locomotor activity which was observed in Experiment 1.

It should also be considered that stressor provoked decreases in mesolimbic DA activity may be secondary, as well as a contributing factor, to enhanced GABA activity. As will be discussed in more detail in Experiment 2, increased DA release within the nucleus accumbens may result in inhibitory GABA mediated feedback to the A10 area from the nucleus accumbens and/or ventral pallidum (Robertson *et al.*, 1995; Kalivas *et al.*, 1993; Heimer *et al.*, 1991). Inhibition of A10 DA neurons by descending GABA afferents to the VTA may have the net effect of disinhibiting GABA neurons projecting from the nucleus accumbens to the ventral pallidum. Accordingly, while enhanced GABAergic activity within the neural circuit containing the VTA, ventral pallidum and nucleus accumbens may promote reductions in locomotor activity, it is not readily apparent whether GABAergic variations are secondary to and/or a contributing factor to diminished mesocorticolimbic DA activity.

While behavioral changes following pharmacological manipulations of central GABA turnover support the proposition that GABA alterations modulate the expression of locomotor activity, footshock induced changes in GABA availability at the level of the A10 area or nucleus accumbens which may contribute to motoric variations remain to be evaluated. However, reductions in locomotor activity following ether or cold exposure have been associated with enhanced GABA concentrations in at least some brain regions, including the hypothalamus and frontal cortex (Acosta *et al.*, 1994). Accordingly, while speculative, a similar increase in GABA release within the A10 region, nucleus accumbens and/or ventral pallidum may be associated with stressor provoked decreases in locomotion. Moreover, in view of the observations that (a) stressor exposure provokes the release of met-enkephalin within the mesocorticolimbic system, and (b) enkephalins modulate DA activity through the inhibition of GABA release, it is conceivable that stressor related release of endogenous enkephalins may contribute to DA and/or locomotor variations through an interaction with central GABA neurons.

Intraventricular administration of the mixed μ and δ receptor agonist, DALA, in Experiment 1 was associated with a partial attenuation of the stressor related decrease in locomotor activity while administration of the selective μ receptor agonist, DAGO, abolished the reduction in activity following footshock exposure. In contrast, administration of the selective δ receptor agonist, DPDPE, had no effect on the stressor provoked decrease in activity. Accordingly, μ rather than δ receptor activation appears to play a role in the stressor related modulation of midbrain DA activity which contributes to changes in locomotor activity. If footshock provoked reductions in locomotor activity are associated with enhanced GABAergic activity in the nucleus accumbens, ventral pallidum and/or the A10 region, μ receptor activation by DAGO may have antagonized stressor related locomotor changes by inhibiting GABA activity. In contrast, DPDPE may have been ineffective in modifying the stressor related decline in locomotor activity owing to a lack of effect of δ receptor activation on GABA neurons within the mesocorticolimbic

system (e.g., Johnson *et al.*, 1992b). It should be emphasized that 1.0 μ g of DPDPE is well within the range of doses which facilitate mesocorticolimbic DA activity (see Devine *et al.*, 1993a). Accordingly, while a 1.0 μ g dose of DPDPE is sufficient to activate mesolimbic DA neurons, it appears to be ineffective in antagonizing footshock induced reductions in locomotor activity when administered prior to stressor application. These data suggest that μ and δ receptors may be linked with different neural circuits which interact with mesencephalic DA neurons during stressor exposure. In effect, opioid receptor subtypes may be differentially involved in the expression of specific stressor associated behavioral pathologies.

The higher dose of DAGO utilized in Experiment 1 increased activity in non-stressed mice while producing no change in the locomotor activity of animals exposed to footshock. In view of the observation that footshock enhances the release of met-enkephalin within the mesocorticolimbic system (Kalivas *et al.*, 1987a), cumulative central opioid receptor activation occasioned by the stressor dependent release of endogenous enkephalins and simultaneous administration of DAGO may have precluded the appearance of hyperactivity ordinarily observed following DAGO administration alone. It has previously been reported that DAMGO increases locomotor activity in familiar, non-stressful environments (e.g., housing cage) while decreasing locomotor activity in anxiety provoking environments (e.g., an open field or elevated plus maze) (Calenco-Choukroun *et al.*, 1991a). Similarly, the high dose of DAGO employed in Experiment 1 enhanced activity in non-stressed animals who were habituated to the locomotor testing apparatus while promoting a moderate although non-significant decrease in activity in mice exposed to footshock. Accordingly, the ability of μ receptor ligands to modulate locomotor activity appears to be dependent upon environmental conditions and potentially reflects the degree of activation of endogenous opioid systems.

Although μ receptor activation attenuated stressor related variations in locomotor activity, intraventricularly administered DALA, DAGO or DPDPE was without effect on

the decrease in rearing observed following uncontrollable footshock. Accordingly, different central mechanisms appear to mediate the horizontal and vertical components of locomotion. Activity and rearing may represent different aspects of motivated behavior, with rearing potentially representing a more sensitive index of exploration while activity reflects more general levels of arousal. Indeed, it has been consistently noted that footshock reduces exploratory head dipping as well as rearing in CD-1 mice, while stressor related alterations in general activity appear to vary as a function of the experimental conditions employed (Mendella, 1994; Dickson, 1993; Zacharko *et al.*, 1983). Accordingly, footshock induced reductions in rearing may represent a more sensitive index of alterations in the emotive aspects of motivated behavior relative to locomotor activity.

In summary, uncontrollable footshock provoked a significant decrease in both horizontal and vertical activity among CD-1 mice immediately following stressor exposure. Intraventricular administration of DAGO eliminated the stressor provoked decrease in horizontal activity but had no effect on rearing. In contrast, intraventricular DPDPE administration was without effect on stressor related decreases in activity or rearing. Accordingly, μ and δ receptor agonists produce differential effects on stressor related alterations in motor activity. In view of the observation that μ but not δ receptor stimulation influences GABA activity, a μ receptor-GABA-DA link at the level of the nucleus accumbens, ventral pallidum and/or A10 region may contribute to the modification of stressor related locomotor changes.

EXPERIMENT 2a-2b:

Modification of Stressor Provoked Reward Alterations From The Dorsal A10 Region Following Intraventricular Enkephalin Administration

It will be recalled that the administration of opioid agonists prior to stressor exposure attenuates the development of subsequent behavioral pathologies, including immobility in the Porsolt swim test (Baamonde *et al.*, 1992; de Felipe *et al.*, 1989), conditioned suppression of motility (Katoh *et al.*, 1990), and decreased responding for brain stimulation from the dorsal A10 region (Wolfe *et al.*, 1990). Such behavioral deficits have been associated with alterations in mesocorticolimbic DA activity (Imperato *et al.*, 1994; Rosetti *et al.*, 1993; Zacharko *et al.*, 1990), suggesting opioid agonists may prevent behavioral pathology through a modulation of stressor provoked midbrain DA variations. Both μ and δ receptor agonists enhance mesencephalic DA activity (Devine *et al.*, 1993a, Spanagel *et al.*, 1992), and the endogenous release of met-enkephalin following stressor exposure appears to facilitate mesocorticolimbic DA turnover (Kalivas *et al.*, 1987a). Therefore, the administration of DALA prior to footshock may attenuate reward alterations from the dorsal VTA through a μ and/or δ receptor linked facilitation of DA activity within the dorsal A10 region. Indeed, stressor related reductions in responding for brain stimulation are not observed from the ventral aspects of the VTA (Zacharko *et al.*, 1990) which contains a more conspicuous distribution of enkephalin relative to the dorsal A10 region (Fallon *et al.*, 1990; Pollard *et al.*, 1989). Accordingly, a higher availability of enkephalin within the ventral VTA during stressor exposure may allow for sustained turnover of DA, precluding footshock provoked reductions in brain stimulation evident from the dorsal A10 region.

It is currently unclear whether the ameliorative influence of DALA on stressor provoked brain stimulation performance deficits from the dorsal A10 region is mediated by the activation of μ or δ receptors. The administration of μ or δ receptor ligands appears to be reinforcing (Singh *et al.*, 1994; Devine *et al.*, 1994; Herz *et al.*, 1992; Bals-Kubik *et*

al., 1990a) and both enhance mesocorticolimbic DA activity (Devine *et al.*, 1993a, Spanagel *et al.*, 1992). However, the distribution of μ and δ receptors within the mesencephalon is heterogeneous, with μ receptors predominating in the A10 region (Speciale *et al.*, 1993; German *et al.*, 1993). In addition, activation of μ and δ receptors differentially influences the horizontal and vertical components of locomotor behavior (e.g., Churchill *et al.*, 1992; Cunningham *et al.*, 1992), as well as the degree of mesocorticolimbic DA release (Devine *et al.*, 1993a). As activation of μ or δ receptors differentially modulated stressor related variations in subcomponents of locomotor activity in Experiment 1, μ and δ receptor stimulation may also differentially influence stressor provoked variations in reward processing. Accordingly, Experiment 2a compared the relative efficacy of intraventricular administration of DAGO or DPDPE prior to uncontrollable footshock in attenuating subsequent reductions in responding for brain stimulation ordinarily observed from the dorsal A10 area.

The central administration of DALA has been noted to produce differential effects on the duration of brain stimulation deficits when administered prior to footshock in contrast to administration following the termination of the stressor. Administration of DALA prior to footshock exposure abolishes reductions in self-stimulation performance from the dorsal VTA typically observed one week following stressor exposure (Wolfe *et al.*, 1990). However, DALA administration following completion of the footshock session induces only a transient recovery of responding for brain stimulation and decrements in self-stimulation are again observed 168-hours following stressor application (Maddeaux *et al.*, 1992). In view of the cascading nature of stressor related neurochemical change, distinct neuromodulatory alterations which influence DA activity may emerge over the course of stressor exposure which are more or less amenable to modulation by μ and/or δ receptor stimulation depending on the time at which opioid administration is accomplished.

It will be recalled that GABA and 5-HT may modulate stressor related alterations in A10 DA activity (e.g., Rasmussen *et al.*, 1994; Kalivas, 1993; Klitenick *et al.*, 1992b).

Interestingly, μ receptors are evident on GABA neurons within the A10 region while δ receptors may be present on 5-HT axon terminals within the A10 region (Arvidson *et al.*, 1995; Sesack *et al.*, 1992). Accordingly, DALA administration prior to stressor exposure may influence DA activity through an opioid receptor interface with specific neural systems which may be independent of the opioid receptor link associated with DA modulation following stressor exposure. In this respect, it is conceivable that (1) the efficacy of selective opioid receptor analogues in attenuating brain stimulation performance deficits may depend on whether the opioids are administered prior to or following stressor exposure, and (2) the immediate and long term effects (i.e., Immediate versus 24-hours and 168-hours post-stressor) of uncontrollable footshock on reward processing may be differentially sensitive to activation of μ and δ receptors. Accordingly, Experiment 2b compared the relative efficacy of intraventricular administration of DAGO and DPDPE following exposure to footshock in reversing immediate and long term stressor provoked alterations in responding for brain stimulation from the dorsal A10 region.

Experiment 2A:

Enkephalin Administration Prior to Footshock Exposure

Procedure

Following a 10 day post-operative recovery period, 48 mice with electrodes implanted in the dorsal VTA were trained to respond for brain stimulation for 15 minutes per day, until stable rates of responding were established. The brain stimulation was delivered at a current intensity of 70 μ A, a frequency of 80 Hz, a pulse width of 0.5 msec, and a pulse duration of .001 seconds. Animals were trained to respond for brain stimulation until stable rates of responding were established (at least 3 consecutive days with less than 10% variation). Baseline self-stimulation rates were determined over a 45 minute test session in which responding for brain stimulation was recorded at three minute

intervals. The animals were matched on baseline self-stimulation performance and subdivided into four groups (n=12/group). Mice received a microinjection of DAGO (0.1, 1.0 ug), DPDPE (1.0 ug) or Vehicle into the lateral ventricle. Following intraventricular drug administration, animals were further subdivided (n=6/group) such that half were exposed to 60 inescapable footshocks while the remaining mice were placed in an identical shock apparatus for an equivalent period of time but shock was withheld. Immediately following the stressor session, mice were returned to the self-stimulation tubs and brain stimulation rates were recorded at three minute intervals over a 45 minute period. All mice were subsequently tested for a 45 minute period of brain stimulation 24-hours and 168-hours following the initial stressor exposure.

Results

Cannula and electrode placements for each of the mice used in Experiment 2a were histologically verified. The behavioral data for any animals exhibiting severe tissue displacement, necrosis, or electrode placements outside the dorsal A10 region were excluded from data analyses. Schematic representations of electrode placements within the dorsal VTA are presented in Figure 2a.

 Insert Figure 2a about here

Baseline Self-Stimulation Performance: An analysis of variance of Baseline brain stimulation performance revealed that the eight sub-groups of mice (n=6) subsequently exposed to the Shock and Drug manipulations did not differ with respect to self-stimulation rates. A main effect for Time was observed, $F(14,560) = 14.83$, $p < .001$. Newman Keuls multiple comparisons revealed that Baseline rates of brain stimulation decreased progressively during the initial 15 minute test interval, remained relatively stable across the

Figure 2a. Schematic representation of electrode placements within the dorsal VTA in CD-1 mice used in Experiment 2a (n=48). Owing to the overlap of electrode placements, several of the 48 placements have been omitted.

- = Vehicle
- = DPDPE (1.0 ug)
- = DAGO (.01 ug)
- = DAGO (1.0 ug)

ensuing 35 minute period, and then declined during the final 9 minute test period (see Figure 2b).

 Insert Figure 2b about here

Post-Shock and Drug Self-Stimulation Performance: An analysis of variance of self-stimulation performance following Shock and Drug administration revealed a main effect for Time, $F(14,560) = 30.17$, $p < .001$, and a Day $\times$ Drug interaction, $F(6,80) = 3.05$, $p < .01$ (see Appendix C). Newman Keuls multiple comparisons of the simple effects associated with the Day $\times$ Drug interaction revealed that at the Immediate test day, mice treated with Vehicle or 1.0 ug of DPDPE exhibited depressed rates of responding for brain stimulation relative to animals administered .01 ug of DAGO, while mice treated with 1.0 ug of DAGO exhibited lower rates of brain stimulation than animals treated with Vehicle or 1.0 ug of DPDPE. At the 24-hour and 168-hour test intervals, mice treated with Vehicle continued to show brain stimulation response deficits relative to subjects treated with either dose of DAGO or DPDPE (see Figure 2c).

 Insert Figure 2c about here

Although the Day $\times$ (Shock $\times$ Drug) interaction failed to reach an appropriate level of statistical significance ($p = .19$), multiple comparisons associated with the higher order interaction were conducted to examine the a priori hypotheses that (a) footshock would reduce the brain stimulation performance of mice treated with Vehicle, and (b) stressor related deficits in self-stimulation would be attenuated in mice administered DAGO and/or DPDPE owing to a μ or δ receptor mediated facilitation of DA activity within the A10

Figure 2b. Mean ($\pm$ S.E.M) Baseline self-stimulation rates among CD-1 mice responding for brain stimulation from the dorsal A10 region in Experiment 2a. Rates of responding for brain stimulation were assessed at three minute intervals over a 45 minute period.

Figure 2c (pages 98-99). Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the dorsal A10 region in Experiment 2a following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition. Rates of responding for ICSS were assessed at three minute intervals over a 45 minute period immediately following footshock and again 24-hours and 168-hours following stressor exposure.

DAGO (.01 UG)

ICSS RATE / 3 MIN.

DAGO (1.0 UG)

ICSS RATE / 3 MIN.

region, respectively. Newman Keuls multiple comparisons revealed that at the Immediate test day, Shocked animals pretreated with Vehicle or DPDPE exhibited a decrease in responding for brain stimulation relative to the respective No Shock conditions. Mice pretreated with a .01 ug dose of DAGO and exposed to the Shock or No Shock conditions exhibited brain stimulation rates comparable to the Non Shocked Vehicle treated animals. Both Shocked and Non Shocked animals administered a 1.0 ug dose of DAGO exhibited a decrease in responding for brain stimulation relative to Non Shocked Vehicle treated subjects. At the 24-hour and 168-hour test days, Shocked animals which had been pretreated with Vehicle continued to exhibit depressed rates of responding for brain stimulation, while stressed mice treated with DPDPE or either dose of DAGO exhibited rates of responding for brain stimulation comparable to those of Non Shocked Vehicle treated subjects (see Figure 2c). Mean self-stimulation rates collapsed across the 15 time intervals are presented in Figure 2d to depict the Day x (Shock x Drug) interaction.

 Insert Figure 2d about here

In summary, animals administered vehicle prior to uncontrollable footshock exhibited a significant decrease in responding for brain stimulation from the dorsal A10 region immediately, 24-hours, and 168-hours following stressor exposure. Pretreatment with DPDPE failed to prevent decreases in responding for brain stimulation immediately following uncontrollable footshock, although responding for brain stimulation was again evident at the 24-hour and 168-hour test intervals. Intraventricular administration of .01 ug of DAGO prior to footshock prevented the expression of brain stimulation performance deficits immediately following stressor exposure, as well as at the 24-hour and 168-hours test days. Administration of 1.0 ug of DAGO was associated with brain stimulation performance deficits in both stressed and non-stressed mice on the immediate test day,

Figure 2d. Mean ($\pm$ S.E.M) self-stimulation performance per three minute interval from the dorsal A10 region in mice administered saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) intraventricularly and exposed to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the 15 time intervals to depict the Day x (Shock x Drug) interaction.

although a recovery of responding for brain stimulation was evident at the 24-hour and 168-hour test intervals.

Experiment 2B: Enkephalin Administration Following Footshock Exposure

Procedure

Following the attainment of stable rates of responding for brain stimulation from the dorsal VTA, 34 mice were matched according to baseline self-stimulation performance and subdivided into three groups ($n=12/\text{group}$ for Vehicle and DAGO; $n=10$ for DPDPE). Half of the animals in each group were exposed to 60 inescapable footshocks while the remaining mice were placed in an identical shock apparatus for an equivalent period of time but shock was withheld. Immediately following the stressor session, brain stimulation performance was assessed for a 15 minute period in which self-stimulation rates were recorded at three minute intervals. Mice were then removed from the tubs and immediately injected intraventricularly with DAGO (0.1 ug), DPDPE (1.0 ug) or Vehicle over a one minute period. Following an additional one minute period to allow for adequate intracerebral diffusion of the drugs, the mice were placed back into the self stimulation tubs and retested for 30 minutes. All mice were subsequently tested for a 45 minute period of brain stimulation 24-hours and 168-hours following initial stressor exposure.

Results

Cannula and electrode placements for each of the mice used in Experiment 2b were histologically verified. As before, the behavioral data for any animals exhibiting necrosis or electrode placements outside the dorsal A10 region were excluded from the data analyses. Schematic representations of electrode placements within the dorsal VTA are presented in Figure 2e.

 Insert Figure 2e about here

Baseline Self-Stimulation Performance: An analysis of variance of Baseline brain stimulation rates revealed that the six groups of mice subsequently placed in the Shock and Drug conditions did not differ in self-stimulation performance (see Appendix D). A main effect for Time was observed, $F(14,560) = 14.83$, $p < .001$. Newman Keuls multiple comparisons revealed that Baseline brain stimulation performance decreased progressively during the first 18 minutes of testing, then remained relatively stable across the remaining test session (see Figure 2f).

 Insert Figure 2f about here

Brain stimulation performance following the Shock and Drug manipulations on the Immediate test day were analyzed in two parts, with the first 15 minute interval following exposure to footshock forming the Immediate Pre-Drug analysis and the final 30 minute interval forming the Immediate Post-Drug analysis. The long term effects (i.e., brain stimulation rates at the 24-hour and 168-hour intervals) were analyzed separately.

Immediate Pre-Drug Self-Stimulation Performance: An analysis of variance of brain stimulation rates following footshock but prior to drug administration revealed a main effect for Shock, $F(1,28) = 27.08$, $p < .001$, a Time x Shock, $F(4,112) = 9.46$, $p < .001$, and Time x (Shock x Drug) interaction, $F(8,112) = 2.27$, $p < .05$ (see Appendix D). Newman Keuls multiple comparisons of the simple effects associated with the Time x (Shock x Drug) interaction revealed that the brain stimulation rates of Shocked animals were significantly depressed relative to the respective No Shock groups. However, while

Figure 2e. Schematic representation of electrode placements within the dorsal A10 region in CD-1 mice used in Experiment 2b (n=34). Owing to the overlap of electrode placements, several of the 34 placements have been omitted.

- = Vehicle
- = DPDPE (1.0 ug)
- = DAGO (.01 ug)

Figure 2f. Mean ($\pm$ S.E.M) Baseline self-stimulation rates among CD-1 mice responding for brain stimulation from the dorsal A10 region in Experiment 2b. Rates of responding for brain stimulation were assessed at three minute intervals over a 45 minute period.

depressed responding for brain stimulation was evident three minutes following completion of footshock for mice which were subsequently treated with Vehicle or DAGO in the Post-drug interval, animals subsequently administered DPDPE did not exhibit a significant depression of brain stimulation rates until nine minutes following stressor exposure. However, at the final test interval prior to drug administration (i.e., 15 minutes following footshock), all three Shock groups exhibited equivalent decreases in responding for brain stimulation relative to the No Shock control groups (see Figure 2g).

 Insert Figure 2g about here

Immediate Post-Drug Self-Stimulation Performance: An analysis of variance of brain stimulation performance following Shock and Drug administration revealed a significant main effect for Shock, $F(1,28) = 16.11$, $p < .001$, and a Time x Shock interaction, $F(4,112) = 2.09$, $p < .05$ (see Appendix D). Newman Keuls multiple comparisons associated with the Time x Shock interaction revealed that the Shocked animals exhibited significantly lower rates of responding for brain stimulation relative to Non Shocked animals across the 30 minute test session. While the Shock x Drug interaction failed to reach an appropriate level of statistical significance ($p = .11$), multiple comparisons associated with the Shock x Drug interaction were conducted to evaluate the *a priori* hypotheses that (1) footshock provoked brain stimulation deficits would persist across the 30 minute test session in Vehicle treated animals, and (2) animals treated with .01 ug of DAGO or 1.0 ug of DPDPE would exhibit a recovery of responding for brain stimulation owing to a reactivation of DA activity via μ or δ receptor stimulation, respectively. Newman Keuls multiple comparisons revealed that Shocked animals treated with Vehicle continued to exhibit lower rates of responding for brain stimulation relative to Non Shocked Vehicle treated mice. The brain

Figure 2g. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the dorsal A10 region in Experiment 2b following exposure to an acute session of uncontrollable footshock or the No Shock condition. Rates of responding for brain stimulation were assessed at three minute intervals over a 15 minute period. Animals were then administered saline, DPDPE (1.0 ug), or DAGO (.01 ug) intraventricularly. The time of drug administration is represented by the broken line. Rates of responding for brain stimulation were again assessed at three minute intervals over an additional 30 minute period. Responding for ICSS was reassessed 24-hours and 168-hours following stressor exposure for a 45 minute session.

IMMEDIATE

24 HOURS

168 HOURS

SHOCK
NO SHOCK

VEHICLE 111

ICSS RATE / 3 MIN.

DPDPE (1.0 UG)

ICSS RATE / 3 MIN.

DAGO (.01 UG)

ICSS RATE / 3 MIN.

stimulation rates of Shocked mice administered .01 ug of DAGO intraventricularly were comparable to Non Shocked subjects treated with DAGO or Vehicle. Shocked animals administered DPDPE intraventricularly exhibited comparable rates of brain stimulation relative to the Non Shocked mice treated with DPDPE, but lower brain stimulation rates relative to Non Shocked animals treated with Vehicle. However, no difference was observed between the brain stimulation rates of Shocked animals treated with DAGO or DPDPE (see Figure 2g).

Long-Term Self-Stimulation Performance: An analysis of variance of brain stimulation performance 24-hours and 168-hours following stressor application revealed significant main effects for Shock, $F(1,28) = 6.01$, $p < .05$, and Drug, $F(2,28) = 4.72$, $p < .05$, as well as a Time x Drug, $F(28,392) = 1.66$, $p < .05$, and a Day x Time interaction, $F(14,392) = 1.77$, $p < .05$ (see Appendix D). While the Shock x Drug interaction failed to reach an appropriate level of statistical significance ($p=.08$), the Shock effect was primarily attributable to the reduced rate of brain stimulation observed in Vehicle treated animals. Newman Keuls multiple comparisons associated with the main effect of Drug revealed that animals treated with DPDPE exhibited higher rates of responding for brain stimulation relative to the subjects administered Vehicle or DAGO irrespective of Shock condition. Multiple comparisons associated with the Time x Drug interaction revealed that brain stimulation performance of Vehicle treated mice was depressed relative to DAGO treated animals during the initial 12 minutes of testing, and again at several intervals throughout the test session (e.g., 21, 24, and 42 minutes following the commencement of behavioral testing). In contrast, with the exception of the six minute Time interval, responding for brain stimulation in subjects administered Vehicle was depressed relative to DPDPE treated animals throughout the test sessions. Animals pretreated with DPDPE also exhibited higher rates of self-stimulation relative to DAGO treated mice, with the exception of the initial six minutes of behavioral testing. Mean self-stimulation rates collapsed across Shock and Day are presented in Figure 2h to depict the Time x Drug interaction.

Insert Figure 2h about here

In summary, animals administered Vehicle following uncontrollable footshock exhibited a significant decrease in responding for brain stimulation from the dorsal A10 region immediately, 24-hours, and 168-hours post-stressor. Intraventricular administration of .01 ug of DAGO or 1.0 ug of DPDPE 15 minutes following the termination of footshock provoked a reversal of brain stimulation response deficits. Pretreatment with DAGO partially attenuated stressor related reductions in self-stimulation ordinarily observed 24-hours and 168-hours post-stressor, while DPDPE administration abolished long term brain stimulation performance deficits.

Discussion

In Experiment 2a, intraventricular administration of a low dose of DAGO prior to uncontrollable footshock abolished brain stimulation performance deficits from the dorsal A10 region immediately following stressor exposure, as well as reductions in responding for brain stimulation ordinarily evident 24-hours and 168-hours following footshock. In contrast to the behavioral effects of DAGO on self-stimulation performance, intraventricular DPDPE administration prior to footshock failed to attenuate the immediate decrease in responding for brain stimulation but prevented brain stimulation performance deficits 24-hours and 168-hours following stressor exposure. In Experiment 2b, intraventricular administration of DAGO or DPDPE following exposure to uncontrollable footshock reversed the immediate disruption in responding for brain stimulation from the dorsal A10 area. However, DPDPE abolished the emergence of long term brain stimulation performance deficits while DAGO administration was associated with a more moderate recovery of responding for self-stimulation one week post-stressor. These data suggest that (1) μ receptor activation plays a more prominent role relative to δ receptor stimulation in

Figure 2h. Mean ($\pm$ S.E.M) self-stimulation performance per three minute interval from the dorsal A10 region in mice administered saline, DPDPE (1.0 ug), or DAGO (.01) intraventricularly. The data shown are from the 24-hour and 168-hour test days to represent the long-term influence of opioid administration on footshock induced brain stimulation variations. The data are collapsed across the Shock conditions and the 24-hour and 168-hour test intervals to depict the Time x Drug interaction.

attenuating the self-stimulation deficits which are typically evident from the dorsal A10 region immediately following stressor exposure, (2) the amelioration of long term disruptions in responding for brain stimulation from the dorsal A10 region following footshock appears to be independent of the attenuation of immediate stressor induced brain stimulation performance deficits, and (3) the cascading neurochemical variations associated with the amelioration of long term brain stimulation deficits may be more closely tied to δ relative to μ receptors.

It should be noted that the high dose of DAGO employed in Experiment 2a depressed brain stimulation performance in stressed and non-stressed mice immediately post-stressor. The self-stimulation measures employed in the current investigation preclude a detection of potential shifts in the current threshold necessary to elicit brain stimulation following intraventricular opioid administration. Therefore, a determination of alterations in the reinforcing efficacy of brain stimulation following the superimposition of the high dose of DAGO on stressor exposure is compromised. The high dose of DAGO may have decreased the rewarding properties associated with brain stimulation or produced significant activation of endogenous reward systems, thereby superceding responding for exogenously reinforcing stimuli. However, other investigations utilizing rate-independent paradigms have demonstrated that high doses of DAGO enhance the threshold for self-stimulation from the lateral hypothalamus (e.g., Johnson *et al.*, 1993), suggesting high doses of μ agonists diminish the rewarding efficacy of brain stimulation. In view of the observation that the high dose of DAGO facilitated locomotor activity in Experiment 1, it is also possible that locomotor hyperactivity may have interfered with the ability of mice to attend to brain stimulation cues following drug administration (i.e., the tub well). It should be emphasized that the .01 ug dose of DAGO and the 1.0 ug dose of DPDPE had no detectable effect on brain stimulation performance or locomotor activity in the absence of stressor application, potentially circumventing the aforementioned confounds.

In view of the established role of mesencephalic DA activity in reward/motivational processing, footshock provoked reductions in responding for brain stimulation from the dorsal A10 region in the absence of opioid administration may be associated with diminished mesocorticolimbic DA activity. It will be recalled that uncontrollable stressors provoke a biphasic pattern of change in DA activity within mesocorticolimbic sites. For example, an increase in DA release is observed from the nucleus accumbens and prefrontal cortex within three to five minutes following exposure to uncontrollable footshock or restraint (Cabib *et al.*, 1994; Imperato *et al.*, 1991; Puglisi-Allegra *et al.*, 1991). However, following 30 minutes of restraint or 60 minutes of uncontrollable footshock, DA release within the nucleus accumbens and prefrontal cortex declines below baseline levels as measured by microdialysis and tissue levels of 3-methoxytyramine (Cabib *et al.*, 1994; Imperato *et al.*, 1991; Puglisi-Allegra *et al.*, 1991). Brief periods of tail pinch (e.g., three minutes) have also been observed to enhance DA release within the A10 region as revealed by in vivo voltammetry, with DA release peaking seven minutes after stressor onset then declining to baseline levels (Zhang *et al.*, 1994). It is conceivable that A10 DA activity may also decline below baseline levels with longer durations of stressor exposure in parallel to the pattern of neurochemical change observed within mesocorticolimbic terminal regions. In view of the observation that at least some stressor related behavioral deficits (e.g., shuttle escape deficits, forced swim induced immobility) are associated with decreased mesocorticolimbic DA activity (Imperato *et al.*, 1994; Rossetti *et al.*, 1993; Anisman *et al.*, 1979), a footshock induced decline in the activity of DA neurons originating in the dorsal A10 region may have contributed to the reduction in self-stimulation performance evident in Experiments 2a and 2b.

It has been suggested that stressor related decreases in neurotransmitter turnover may reflect not only a depletion of releasable pools of the transmitter (see Anisman *et al.*, 1992a), but also the inhibitory influence of feedback mechanisms (Cabib *et al.*, 1994; Imperato *et al.*, 1991). Indeed, the enhanced release of DA within mesocorticolimbic

terminal sites associated with stressor exposure may result in inhibitory feedback to the A10 area from brain regions innervated by A10 DA neurons. For example, intra-nucleus accumbens application of D₁ receptor agonists increases Fos immunoreactivity in GABA containing neurons which project from the nucleus accumbens to the VTA (Robertson *et al.*, 1995). In view of the observation that GABA containing neurons from the ventromedial aspects of the ventral pallidum innervate A10 DA neurons (Kalivas *et al.*, 1993; Heimer *et al.*, 1991), enhanced release of GABA from pallidal efferents to the A10 region may also contribute to the inhibition of mesocorticolimbic DA neurons during stressor exposure. The release of GABA from A10 afferents appears to decrease DA cell activity by stimulating GABA_B receptors located on A10 DA cells (Sugita *et al.*, 1992). Feedback regulation from prefrontal cortex neurons containing excitatory amino acids may also inhibit mesocorticolimbic DA activity by facilitating GABA release within the A10 region via activation of non-NMDA receptors (Wang *et al.*, 1995). In addition, somatodendritic DA release appears to facilitate the release of GABA within the A10 region through activation of D₁ receptors located on GABA interneurons (Rice *et al.*, 1994; Cameron *et al.*, 1993). Accordingly, enhanced release of DA within the A10 region and within mesocorticolimbic terminal regions may result in compensatory feedback regulation to the A10 area. Conversely, as noted in Experiment 1, a decrease in DA release from A10 terminal regions may also result in disinhibition of GABA neurons within the nucleus accumbens (e.g., Retaux *et al.*, 1994), which appear to project to both the ventral pallidum and A10 region (Kalivas *et al.*, 1993; Heimer *et al.*, 1991). Accordingly, enhanced release of GABA from A10 interneurons, as well as GABA mediated feedback from mesocorticolimbic terminal regions may decrease A10 DA activity following stressor exposure. In this respect, brain stimulation performance deficits induced by uncontrollable footshock may reflect diminished A10 DA activity owing to direct effects on amine availability as well as to the indirect influence of GABA mediated inhibition of midbrain DA activity.

Intraventricular DAGO but not DPDPE administration prior to footshock attenuated brain stimulation performance deficits ordinarily observed immediately following stressor exposure. Accordingly, μ receptor activation may have antagonized the decrease in mesocorticolimbic DA activity potentially associated with the deterioration of self-stimulation performance. There are data which suggest that an interaction between μ receptors and central GABA activity may contribute to DA variations observed in response to stressor application. Met-enkephalin release within the VTA following uncontrollable footshock has been associated with a naloxone reversible increase in DA turnover within the prefrontal cortex and nucleus accumbens (Kalivas *et al.*, 1987a; Miller *et al.*, 1984). Intra-VTA administration of DAGO or met-enkephalin produces a μ receptor mediated enhancement of extracellular DA level within the VTA and nucleus accumbens by releasing DA neurons from the tonic inhibitory influence of GABA interneurons (Klitnick *et al.*, 1992b; Johnson *et al.*, 1992b; Kalivas *et al.*, 1990). Accordingly, the release of met-enkephalin following exposure to uncontrollable stressors may facilitate A10 DA activity through a μ receptor mediated inhibition of GABAergic interneurons. However, while midbrain GABA release may initially be inhibited by a stressor provoked release of met-enkephalin, a subsequent decline in enkephalin levels may contribute to the disinhibition of central GABA activity and therefore, inhibition of A10 DA activity. Indeed, met-enkephalin levels within the VTA show a time dependent decrease 5 to 20 minutes following exposure to uncontrollable footshock (Kalivas *et al.*, 1987a). Interestingly, it has been reported that extracellular levels of DA within the prefrontal cortex and nucleus accumbens peak approximately 15 to 20 minutes following footshock onset, then DA levels progressively decline (Sorg *et al.*, 1993; Imperato *et al.*, 1991; Abercrombie *et al.*, 1989). Accordingly, it is conceivable that footshock provoked brain stimulation deficits may coincide with the time dependent reduction in the availability of endogenous levels of met-enkephalin, resulting in the disinhibition of GABA neurons and an inhibition of mesencephalic DA activity.

Intraventricular DAGO application prior to stressor exposure in Experiment 2a may have ameliorated brain stimulation deficits by prolonging the duration of DA neuronal activation ordinarily occasioned by endogenous met-enkephalin release. In view of the observation that DAGO interacts with μ receptors on GABAergic interneurons and/or A10 afferents originating in the nucleus accumbens and ventral pallidum, DAGO may have sustained the inhibition of GABA release within the A10 region, thereby prolonging DA activity and preventing self-stimulation alterations associated with the inhibition and/or depletion of mesocorticolimbic DA release. A schematic representation of (a) the potential interactions between enkephalin, GABA, and mesocorticolimbic DA activity in response to uncontrollable footshock and (b) the modulatory influence of μ receptor stimulation by DAGO on A10 DA activity is presented in Figure A.

It should be noted that despite the distribution of both met-and-leu enkephalin within the mesencephalon (e.g., Zahm *et al.*, 1985), the release of leu-enkephalin within the A10 region following stressor exposure has not been examined. In addition, obtaining selective measurements of met- and leu-enkephalin may be problematic due to cross-sensitivity between the antisera raised against met- and leu-enkephalin (see Sesack *et al.*, 1992; Fallon *et al.*, 1990). Accordingly, it is presently unclear whether enkephalin release within the A10 region following stressor exposure is limited to the met-enkephalin peptide sequence. Moreover, met- and leu-enkephalin interact with both μ and δ receptors (see Adler *et al.*, 1990), thus the stressor related activation of DA neurons secondary to μ receptor stimulation may not be limited to the effects of stressor provoked met-enkephalin release. Therefore, while DAGO may facilitate DA activity by activating μ receptors, it is unclear whether DAGO simulates the effects of endogenous met-and/or leu-enkephalin on A10 DA functioning.

In addition to modulation by met-and/or leu enkephalin, stressor related mesocorticolimbic DA variations may be associated with enhanced release of the endogenous κ receptor ligand, dynorphin. Dynorphin is extensively co-distributed with

Figure A: Modulatory Role of Footshock Provoked Release of Enkephalin on A10 DA Activity

A. Footshock may increase the release of met-enkephalin within the A10 area from neurons originating in multiple brain regions (1) resulting in the stimulation of post-synaptic μ receptors on A10 GABA interneurons (2). The release of GABA from A10 interneurons is decreased by μ receptor activation, reducing the GABA_A receptor mediated inhibition of DA neurons (3).

B. Met-enkephalin is also colocalized in GABA containing afferents to the A10 region originating in the nucleus accumbens and ventral pallidum (4). The release of met-enkephalin from A10 afferents may also act at pre-synaptic μ receptors to inhibit GABA release from descending GABA neurons which innervate the A10 region (5) and ordinarily inhibit DA activity via stimulation of GABA_B receptors located on A10 DA cells (6). The net effect of enkephalin release within the A10 area is enhanced DA activity owing to disinhibition.

C. Activation of A10 DA neurons secondary to GABA inhibition by μ receptor activation facilitates the release of DA at mesocorticolimbic sites, including the nucleus accumbens and ventral pallidum (7). Stimulation of D1 or D2 receptors on mesocorticolimbic terminal regions may activate GABA mediated feedback from the ventral pallidum and nucleus accumbens (8), decreasing A10 DA activity via GABA_B receptor stimulation (6).

D. DAGO administration prior to footshock exposure may have ameliorated brain stimulation performance deficits by extending the duration of DA activation associated with footshock, which may be diminished following a reduction in the availability of endogenous met-enkephalin and/or enhanced GABA mediated feedback from A10 terminal regions. DAGO may have inhibited GABA mediated inhibition of DA neurons by stimulating μ receptors on GABA interneurons (2) and/or descending GABA neurons which innervate A10 DA cells (6).

met- and leu-enkephalin in the central nervous system and considerable overlap in enkephalin and dynorphin containing fibers and cell bodies has been detected within A10 terminal regions, including the nucleus accumbens, striatum, amygdala, hypothalamus, and dorsal raphe (see Fallon, 1990). The enkephalins and dynorphin appear to exert an antagonistic influence on analgesia, respiratory depression, temperature regulation, sexual behavior, and neuroendocrine functions, suggesting dynorphin exerts a modulatory role on proenkephalin derived peptide systems (discussed in Fallon *et al.*, 1990; Smith *et al.*, 1988). Increases in central dynorphin activity have been reported following stressor exposure (Nabeshima *et al.*, 1992; Smith *et al.*, 1988). Accordingly, it is conceivable that a stressor provoked release of dynorphin may antagonize the facilitation of mesocorticolimbic DA activity induced by met- and/or leu-enkephalin. In support of such a proposition, administration of the selective κ receptor agonists, U50,488, reduces mesolimbic DA turnover following prior activation of DA neurons (e.g., following haloperidol or morphine administration), while having no effect on DA activity under basal conditions (Narita *et al.*, 1993; Manzanares *et al.*, 1991). The activation of κ receptors has been linked with presynaptic inhibition of DA release in the nucleus accumbens (see Spanagel *et al.*, 1992), thus dynorphin may indirectly oppose midbrain DA activation during stressor exposure by interacting with κ receptors at mesocorticolimbic terminal regions. It has previously been demonstrated in this laboratory that administration of the selective κ receptor agonist, U50,488, immediately following uncontrollable footshock antagonizes self-stimulation deficits ordinarily evident from the dorsal A10 region (Dickson, 1993). Accordingly, κ receptor stimulation may attenuate overactivation of the mesencephalic DA system in response to stressor exposure, thereby precluding the reduction in responding for brain stimulation associated with footshock provoked midbrain DA variations.

In contrast to the ameliorative influence of U50,488 on brain stimulation deficits (Dickson, 1993), other studies have demonstrated that administration of dynorphin or κ receptor agonists exacerbates stressor provoked behavioral pathology. For example,

administration of dynorphin or U69,593 prior to stressor exposure potentiates the emergence of conditioned suppression of motility (Nabeshima *et al.*, 1988). Discrepant findings with respect to the role of dynorphin in modulating stressor related pathology may be accounted for by the relative selectivity of dynorphin analogues for κ relative to μ receptors. While dynorphin is approximately 400 times more selective for κ relative to δ receptors, dynorphin is only 64 times more selective for κ relative to μ receptors (Emmerson *et al.*, 1994). In contrast, U50,488 is 900 times more selective for κ relative to μ receptors (Emmerson *et al.*, 1994). Accordingly, in addition to its action at κ receptors, endogenous dynorphin (but not selective κ receptor agonists) may regulate the met- and/or leu-enkephalin mediated facilitation of mesocorticolimbic DA activity through an antagonistic action at μ receptors within the A10 area. Indeed, central administration of dynorphin antagonizes the effects of selective μ receptor agonists in preventing conditioned suppression of motility and forced swim induced immobility (Katoh *et al.*, 1990). Accordingly, it is conceivable that dynorphin release may initially have an adaptive influence owing to a κ receptor mediated attenuation of mesocorticolimbic activation, while higher levels of dynorphin may result in antagonism of μ receptors and ensuing pathology. In this respect, stressor provoked dynorphin release may antagonize the μ receptor mediated facilitation of A10 DA activity by met-enkephalin, thereby contributing to the emergence of brain stimulation performance deficits. Considering central administration of dynorphin analogues produces conditioned place aversion (Bals-Kubik *et al.*, 1990b; Shippenberg *et al.*, 1987; Mucha *et al.*, 1985), endogenous dynorphin release may be associated with a state of dysphoria. Moreover, the demonstration that augmentation of μ receptor stimulation prior to or following footshock in Experiments 2a-2b prevented self-stimulation deficits suggests that the antagonistic influence of dynorphin on μ receptors may be associated with pathology rather than serving as an adaptive homeostatic mechanism. Accordingly, intraventricular DAGO administration prior to stressor exposure in Experiment 2a may have saturated μ receptors such that dynorphin was ineffective in

suppressing midbrain DA activity through an antagonistic action at μ receptors, thereby attenuating brain stimulation performance deficits.

Although the administration of DAGO or DPDPE prior to footshock abolished the emergence of long term deficits in responding for brain stimulation, the self-stimulation performance of animals treated with DAGO 15 minutes following completion of the footshock session was depressed relative to DPDPE treated animals 24- and 168-hours post-stressor. While intraventricular DAGO administration prior to footshock may have attenuated reductions in self-stimulation by sustaining DA activation, midbrain DA depletion and/or inhibition may have already ensued when DAGO was administered 15 minutes following the termination of footshock, owing to a reduction in the availability of met- and/or leu-enkephalin and/or enhanced dynorphin activity. Accordingly, it is conceivable that DAGO did not provoke a complete attenuation of long term brain stimulation performance deficits in Experiment 2b owing to an occupation of at least some populations of μ receptors by dynorphin. In effect, endogenous dynorphin may have antagonized the DAGO mediated inhibition of GABA release via μ receptor blockade. The time dependent release of enkephalin and/or dynorphin may therefore influence the efficacy of DAGO in ameliorating reward deficits when administered post-stressor as opposed to administration prior to footshock exposure. A schematic representation of (a) the potential mechanisms by which stressor provoked dynorphin release may antagonize mesocorticolimbic DA activation following μ receptor stimulation by met-enkephalin and (b) the modulatory role of DAGO administration on dynorphin mediated reductions in A10 DA activity is presented in Figure B.

Intraventricular administration of DPDPE prior to stressor exposure was without effect on the immediate footshock induced brain stimulation performance deficits ordinarily observed from the dorsal VTA. Similarly, pretreatment with DPDPE failed to attenuate footshock induced decreases in locomotor activity and rearing in Experiment 1. However, DPDPE administration post-stressor reversed self-stimulation deficits from the dorsal A10

Figure B: Modulatory Role of Footshock Provoked Release of Dynorphin on A10 DA Activity

A. The stressor provoked release of dynorphin from multiple brain regions (1) may act at κ receptors located on mesocorticolimbic DA terminals to presynaptically inhibit DA release (2). Activation of κ receptors may prevent overactivation of the mesocorticolimbic system, thereby precluding the development of brain stimulation performance deficits.

B. Dynorphin may also act directly at μ receptors on descending GABA efferents (3) or μ receptors on GABA interneurons (4) to antagonize the continued facilitation of DA activity induced by met-enkephalin mediated μ receptor stimulation within the A10 region. The antagonistic action of dynorphin at μ receptors may promote behavioral pathology.

C. The administration of DAGO prior to stressor exposure may have saturated μ receptors (3,4) such that dynorphin was ineffective in antagonizing the met-enkephalin mediated facilitation of A10 DA activity, precluding the development of brain stimulation deficits from the dorsal A10 region. In contrast, DAGO administration post-stressor may have been only partially effective in ameliorating brain stimulation deficits if dynorphin occupied at least some populations of μ receptors (3 or 4), antagonizing the DAGO mediated inhibition of GABA release and therefore, reactivation of A10 DA neurons.

region. In addition, the administration of a comparable dose of DPDPE following uncontrollable footshock has previously been noted to reverse stressor associated reductions in locomotor activity (Mendella, 1994). Accordingly, the ameliorative influence of δ receptor stimulation on stressor related behavioral pathology appears to be dependent upon prior activation of the mesocorticolimbic DA system and the emergence of additional neurochemical variations (e.g., enhanced GABA and/or dynorphin activity) which may interact with midbrain DA neurons to produce behavioral pathology.

The potential mechanisms of action by which δ receptors modulate midbrain DA activity remain speculative. Central δ receptors have not been identified on either DA or GABA containing neurons in the A10 region (Churchill *et al.*, 1992b; Johnson *et al.*, 1992b; Dils *et al.*, 1990). However, within other brain regions, including the hypothalamus, brain stem and spinal cord, δ receptors have been detected on the axon terminals of 5-HT neurons originating in the raphe nuclei (Arvidsson *et al.*, 1995; Allen *et al.*, 1993). It is conceivable that δ receptors within the A10 region may also be localized on serotonergic terminals within the A10 region. Indeed, both the dorsal and ventral A10 regions are innervated by 5-HT neurons from the dorsal and medial raphe nuclei (Halliday *et al.*, 1989; Herve *et al.*, 1987) and there appears to be a positive correlation between the density of δ receptors and the distribution of 5-HT terminals within the A10 area (Delay-Goyet *et al.*, 1990; Halliday *et al.*, 1989). It has been suggested that δ receptors may presynaptically facilitate 5-HT release (see Arvidsson *et al.*, 1995). Accordingly, it is possible that DPDPE administration may have enhanced 5-HT release from serotonergic axons within the A10 region. Augmentation of 5-HT within the A10 area has been noted to facilitate DA activity via 5-HT_{1B} receptor stimulation (Johnson *et al.*, 1992c). Interestingly, microinjections of 5-HT or the 5-HT releasing agent, fenfluramine, into the A10 region reduces the GABA_B receptor mediated inhibition of A10 DA activity (Cameron *et al.*, 1994). Accordingly, stimulation of 5-HT_{1B} receptors may antagonize GABA mediated inhibition of DA activity within the mesencephalon. In this respect, DPDPE administration

post-stressor may have reinstated responding for brain stimulation from the dorsal A10 region through a δ receptor mediated augmentation of 5 HT release, thereby reducing the inhibitory influence of GABA on A10 DA neurons.

The efficacy of δ receptor activation in modulating immediate stressor related brain stimulation deficits when administered post-stressor but not prior to footshock may be accounted for by the populations of GABA neurons with which δ receptors interface. It has been reported that intra-VTA administration of DPDPE fails to hyperpolarize GABA interneurons within the A10 region (Johnson *et al.*, 1992b). However, owing to the finding that 5-HT inhibits GABAB but not GABAA receptor mediated inhibitory synaptic potentials (Cameron *et al.*, 1994; Johnson *et al.*, 1992c), a δ receptor mediated facilitation of 5-HT activity likely occurs secondary to diminished GABA release from A10 afferents rather than GABA interneurons. Moreover, 5-HT_{1B} agonists enhance A10 DA cell firing in response to stimulatory pulses while having no effect on basal A10 DA activity (Prisco *et al.*, 1994; Johnson *et al.*, 1992c). Accordingly, it is conceivable that A10 DA activity may be amenable to δ receptor stimulation only after the initiation of enhanced GABA mediated feedback to the A10 region. In support of this proposition, there is evidence that δ receptors play a minor role in the tonic activation of A10 DA neurons by endogenous met- and/or leu-enkephalin relative to μ receptor stimulation. For example, intra-VTA administration of the selective μ receptor antagonist, CTOP, significantly decreases DA release from the nucleus accumbens (Spanagel *et al.*, 1992; but see Devine *et al.*, 1993c), while intra-VTA administration of the δ receptor antagonist, natrindole, produces only a marginal decline in accumbens DA release (Devine *et al.*, 1993a). In addition, intraventricular administration of natrindole has no effect on mesocorticolimbic turnover (Manzanares *et al.*, 1993). In view of the observation that μ receptors modulate the activity of both GABA interneurons and GABA containing efferents from the nucleus accumbens (Sugita *et al.*, 1992; Johnson *et al.*, 1992b; Dilts *et al.*, 1989), μ receptors may be involved in both tonic and phasic A10 DA regulation. In contrast, δ receptors may be primarily

associated with the modulation of phasic changes in DA activity. In this respect, DPDPE may have been relatively ineffective in modulating A10 DA activity when administered prior to stressor application owing to a lack of effect of δ receptor activation on GABA interneurons. In contrast, DPDPE administration post-stressor may have stimulated DA activity by diminishing GABA release from A10 afferents, secondary to an enhanced release of 5-HT. A schematic representation of (a) the potential modulatory influence of δ receptor stimulation on mesocorticolimbic DA activity, and (b) possible mechanisms by which DPDPE may have reversed brain stimulation performance deficits when administered post-stressor is presented in Figure C.

Interestingly, intraventricular DPDPE administration prior to footshock abolished the brain stimulation performance deficits ordinarily observed 24-hours and 168-hours post-stressor despite having no ameliorative effect on immediate stressor induced behavioral pathology. In addition, DPDPE administration post-stressor completely attenuated long term self-stimulation deficits relative to the marginal effect of DAGO in ameliorating self-stimulation deficits 168-hours post stressor. In effect, δ receptor activation appears to antagonize the cascading neural changes which contribute to the development of persistent brain stimulation performance deficits following footshock. The mechanisms through which δ receptor stimulation may oppose the emergence of long term behavioral pathology is presently unclear. However, it appears that the activation of μ , δ and κ receptors may exert a time dependent influence in the modulation of stressor related DA changes within the A10 region. The stimulation of μ receptors by met-and/or leu-enkephalin may initially facilitate DA activity in response to stressor exposure while the interaction of dynorphin with κ or μ receptors may oppose DA activation, potentially contributing to pathology. In contrast, activation of δ receptors appears to override the inhibitory influence of dynorphin release on DA activity. For example, it has been observed that intraventricular dynorphin administration fails to antagonize the ameliorative effect of selective δ but not μ receptor agonists in preventing stressor induced behavioral pathologies

(e.g., forced swim induced immobility) (Katoh *et al.*, 1990). Accordingly, while speculative, a dynorphin mediated reduction in DA activity secondary to μ receptor antagonism may contribute to delayed or proactive neurochemical variations associated with more long term deficits in responding for brain stimulation. Pharmacological stimulation of δ receptors may contribute to adaptive neurochemical changes which preclude long term pathology, potentially through an activation of central 5-HT activity.

In summary, intraventricular DAGO but not DPDPE administration prior to uncontrollable footshock abolished the brain stimulation performance deficits ordinarily evident from the dorsal A10 region immediately following stressor exposure. Intraventricular DAGO may have prevented reductions in responding for self-stimulation by sustaining the stressor induced activation of mesocorticolimbic DA activity through a μ receptor mediated inhibition of GABA release from A10 interneurons and/or afferents to the A10 region. Both DAGO and DPDPE attenuated immediate reductions in responding for self-stimulation when administered post-stressor. The stimulation of μ receptors by DAGO may have directly inhibited GABA release from interneurons or A10 afferents, while δ receptor stimulation may have decreased GABA release from A10 afferents secondary to enhancing the release of 5-HT from terminals in the A10 area. Administration of DPDPE ameliorated long term self-stimulation deficits regardless of the time of administration relative to stressor imposition, while moderate brain stimulation performance deficits were evident 168-hours post-stressor when DAGO was administered after footshock. Activation of δ receptors may antagonize the stressor provoked neurochemical cascade which contributes to long term brain stimulation deficits through a 5-HT mediated modulation of A10 DA activity.

EXPERIMENT 3:

Brain Stimulation Performance From The Ventral A10 Region Following Uncontrollable Footshock and Intraventricular Opioid Administration

It will be recalled that stressor provoked neurochemical variations are brain region specific and dependent upon the qualitative and quantitative characteristics of the stressor employed. The mesocorticolimbic DA system appears to be particularly sensitive to mild stressor application while more intense stressors are required to activate noradrenergic and serotonergic systems (Inoue *et al.*, 1995; Willner *et al.*, 1992; Anisman *et al.*, 1992a; Zacharko *et al.*, 1991). However, even within the mesocorticolimbic system, considerable variability is observed with respect to the vulnerability of DA neurons to stressor application. The prefrontal cortex appears to be selectively activated by mild stressor exposure (e.g., Sorg *et al.*, 1993; Deutch *et al.*, 1991; D'Angio *et al.*, 1990) while more intense stressors are required to enhance DA turnover and release within the nucleus accumbens and other A10 terminal regions (Inoue *et al.*, 1995; Shanks *et al.*, 1991; Bertolucci-D'Angio *et al.*, 1990; Abercrombie *et al.*, 1989). In contrast, the nigrostriatal system is relatively insensitive to stressor exposure, exhibiting elevated DA turnover only following particularly severe or motorically demanding stressors (Bertolucci-D'Angio *et al.*, 1990; Abercrombie *et al.*, 1989). In addition to inter-regional variations in response to stressor exposure, intra-regional differences in the sensitivity to stressor induced DA alterations are observed within mesocorticolimbic sites. For example, subpopulations of neurons within the prefrontal cortex appear to be selectively activated by mild stressors while other neurons appear to be inhibited or show no change in firing pattern in response to stressor application (Mantz *et al.*, 1989). Within the nucleus accumbens, mild stressors selectively enhance DA turnover within the shell but not the core region of the accumbens (Deutch *et al.*, 1992). Electrophysiological and neurochemical data have also demonstrated that a subgroup of A10 DA neurons which originate in the dorsal and medial regions of the VTA (i.e., the paratrachial, interfascicular, and caudal linear nuclei) and project to the

prefrontal cortex are preferentially sensitive to mild stressors relative to more ventral regions of the A10 area (i.e., the paranigral nucleus) (Deutch *et al.*, 1991). Accordingly, discreet subregions of the mesocorticolimbic system appear to be more susceptible to mild stressor induced DA activation while other DA containing areas are more resistant to stressor application. Owing to the proposed role of neuropeptides in modulating stressor provoked DA activity (see Kalivas, 1993), regional variations in peptide distribution may contribute, at least in part, to the vulnerability or resistance of subregions of the mesocorticolimbic system to stressor related neurochemical variations

Inter-and intra-regional variations in stressor provoked DA variations are paralleled at a behavioral level by region specific alterations in responding for brain stimulation following uncontrollable stressor exposure. For example, footshock provoked brain stimulation deficits are not observed from the substantia nigra which is relatively resistant to stressor induced increases in DA turnover (Zacharko *et al.*, 1983). In contrast, stressor provoked reductions in self-stimulation performance are observed from mesocorticolimbic terminal regions which exhibit DA variations following stressor application, including the prefrontal cortex and nucleus accumbens (Zacharko *et al.*, 1991; Wolfe *et al.*, 1990; Bowers *et al.*, 1987). Uncontrollable footshock has also been observed to provoke different durations of brain stimulation performance deficits across subregions of the prefrontal cortex, with transient deficits in responding for brain stimulation being observed from some aspects of the prefrontal cortex while self-stimulation deficits are still apparent one week post-stressor from other cortical regions (Wolfe *et al.*, 1991). In addition, reductions in responding for brain stimulation following uncontrollable footshock are evident from the dorsal but not the ventral aspects of the A10 region (Zacharko *et al.*, 1990). In view of the observation that the ventral A10 region contains a more conspicuous distribution of enkephalin activity, the distribution of opioid peptides within subregions of the mesocorticolimbic system may contribute to the resistance of some DA regions to stressor provoked behavioral pathology.

The vulnerability of subregions of the A10 area to stressor related brain stimulation performance deficits can be modified by intraventricular opioid administration. As demonstrated in Experiment 2a and in a previous investigation (Wolfe *et al.*, 1990), administration of the mixed μ/δ receptor ligand, DALA, or selective μ and δ receptor analogues (i.e., DAGO, DPDPE) prior to footshock ameliorates the immediate and/or long term variations in brain stimulation performance observed from the dorsal A10 region. In contrast, administration of DALA prior to stressor exposure has been noted to promote decreases in responding for brain stimulation from the ventral A10 region reminiscent of the self-stimulation performance deficits induced by uncontrollable footshock from the dorsal A10 area (Wolfe *et al.*, 1991b). Accordingly, while footshock alone does not provoke brain stimulation performance deficits from the ventral VTA, superimposition of μ and/or δ receptor stimulation on stressor related DA alterations may provoke neurochemical variations within the ventral A10 region consistent with behavioral pathology. Experiment 3 evaluated the extent to which intraventricular administration of the doses of DAGO and DPDPE utilized in Experiments 2a-2b influenced self-stimulation performance from the ventral A10 region when administered prior to uncontrollable footshock.

Procedure

Following a 10 day postoperative recovery period, 48 animals with electrodes implanted in the ventral VTA were trained to respond for brain stimulation for 15 minutes per day, employing the brain stimulation parameters detailed in Experiments 2a-2b. Baseline self-stimulation rates were determined over a 45 minute test session in which responding for brain stimulation was recorded at three minute intervals. Mice were subsequently matched on the basis of baseline brain stimulation performance and subdivided into four groups ($n=12/\text{group}$). Mice received a microinjection of DAGO (0.1, 1.0 μg), DPDPE (1.0 μg) or Vehicle into the lateral ventricle. The mice were then further subdivided ($n=6/\text{group}$) such that half of the animals were exposed to 60 inescapable

footshocks while the remaining mice were placed in an identical shock apparatus for an equivalent period of time but shock was withheld. Immediately following the stressor session, mice were returned to the self-stimulation tubs and brain stimulation rates were recorded at three minute intervals over a 45 minute period. All mice were subsequently tested for a 45 minute period of brain stimulation 24-hours and 168-hours following the initial stressor exposure.

Results

Cannula and electrode placements for each of the mice used in Experiment 3 were histologically verified. Data for any animals exhibiting severe tissue displacement, necrosis, or electrode placements outside the boundaries of the ventral A10 region were excluded from the experiment. A schematic representation of electrode placements within the ventral A10 area is presented in Figure 3a.

 Insert Figure 3a about here

Baseline Self-Stimulation Performance: An analysis of variance of Baseline responding for brain stimulation from the ventral A10 area revealed a main effect for Time, $F(14,560) = 13.38$, $p < .001$, and a Time x Shock interaction, $F(14,560) = 2.15$, $p < .01$ (see Appendix E). Newman Keuls multiple comparisons associated with the Time effect revealed that Baseline self-stimulation rates decreased progressively during the initial 21 minutes of behavioral testing, then remained relatively stable during the remaining 24 minutes of the test interval. Multiple comparisons associated with the Time x Shock interaction revealed that 18 to 24 minutes following commencement of self-stimulation, animals randomly assigned to the No Shock condition exhibited higher rates of responding for brain stimulation relative to mice subsequently placed in the Shock condition. However, as cumulative responding for brain stimulation over the entire 45 minute interval did not differ

Figure 3a. Schematic representation of electrode placements within the ventral A10 region in CD-1 mice used in Experiment 3 (n=48). Owing to the overlap of electrode placements, several of the 48 placements have been omitted.

- = Vehicle
- = DPDPE (1.0 ug)
- = DAGO (.01 ug)
- = DAGO (1.0 ug)

between the subjects subsequently assigned to the Shock and No Shock conditions, it is unlikely that the minor difference in self-stimulation rates observed at the three Time intervals led to any systematic bias following stressor application (see Figure 3b).

Insert Figure 3b about here

Post-Shock and Drug Self-Stimulation Performance: An analysis of variance of self-stimulation performance following the Shock and Drug manipulations revealed a significant Day x Drug, $F(6,80) = 3.19$, $p < .01$, and a Time x (Shock x Drug) interaction, $F(42,560) = 1.78$, $p < .01$ (see Appendix E). Newman Keuls multiple comparisons of the simple main effects associated with the Day x Drug interaction revealed that on the Immediate test day, both the Shocked and Non Shocked mice administered DPDPE or the 1.0 ug or .01 ug dose of DAGO exhibited a decrease in responding for brain stimulation relative to Vehicle treated animals. At the 24-hour test interval, animals administered the .01 or 1.0 ug dose of DAGO continued to exhibit brain stimulation performance deficits while the self-stimulation rates of mice pretreated with DPDPE were comparable to the Vehicle treated animals. At the 168-hour test interval, self-stimulation performance among mice administered DAGO or DPDPE was equivalent to Vehicle treated animals, although brain stimulation rates in mice pretreated with DPDPE were higher relative to mice treated with the .01 or 1.0 ug dose of DAGO (see Figure 3c). Mean self-stimulation performance collapsed across the 15 time intervals are presented in Figure 3d to depict the Day x Drug interaction.

Insert Figures 3c-3d about here

Figure 3b. Mean ($\pm$ S.E.M) Baseline self-stimulation rates among CD-1 mice responding for brain stimulation from the ventral A10 region in Experiment 3. Rates of responding for brain stimulation were assessed at three minute intervals over a 45 minute period.

Figure 3c (pages 141-142). Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the ventral A10 region following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition. Rates of responding for ICSS were assessed at three minute intervals over a 45 minute period immediately following footshock and again 24-hours and 168-hours following stressor exposure.

VEHICLE

ICSS RATE / 3 MIN.

DF-DPE (1.0 UG)

ICSS RATE / 3 MIN.

Figure 3d. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the ventral A10 region following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the 15 time intervals to depict the Day $\times$ Drug interaction.

Newman Keuls multiple comparisons of the simple main effects associated with the Time x (Shock x Drug) interaction (i.e., when the data were collapsed across the three test days) revealed that self stimulation performance among Shocked animals pretreated with Vehicle was elevated relative to Non Shocked Vehicle treated mice 15 to 45 minutes following the commencement of behavioral testing. Responding for brain stimulation among Shocked mice treated with DPDPE was comparable to the Non Shocked Vehicle treated mice for the duration of behavioral testing, but depressed relative to Shocked Vehicle treated animals for the 12 to 45 minute interval of behavioral testing. Brain stimulation performance among Shocked animals pretreated with the .01 ug dose of DAGO was depressed relative to Shocked mice treated with Vehicle or DPDPE for the entire 45 minute period of behavioral testing, and to the Non Shocked animals administered .01 ug of DAGO for the 30 to 45 minute interval of behavioral testing. Shocked mice treated with 1.0 ug of DAGO exhibited lower rates of brain stimulation relative to Shocked mice treated with Vehicle or DPDPE, as well as to the Non Shocked mice administered 1.0 ug of DAGO for the entire 45 minute period of behavioral testing. Mean self-stimulation performance collapsed across the three test days are presented in Figure 3e to depict the Time x (Shock x Drug) interaction.

Insert Figures 3e about here

In summary, uncontrollable footshock provoked an increase in brain stimulation performance from the ventral A10 region in Vehicle treated animals. Intraventricular administration of DPDPE prior to footshock produced a moderate decrease in self-stimulation in both Shocked and Non Shocked animals on the Immediate test day. Intraventricular administration of .01 or 1.0 ug of DAGO promoted a reduction in

Figure 3e. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the ventral A10 region following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the Immediate, 24-hour and 168-hour test days to depict the Time x (Shock x Drug) interaction.

responding for brain stimulation at the Immediate and 24-hour test days in both Shocked and Non Shocked animals. When the data were collapsed across the three test days, it was evident that brain stimulation performance in Shocked mice administered DPDPE was comparable to Non Shocked animals administered Vehicle or DPDPE. However, Shocked mice which had been pretreated with either the .01 or 1.0 dose of DAGO exhibited depressed responding for self-stimulation relative to Non Shocked DAGO treated animals, as well as to Shocked or Non Shocked mice treated with Vehicle or DPDPE.

Discussion

In contrast to the stressor related brain stimulation deficits which were evident from the dorsal A10 region in Experiments 2a-2b, uncontrollable footshock was observed to enhance responding for brain stimulation from the ventral A10 region in vehicle treated mice. It should be noted that a previous investigation in this laboratory failed to detect a difference in brain stimulation performance from the ventral A10 region in stressed and non-stressed mice (Zacharko *et al.*, 1990). However, the increase in responding for self-stimulation from the ventral A10 area detected in the current investigation was evident only during the last 30 minutes of behavioral testing. In view of the observation that Zacharko *et al.* (1990) evaluated brain stimulation rates for only a 15 minute period, the detection of a stressor related enhancement of self-stimulation which may have emerged with a longer duration of behavioral testing was precluded. Accordingly, footshock appears to provoke a moderate facilitation of responding for brain stimulation from the ventral A10 region, in direct contrast to the stressor induced brain stimulation performance deficits observed from the dorsal aspects of the A10 area.

It will be recalled that uncontrollable stressors increase DA turnover, DA release, and the expression of tyrosine hydroxylase, Fos and *c-fos* within the A10 region (Beck *et al.*, 1995; Zhang *et al.*, 1994; Deutch *et al.*, 1991; Hirada *et al.*, 1989; Herman *et al.*, 1988). However, discrete subregions of the A10 region are selectively activated depending on the qualitative characteristics of the stressor parameters employed. For example, mild

stressors (e.g., 15 minutes of restraint) increase Fos expression to a greater extent in the dorsal and medial aspects of the A10 area relative to the ventral A10 region (Deutch *et al.*, 1991). Accordingly, stressors of a more intense and/or prolonged nature may be required in order to globally activate A10 DA neurons. It is well established that while mild stressors selectively enhance DA turnover in the prefrontal cortex (Deutch *et al.*, 1990; Dunn *et al.*, 1988), increasing the intensity of the stressor provokes more widespread activation of mesocorticolimbic terminal fields (e.g., Shanks *et al.*, 1991; Bertolluci-D'Angio *et al.*, 1990; Abercrombie *et al.*, 1989; Dunn *et al.*, 1988). It has also been noted that increasing the duration of stressors which selectively enhance DA turnover in the prefrontal cortex, without increasing the intensity (e.g., 15 relative to 30 minutes of footshock), results in enhanced DA turnover in additional mesocorticolimbic regions (see Roth *et al.*, 1988; Dunn *et al.*, 1988). In this respect, it is conceivable that the footshock parameters utilized in Experiments 2-3 resulted in a rapid activation of the dorsal A10 region, with ventral DA neurons being recruited later in the footshock session. Owing to the delayed activation of the ventral A10 region, ventral DA neurons may still have been in an enhanced state of activity during behavioral testing, in contrast to the potential depletion and/or inhibition of DA activity within the dorsal A10 DA region. Indeed, the stressor related increase in brain stimulation performance from the ventral A10 region supports the proposition that the stressor provoked facilitation of ventral A10 DA neurons was sustained following the termination of footshock.

The differential neuropeptide distribution throughout the A10 region may also contribute to the varied sensitivity of subregions of midbrain DA neurons to stressor related pathology. The ventral A10 region contains a more conspicuous distribution of met-enkephalin and a higher density of both μ and δ receptors relative to the dorsal A10 region (German *et al.*, 1993; Delay-Goyet *et al.*, 1990; Fallon *et al.*, 1990). In view of the observation that opioid administration attenuates stressor related behavioral alterations, endogenous enkephalin release may exert a prophylactic influence on stressor induced

neurochemical variations contributing to pathology. In this respect, a higher degree of enkephalin activation within the ventral VTA during stressor exposure may preclude footshock provoked alterations in ventral A10 DA activity which contribute to brain stimulation deficits. However, the contributing influence of additional neuropeptides (e.g., substance P, neurotensin, cholecystokinin), and the distribution of DA, GABA, and 5-HT receptors, amongst others, to the stressor induced behavioral alterations observed from subregions of the A10 region cannot be dismissed.

Intraventricular administration of the high dose of DAGO in Experiment 2a and Experiment 3 elicited a decrease in responding for self-stimulation from both the dorsal and ventral aspects of the A10 region in the absence of stressor exposure. The inhibition of brain stimulation performance by 1.0 ug of DAGO is consistent with previous investigations demonstrating that high doses of morphine or DAGO reduce responding for brain stimulation from the lateral hypothalamus in both rate-dependent and rate-independent paradigms (e.g., Johnson *et al.*, 1993; Heidbreder *et al.*, 1992; De Witte *et al.*, 1989; Rompre *et al.*, 1989a). However, while .01 ug of DAGO and 1.0 ug of DPDPE had no discernible influence on responding for brain stimulation from the dorsal A10 region in non stressed animals in Experiments 2a-2b, both DAGO and DPDPE provoked a transient depression in responding for brain stimulation from the ventral A10 area. The depression of self-stimulation performance following administration of DPDPE or the low dose of DAGO was unexpected in view of the previous observation that a 1.0 ug dose of DALA had no influence on brain stimulation performance from the dorsal or ventral A10 regions (Wolfe *et al.*, 1991b). Accordingly, brain stimulation performance following intraventricular opioid administration appears to be dependent on (a) the dose employed, (b) the opioid receptor subtype which is activated, and (c) the central site from which self-stimulation is attained. The differential distribution of opioid receptors within the brain regions from which self-stimulation is derived may influence the degree of DA activation

associated with selective opioid receptor agonists and therefore, brain stimulation performance.

Due to the depression of brain stimulation in non-stressed animals administered DPDPE or DAGO, the synergistic effect of opioid administration and footshock in provoking brain stimulation deficits from the ventral A10 region was not as evident as previously noted following DALA administration (Wolfe *et al.*, 1991b). However, the self-stimulation performance of stressed mice administered DPDPE was not significantly different from non-stressed animals treated with DPDPE. In contrast, brain stimulation performance in stressed mice pretreated with either .01 or 1.0 ug of DAGO was depressed relative to non-stressed animals administered Vehicle or DAGO. Accordingly, activation of μ but not δ receptors appeared to have an additive effect with footshock exposure, provoking a decline in responding for self-stimulation from the ventral A10 area which was greater than the decrease in self-stimulation observed following opioid administration alone.

The more pronounced self stimulation deficits observed in stressed relative to non-stressed animals following DAGO administration may reflect the combined influence of endogenous enkephalin release and exogenous stimulation of μ receptors on ventral A10 DA activity. In effect, the combined influence of opioid administration and footshock may be analogous to further increasing the intensity or duration of the stressor, potentially indicating that the ventral A10 region may be susceptible to the neurochemical variations contributing to behavioral pathology with sufficient activation of DA neurons. However, the mechanism of action by which depressed DA activity may develop following opioid administration is unclear. As noted in Experiments 2a-2b, sustained activation of A10 DA neurons provoked by stressor exposure may result in subsequent DA depletion and/or inhibitory feedback from GABA and/or dynorphin containing afferents to the A10 region. Alternatively, the inhibition of ventral A10 DA activity may represent a mechanism of action distinct from that involved in stressor mediated reductions in brain stimulation from

the dorsal A10 region. In this respect, it has been demonstrated that pharmacological manipulations which promote sustained activation of DA cells induce depolarization inactivation, a state of neural quiescence owing to excessive membrane depolarization (Mereu *et al.*, 1995; Bunney *et al.*, 1991; Debonnel *et al.*, 1990). For example, the superimposition of a stressor (e.g., tail pinch) or intra-VTA morphine application following prior activation of the mesocorticolimbic system with acute haloperidol or pimozide administration provokes depolarization inactivation of A10 DA neurons (Zhang *et al.*, 1994; Henry *et al.*, 1992; Rompre *et al.*, 1989b). Depolarization block of A10 DA neurons has been associated with a reduction in responding for brain stimulation from the lateral hypothalamus and central gray region (Rompre *et al.*, 1989a; 1989b; Wise *et al.*, 1989). Similarly, the stressor provoked release of enkephalin and the ensuing increase in ventral A10 DA activity may have had an additive effect with the DAGO mediated stimulation of μ receptors, leading to inhibition of A10 DA neurons and subsequent reductions in brain stimulation performance.

Decreased A10 DA activity owing to depolarization inactivation appears to be distinct from inhibition of DA cell activity secondary to enhanced feedback regulation. For example, pharmaceutical agents which typically facilitate DA cell activity, including morphine and glutamate, prolong rather than reverse depolarization block of A10 DA neurons (Wise *et al.*, 1989). In addition, morphine and glutamate are ineffective in reversing brain stimulation performance deficits from the medial forebrain bundle associated with depolarization inactivation (Rompre *et al.*, 1989; Wise, 1989). However, intra-VTA administration of the GABA agonists, muscimol or baclofen, reactivates DA cell firing (Henry *et al.*, 1992; Wise, 1989) and reinstates responding for brain stimulation following induction of depolarization block by haloperidol and/or morphine (Rompre *et al.*, 1989). These findings are in direct contrast to the reversal of brain stimulation deficits by DAGO or DPDPE in Experiment 2b, both which appear to antagonize GABA release within the A10 region. Accordingly, brain stimulation performance deficits from the ventral

A10 region associated with footshock and μ receptor stimulation may represent reduced A10 DA activity, although as a result of a different mechanism of action relative to potential stressor provoked decreases in dorsal A10 DA activity.

The demonstration that responding for brain stimulation from the dorsal and ventral aspects of the A10 region is differentially sensitive to stressor exposure and opioid administration emphasizes the heterogeneous nature of the responsiveness of A10 DA neurons to environmental stimuli. In consideration of the reciprocal innervations between the A10 area and cortical, limbic, brain stem and midbrain regions, the A10 region may best be characterized as a convergence point or part of a complex circuit system which organizes adaptive behavioral responses to stimuli (Oades *et al.*, 1987). In this respect, the A10 region may assimilate information from diverse inputs from multiple brain regions and gate the propagation of information to other levels of the mesocorticolimbic system (Le Moal *et al.*, 1991; Oades *et al.*, 1987). Subregions of the mesocorticolimbic DA system, including the dorsal A10 area and the medial prefrontal cortex, appear to be preferentially sensitive to mild stressors (Deutch *et al.*, 1991; 1990; Mantz *et al.*, 1989) and may be involved in the initial cognitive evaluation of environmental stimuli (Bertolucci-D'Angio *et al.*, 1990). With stressors of a longer duration, the shell region of the nucleus accumbens, which innervates the ventromedial pallidum and ventral A10 region, is also enhanced (Deutch *et al.*, 1992). With more intense or prolonged stressors, mesocorticolimbic DA activation appears to become more global, potentially reflecting the communication of information to other brain regions and the recruitment of additional neural systems necessary for adaptive behavioral responding. For example, activation of the core region of the nucleus accumbens may result in adaptive motoric responses to stimuli owing to interconnections with the dorsolateral pallidum and nigrostriatal system (see Hooks *et al.*, 1995; Deutch *et al.*, 1992). Indeed, enhanced activity within the neural circuit containing the A10 region, nucleus accumbens, and ventral pallidum appears to be involved in the organization of adaptive behavioral responses to stressors through an integration of limbic

and motor systems (Hooks *et al.*, 1995; Salamone, 1994; Kiyatkin *et al.*, 1993). Accordingly, differential activation of subregions of the A10 area and mesocorticolimbic terminal sites in response to stressors may fundamentally be expressed in terms of variations in motivational processing and the emergence of behavioral coping and/or pathology.

In summary, uncontrollable footshock was associated with an increase in responding for brain stimulation from the ventral aspects of the A10 region. Intraventricular administration of DPDPE or DAGO provoked a transient decrease in responding for brain stimulation in both stressed and non-stressed animals. However, only DAGO administration appeared to have an additive effect with stressor exposure, provoking more substantial decreases in responding for brain stimulation in stressed relative to non-stressed mice. Footshock may have further exacerbated brain stimulation deficits in DAGO treated animals owing to the combined stimulatory effect of endogenous release of enkephalin and DAGO on μ receptors. The differential sensitivity of subregions of the A10 area to stressor exposure and opioid receptor stimulation suggests that DA systems originating in the dorsal and ventral A10 region may be differentially activated in response to environmental stimuli and differentially contribute to stressor related alterations in motivational processing.

EXPERIMENT 4

Brain Stimulation Performance From The Interpeduncular Nucleus: Modification By Uncontrollable Footshock and Intraventricular Opioid Administration

Though the mesocorticolimbic DA system has been implicated as playing a central role in reward/motivational processing, responding for brain stimulation is not limited to the A10 region within the mesencephalon. For example, responding for brain stimulation can also be attained from the substantia nigra and the red nucleus (Zacharko *et al.*, 1990c; Bowers *et al.*, 1987). Early investigations into the neural substrates underlying rewarding brain stimulation (e.g., Crow, 1975) revealed that self-stimulation can also be elicited from the interpeduncular nucleus, a mesencephalic brain structure situated along the midline, ventromedial to the A10 nuclei. Preliminary analyses in this laboratory have confirmed that significant rates of responding for brain stimulation are evident from the interpeduncular nucleus which are comparable to or exceed the rates of self-stimulation observed from the A9 and A10 regions. In contrast to the dopaminergic substrate associated with self-stimulation performance from the dorsal and ventral VTA, as well as the A9 region, the interpeduncular nucleus is largely devoid of DA containing somata or axon terminals. Cell bodies within the interpeduncular nucleus contain primarily 5-HT, and at least a subregion of the interpeduncular nucleus appears to be continuous with the medial raphe (Hamill *et al.*, 1984a). Several investigators have noted that the interpeduncular nucleus projects to the A10 region (Tork *et al.*, 1984; Contestabile *et al.*, 1981), and that the dendritic fields of neurons within the paranigral A10 area and the interpeduncular nucleus overlap (Oades *et al.*, 1987). Extensive reciprocal interconnections also exist between the interpeduncular nucleus and the habenula, which in turn forms reciprocal interconnections with the A10 region (Iwahori *et al.*, 1993a; Oades *et al.*, 1987; Artymyshyn *et al.*, 1985). Accordingly, it is conceivable that brain stimulation to the interpeduncular nucleus may derive its

reinforcing properties owing to a direct or indirect activation of DA neurons within the A10 region.

As demonstrated in Experiment 3 and in previous investigations, stressor provoked brain stimulation deficits are not evident for the ventral VTA or the substantia nigra (Zacharko *et al.*, 1991b; 1990c, 1983). The ventral A10 and A9 regions appear to be more resistant to stressor provoked DA variations which contribute to the expression of behavioral pathology relative to the dorsal A10 area. As previously noted, one of the potential factors contributing to the resistance of mesencephalic DA regions to stressor provoked neurochemical alterations associated with pathology may be the relative distribution of enkephalin and/or other neuropeptides. The interpeduncular nucleus contains a dense distribution of met- and leu-enkephalin containing perikarya as well as enkephalin containing axon terminals (Iwahori *et al.*, 1993b; Liang *et al.*, 1993; Tiu *et al.*, 1993; Shinoda *et al.*, 1986; Hamill *et al.*, 1984b). In addition, a conspicuous distribution of μ and δ receptor binding has been noted in the interpeduncular nucleus (Mansour *et al.*, 1994; Speciale *et al.*, 1993; German *et al.*, 1993). Accordingly, the interpeduncular region exhibits at least some anatomical similarities to that of the ventral relative to the dorsal A10 region. Preliminary investigations have revealed that self-stimulation performance from the interpeduncular nucleus is comparable to that elicited from the ventral A10 region (approximately 1800 responses/15 minutes) in contrast to the marginally lower rates of self-stimulation observed from the dorsal A10 region (approximately 1500 responses/15 minutes). The latency to acquire significant rates of responding for self-stimulation (i.e., exceeding 50 responses/15 minutes) from the interpeduncular nucleus is also comparable to that of the ventral A10 region, with an average of two to three days required to elicit responding, in contrast to the latency of approximately seven to eight days to obtain responding for brain stimulation from the dorsal A10 region. Owing to the similar brain stimulation performance and opioid distribution evident between the ventral A10 region and the interpeduncular nucleus, it might be expected that the interpeduncular nucleus would

likewise be resistant to stressor provoked brain stimulation deficits. However, paralleling the effects evident from the ventral A10 region, μ and/or δ receptor stimulation in combination with footshock may provoke self-stimulation deficits from the interpeduncular region. Accordingly, Experiment 4 evaluated brain stimulation performance from the interpeduncular nucleus following uncontrollable footshock, and assessed whether intraventricular administration of DALA, DAGO or DPDPE prior to footshock would modify responding for brain stimulation from the interpeduncular region comparable to that evident from the ventral A10 region.

Procedure

Following a 10 day postoperative recovery period, 50 animals with electrodes implanted in the interpeduncular nucleus were trained to respond for brain stimulation for 15 minutes per day, employing the brain stimulation parameters detailed in Experiments 2a-2b. Baseline self-stimulation rates were determined over a 45 minute test session and responding for brain stimulation was recorded at three minute intervals. The mice were then matched on Baseline self-stimulation performance and subdivided into five groups ($n=10/\text{group}$). Mice received a microinjection of DALA (1.0 μg), DAGO (0.1, 1.0 μg), DPDPE (1.0 μg) or Vehicle into the lateral ventricle. The mice were then further subdivided ($n=5/\text{group}$) such that half the mice were exposed to 60 inescapable footshocks while the remaining mice were placed in an identical shock apparatus for an equivalent period of time but shock was withheld. Immediately following the stressor session, mice were returned to the self-stimulation tubs and brain stimulation rates were recorded at three minute intervals over a 45 minute period. All mice were subsequently tested for a 45 minute period of brain stimulation 24-hours and 168-hours following initial stressor exposure.

Results

Cannula and electrode placements for each of the mice used in Experiment 4 were histologically verified. Data for any animals exhibiting severe tissue displacement,

necrosis, or electrode localization outside of the interpeduncular nucleus were excluded from the experiment. A schematic representation of electrode placements within the interpeduncular nucleus is presented in Figure 4a.

 Insert Figure 4a about here

Baseline Self-Stimulation Performance: An analysis of variance of Baseline responding for brain stimulation from the interpeduncular nucleus revealed that the ten groups of mice ($n=5$) subsequently assigned to the Shock and Drug conditions did not differ in self-stimulation performance. A main effect of Time was observed, $F(14,560) = 14.83$, $p < .001$ (see Appendix F). Newman Keuls multiple comparisons revealed that self-stimulation rates remained relatively stable throughout behavioral testing, with a moderate decline in brain stimulation performance evident during the final six minutes of the test session (see Figure 4b).

 Insert Figure 4b about here

Post-Shock and Drug Self-Stimulation Performance: An analysis of variance of self-stimulation performance following Shock and Drug administration revealed a Day x Shock, $F(2,80) = 9.15$, $p < .001$, a Day x Time, $F(28,1120) = 1.75$, $p < .001$, and a Day x (Shock x Drug) interaction, $F(8,80) = 1.98$, $p = .052$ (see Appendix F). Newman Keuls multiple comparisons of the simple effects associated with the Day x (Shock x Drug) interaction revealed that on the Immediate test day, brain stimulation performance of Shocked mice pretreated with Vehicle was depressed relative to the Non Shocked animals administered Vehicle. Self-stimulation deficits were also evident in Shocked mice administered DPDPE

Figure 4a. Schematic representation of electrode placements within the interpeduncular nucleus in CD-1 mice used in Experiment 4. Two representative electrode placements are depicted for each group.

- = Vehicle
- ▲ = DALA (1.0 ug)
- = DPDPE (1.0 ug)
- = DAGO (.01 ug)
- = DAGO (1.0 ug)

Figure 4b. Mean ($\pm$ S.E.M) Baseline self-stimulation rates among CD-1 mice responding for brain stimulation from the interpeduncular nucleus in Experiment 4. Rates of responding for brain stimulation were assessed at three minute intervals over a 45 minute period.

or the .01 ug dose of DAGO relative to the respective Non Shocked control groups treated with DPDPE or DAGO, as well as the Non Shocked Vehicle treated animals. Both Shocked and Non Shocked animals which had been administered the 1.0 ug dose of DAGO exhibited a decrease in responding for brain stimulation relative to the Non Shocked Vehicle treated mice. The self-stimulation performance of both Shocked and Non Shocked animals pretreated with DALA was comparable to the Non Shocked Vehicle treated mice. At the 24-hour test interval, the brain stimulation performance of Shocked animals treated with Vehicle or the .01 ug dose of DAGO was equivalent to Non Shocked Vehicle treated mice. In contrast, the brain stimulation performance of Shocked mice administered DALA or DPDPE was depressed relative to the respective Non Shocked control groups as well as to the Non Shocked Vehicle treated mice. Animals treated with the 1.0 ug dose of DAGO continued to exhibit depressed rates of responding for brain stimulation irrespective of Shock condition. At the 168-hour test interval, the self-stimulation performance of Shocked mice pretreated with DALA or the 1.0 ug dose of DAGO was comparable to Shocked Vehicle treated mice, but depressed relative to the Shocked animals administered DPDPE or the .01 ug dose of DAGO (see figure 4c). Mean self-stimulation rates collapsed across the 15 time intervals are presented in Figure 4d to depict the Day x (Shock x Drug) interaction.

 Insert Figures 4c-4d about here

Newman Keuls multiple comparisons of the simple effects associated with the Day x Time interaction revealed that on the Immediate and 24-hour test days, animals exhibited a decrease in brain stimulation performance within the initial six minutes of behavioral testing. At the 168-hour test day, brain stimulation rates remaining relatively stable across the first 21 minutes of behavioral testing, followed by a moderate decline in responding across the ensuing test interval (see figure 4c).

Figure 4c (pages 165-166). Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the interpeduncular nucleus following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition. Rates of responding for brain stimulation were assessed at three minute intervals over a 45 minute period immediately following footshock and again 24-hours and 168-hours following stressor exposure.

168 HOURS

ICSS RATE / 3 MIN.

ICSS RATE / 3 MIN.

ICSS RATE / 3 MIN.

SHOCK
NO SHOCK

Figure 4d. Mean ($\pm$ S.E.M) self-stimulation performance among CD-1 mice responding for brain stimulation from the interpeduncular nucleus following intraventricular administration of saline, DPDPE (1.0 ug), or DAGO (.01, 1.0 ug) and exposure to an acute session of uncontrollable footshock or the No Shock condition. The data are collapsed across the 15 Time intervals to depict the Day $\times$ (Shock $\times$ Drug) interaction.

In summary, footshock decreased responding for brain stimulation from the interpeduncular nucleus in Vehicle treated mice immediately following uncontrollable footshock exposure. Intraventricular administration of DALA, but not DPDPE or DAGO, prior to footshock ameliorated the immediate decrease in self-stimulation performance. Significant decreases in responding for self-stimulation were evident in stressed animals pretreated with DALA or DPDPE 24-hours post-stressor. The 1.0 ug dose of DAGO promoted a decrease in brain stimulation performance in both stressed and non-stressed animals at the Immediate and 24-hour test intervals. At the 168-hour test interval, differences in responding for brain stimulation were no longer observed between stressed and non-stressed mice, although animals treated with DPDPE or .01 ug of DAGO exhibited higher self-stimulation rates relative to mice pretreated with DALA or 1.0 ug of DAGO.

Discussion

Consistent with preliminary investigations, significant rates of brain stimulation were elicited from the interpeduncular nucleus which were comparable to those noted from the ventral A10 region. However, the nature of the neural substrate associated with self-stimulation from the interpeduncular region is largely unknown. In some instances, stimulation of brain regions other than mesocorticolimbic sites is reinforcing owing to afferent connections with the A10 region. For example, while brain stimulation from the medial forebrain bundle is believed to primarily activate descending cholinergic neurons (Rompre *et al.*, 1985; Yeomans *et al.*, 1985), these neurons synapse on DA cells within the A10 region. Accordingly, activation of the mesocorticolimbic system may be the second stage of processing of the "reward signal" and represent a common pathway mediating the reinforcing effects of rewarding stimuli (see Wise *et al.*, 1989; 1982). Indeed, pharmacological inhibition of A10 DA neurons is associated with the cessation of responding for brain stimulation from the medial forebrain bundle (Fcuriezios *et al.*, 1992; Rompre *et al.*, 1989a). However, there is also evidence for reward systems independent of the DA neurons originating in the A10 region. For example, 6-OHDA lesions of the A10

area do not completely abolish self-stimulation from the nucleus accumbens, frontal cortex, and substantia nigra, and no direct correspondence appears to exist between the degree of DA innervation and brain stimulation performance from the septum, amygdala, frontal cortex, nucleus accumbens, and the caudate (Wise *et al.*, 1989; Phillips, 1984). In view of the paucity of research on the role of the interpeduncular nucleus in brain stimulation, the extent to which stimulation to the interpeduncular nucleus is reinforcing due to afferent connections with A10 DA neurons or activation of a reward system independent of the mesocorticolimbic system has not been examined.

The results of Experiment 4 suggest that responding for brain stimulation from the interpeduncular nucleus may be independent of activation of the A10 region. In particular, the transient disruption in brain stimulation performance observed from the interpeduncular nucleus following footshock did not appear to parallel the footshock provoked alterations in self-stimulation observed from either the dorsal or ventral A10 regions in Experiments 2 and 3. In contrast to the increase in self-stimulation observed from the ventral A10 region immediately post-stressor, significant decreases in brain stimulation were observed from the interpeduncular nucleus following footshock. However, in contrast to the rapid deterioration in self-stimulation performance observed from the dorsal A10 region on the immediate test day, a moderate reduction in responding for brain stimulation was observed from the interpeduncular nucleus for the initial 15 minute post-stressor interval, followed by a profound depression of self-stimulation for the remaining 30 minutes of behavioral testing. In addition, brain stimulation deficits were absent 24-hours post-stressor from the interpeduncular region in contrast to the self-stimulation deficits observed 24- and 168-hours post-stressor from the dorsal A10 region. Accordingly, footshock provoked alterations in responding for brain stimulation from the interpeduncular region do not parallel the stressor related variations in self-stimulation evident from either the dorsal or ventral A10 regions.

The influence of intraventricular opioid administration on brain stimulation performance from the interpeduncular nucleus is also distinct from the pattern of change in brain stimulation elicited from the A10 DA activity following opioid administration. Intraventricular administration of DPDPE or the .01 μg dose of DAGO in non-stressed animals had no influence on self-stimulation performance from the interpeduncular region while causing a depression of responding from the ventral A10 area. Moreover, in contrast to the previously demonstrated reduction in brain stimulation performance from the ventral A10 region following footshock and DALA administration (Wolfe *et al.*, 1991), DALA attenuated the stressor provoked brain stimulation deficits from the interpeduncular nucleus ordinarily evident immediately post-stressor. However, in contrast to the ameliorative influence of DALA on brain stimulation deficits from the dorsal A10 region 24- and 168-hours post-stressor (Wolfe *et al.*, 1990), DALA administration was associated with a depression of responding for brain stimulation 24-hours post-stressor from the interpeduncular nucleus. Administration of .01 μg of DAGO prior to footshock had no influence on brain stimulation performance deficits from the interpeduncular region, while abolishing brain stimulation performance deficits from the dorsal A10 region, and reducing responding for brain stimulation from the ventral A10 region. In addition, administration of DPDPE exacerbated self-stimulation deficits 24-hour post-stressor from the interpeduncular region, while ameliorating brain stimulation performance deficits 24-hours and 168-hours post-stressor from the dorsal A10 region, and depressing self-stimulation from the ventral A10 region immediately post-stressor. Accordingly, the influence of μ or δ receptor stimulation on stressor provoked brain stimulation performance deficits from the interpeduncular nucleus does not parallel the cumulative effects of opioid administration and footshock from either the dorsal or ventral A10 regions.

It was of interest that activation of both μ and δ receptors (i.e., DALA administration) had an ameliorative influence on self-stimulation deficits evident immediately post-stressor from the interpeduncular nucleus while the more selective μ and

δ receptor ligands, DAGO and DPDPE, had no influence on brain stimulation performance deficits. Moreover, activation of δ receptors with DALA or DPDPE appeared to delay or interfere with the recovery of behavioral responding evident in vehicle treated animals 24-hours post-stressor. In effect, δ receptor stimulation appeared to oppose neurochemical adaptation within the interpeduncular region, in direct contrast to the delayed therapeutic effect of δ receptor stimulation in ameliorating brain stimulation deficits from the dorsal A10 region. However, owing to a lack of research on stressor provoked neurochemical variations within the interpeduncular nucleus, the mechanisms of action by which μ and δ receptors may modulate neural functioning within the interpeduncular region during stressor exposure, and therefore reward processing, remain relatively unknown. Nevertheless, opioid peptides clearly do not exert homogeneous effects on the motivational alterations associated with stressor exposure from various mesencephalic sites.

Although self-stimulation alterations from the interpeduncular region following footshock appear to be distinct from the stressor provoked variations in brain stimulation noted from the A10 region, neurochemical variations within the interpeduncular nucleus appears to influence mesocorticolimbic DA activity to some extent. Descending cholinergic neurons originating in the septum, mammillary bodies, suprachiasmatic nucleus, nucleus of the diagonal band, and magnocellular preoptic area which innervate the interpeduncular region (see Iwahori *et al.*, 1993a; Kawaya *et al.*, 1988; Woolf *et al.*, 1985) appear to exert an indirect, tonic inhibitory influence on mesocorticolimbic DA activity. For example, administration of atropine into the interpeduncular nucleus increases DA turnover in the prefrontal cortex and nucleus accumbens (Nishikawa *et al.*, 1986). In addition, lesions of the fascicular retroflex, the descending fiber pathway which channels cholinergic neurons from the forebrain to the interpeduncular nucleus, increases DA turnover in the prefrontal cortex and nucleus accumbens and enhances locomotor activity (Thornton *et al.*, 1994; Nishikawa *et al.*, 1986). Interestingly, naloxone administration into the interpeduncular nucleus increases DA turnover in the nucleus accumbens and olfactory tubercle but not the

prefrontal cortex (Nishikawa *et al.*, 1986). In view of the observation that descending cholinergic neurons may tonically activate enkephalin containing interneurons (Tiu *et al.*, 1993), enkephalin activity within the interpeduncular nucleus may inhibit at least a subpopulation of mesocorticolimbic DA neurons. Accordingly, endogenous opioid peptides appear to not only integrate the input of various neurotransmitter systems within brain regions involved in reward (i.e., the A10 region) but appear to be involved in communication, and possibly integration, between distinct reward systems involved in motivation.

In summary, uncontrollable footshock was observed to induce transient disruptions in responding for brain stimulation from the interpeduncular nucleus. Administration of DALA prior to stressor exposure prevented the emergence of brain stimulation deficits immediately post-stressor, while DAGO and DPDPE administration had no effect on immediate stressor provoked alterations in self-stimulation performance. The stimulation of δ receptors associated with DALA and DPDPE administration interfered with the recovery of responding for brain stimulation which was evident in vehicle treated animals 24-hours post-stressor. Brain stimulation from the interpeduncular nucleus appears to represent activation of a reward system which is independent of the A10 DA region. In addition, motivational processing appears to be modulated by opioid peptides in a fundamentally different way in the interpeduncular nucleus in contrast to that observed in either the dorsal or ventral A10 regions. Accordingly, motivational alteration following stressor exposure, does not appear to be reducible to a homogeneous neurochemical substrate or to a single brain region within the mesencephalon.

General Discussion

Stressor provoked variations in mesocorticolimbic DA activity have been associated with a diverse range of behavioral disturbances in infrahuman subjects which may simulate symptoms of clinical depression. In some animal models of depression, it is difficult to differentiate between the motoric and the motivational components of stressor related behavioral deficits associated with variations in mesocorticolimbic DA activity (e.g., shuttle escape deficits, forced swim induced immobility). However, other behavioral paradigms appear to be of heuristic value in defining the motivational changes associated with uncontrollable stressor exposure. In this respect, the present investigation assessed variations in responding for brain stimulation from the mesencephalon following stressor imposition. Stressor provoked patterns of change in self-stimulation performance from the dorsal A10 area, the ventral A10 area, and the interpeduncular nucleus could clearly be differentiated from one other. Brain stimulation performance deficits lasting for at least one week period were observed from the dorsal A10 region, while relatively transient reductions in responding for brain stimulation were detected from the interpeduncular nucleus, and self-stimulation deficits were not evident from the ventral A10 region. These data revealed that (1) although decreases in locomotor activity and rearing are provoked by footshock, motoric variations cannot account for the expression of brain stimulation performance deficits, (2) different portions of the mesencephalon are vulnerable to stressor provoked neurochemical variations associated with behavioral pathology while other regions are resistant, and (3) the neurochemical changes which contribute to reward and/or motivational changes are not limited to DA variations within the mesocorticolimbic system.

The stressor provoked behavioral deficits which were detected following uncontrollable footshock could be differentiated with respect to the duration of behavioral pathology. The stressor related decreases in locomotor activity and rearing which were observed immediately following footshock were no longer evident 24-hours post-stressor. In addition, reduced responding for brain stimulation was noted from both the

interpeduncular nucleus and the dorsal A10 region immediately following footshock, but self-stimulation deficits were detected only from the dorsal A10 region 24-hours and one week post-stressor. Accordingly, the neurochemical variations associated with specific behavioral changes following footshock appear to be heterogeneous with respect to the capacity to adapt to the acute perturbations provoked by stressor exposure. The pattern of behavioral change following footshock may be reminiscent of the tendency for some symptoms of psychological disturbance which develop following aversive experiences to rapidly abate while others are more persistent in nature. However, it should also be considered that although some stressor provoked behavioral variations may be relatively transient in nature, neurochemical alterations which emerge immediately post-stressor have proactive effects on behavior. In this respect, even brief behavioral changes may reflect the initiation of neurochemical changes which may have long term repercussions in terms of vulnerability to the subsequent development of psychopathology. Indeed, in clinical populations, neurochemical alterations contributing to depression are likely dynamic in nature and may develop gradually over months or years, owing to early childhood circumstances, stressful life experiences, and ruminations over past events.

It was of interest that brain stimulation performance deficits from the dorsal A10 region was the only behavioral change which was maintained one week post-stressor. Intuitively, it is appealing to speculate that a dysregulation of DA activity within subregions of the mesocorticolimbic system may contribute to relatively enduring changes in motivational states. However, the observation that footshock provoked self-stimulation deficits from the interpeduncular region emphasizes that the development of anhedonia cannot be ascribed to any one brain system, despite the vast body of literature supporting the significant role of the A10 region in reward processing. It should also be noted that a decrease in brain stimulation from the dorsal A10 region or the interpeduncular nucleus following a single session of footshock does not represent an absolute loss of the ability to respond to rewarding stimuli. Indeed, animals are still responsive to the reinforcing

efficacy of brain stimulation from other mesencephalic sites following footshock, including the ventral A10 region and the substantia nigra. However, the temporal pattern observed in self-stimulation performance across brain regions following stressor imposition may simulate the tendency for the severity and pervasiveness of anhedonia to vary over time in depressed individuals. Moreover, the expression of anhedonia likely reflects a collection of motivational alterations associated with neurochemical changes within multiple brain systems, the development of which may vary as a function of the severity, predictability, duration, and controllability of the stressors which are encountered. Accordingly, certain environmental conditions may favor the development of more widespread neurochemical changes associated with motivational variations, ultimately influencing the degree, duration, and/or type of anhedonia expressed.

The demonstration that opioid receptor ligands ameliorated at least some of the behavioral deficits provoked by footshock suggests that endogenous opioid peptide release may contribute to the immediate ability to cope with aversive stimuli, as well as to protect the organism from the development of protracted pathology. Pretreatment with a low dose of the μ receptor agonist, DAGO, prior to uncontrollable footshock prevented the reductions in locomotor activity ordinarily evident immediately post-stressor and abolished the development of brain stimulation deficits from the dorsal A10 region. In essence, μ receptor stimulation prior to stressor exposure may mimic the neural variations associated with successful behavioral coping in response to controllable stressors, or the neurochemical profile of individuals who are resistant to depression. In contrast, stressor provoked neurochemical variations associated with the emergence of brain stimulation deficits may simulate the neurochemical changes occurring in individuals at risk for the development of psychopathology, owing to a history of stressor exposure and/or a genetic susceptibility to depression. In contrast to DAGO administration, pretreatment with the selective δ receptor agonist, DPDPE, prior to footshock did not attenuate stressor provoked reductions in activity or responding for brain stimulation from the dorsal A10 region

immediately post-stressor, but instead, prevented the emergence of brain stimulation deficits from the dorsal A10 region 24-hours and 168-hours post stressor. Accordingly, δ receptor activation appeared to antagonize the cascading and/or proactive neurochemical changes which may contribute to prolonged behavioral pathology. It is conceivable that the release of endogenous δ receptor ligands may reverse stressor related neurochemical perturbations associated with pathology, contributing to the tendency for some individuals to recover rapidly from aversive experiences while others exhibit more prolonged affective and/or motivational changes in response to stressors.

The observation that intraventricular opioid administration reversed brain stimulation deficits from the dorsal A10 region when administered after footshock suggests that opioids may have therapeutic effects on behavioral pathology. Both μ and δ receptor agonists reversed the brain stimulation deficits which were evident from the dorsal A10 region immediately following footshock exposure. In addition, DPDPE administration resulted in the abolishment of brain stimulation performance deficits one week post-stressor while moderate brain stimulation deficits were still apparent in mice treated with DAGO. The rapid reversal of behavioral pathology following acute opioid administration is impressive in view of the chronic regimens required to reverse brain stimulation deficits with antidepressant agents. For example, at least 10 days of mianserin administration is required to attenuate stressor induced brain stimulation deficits from the A10 region (Moreau *et al.*, 1994), and 15 days of desmethylinipramine administration is necessary to reverse brain stimulation deficits from the prefrontal cortex (Wolfe *et al.*, 1991a). Accordingly, opioid agonists, and δ receptor ligands in particular, appear to exert rapid and persistent antidepressant effects with acute administration.

The functional interaction which may exist between δ receptors and 5-HT release is particularly intriguing in view of the well documented role of 5-HT in depression and the therapeutic efficacy of pharmacological agents selective to 5-HT receptors in ameliorating depressive symptomatology. Indeed, there is evidence that the antidepressant properties of

agents which influence 5-HT activity may be attributable, at least in part, to an interaction between 5-HT and DA in the A10 region. For example, chronic administration of selective 5-HT reuptake inhibitors, such as fluoxetine, enhance extracellular DA levels in the prefrontal cortex and nucleus accumbens (e.g., Tanda *et al.*, 1994). Administration of selective 5-HT_{1C} receptor antagonists directly to the A10 region enhances DA cell activity (Prisco *et al.*, 1994), and numerous antidepressants appear to exert an antagonistic action at 5-HT_{1C} receptors, including the selective 5-HT reuptake inhibitors, sertraline, fluvoxamine, and citalopram, as well as conventional tricyclic antidepressants and monoamine oxidase inhibitors (Jenck *et al.*, 1994). Moreover, systemic administration of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, which has been demonstrated to have mixed antidepressant/anxiolytic properties in clinical populations, enhances the firing rate of subpopulations of A10 DA neurons, enhances DA levels in the prefrontal cortex and nucleus accumbens, and has rewarding properties as assessed in conditioned place preference paradigms (Prisco *et al.*, 1994; Rasmussen *et al.*, 1994; Papp *et al.*, 1991). Finally, 8-OH-DPAT and other 5-HT_{1A} agonists (e.g., buspirone, ipsapirone, gepirone) provoke antidepressant effects in several animal models of depression following acute administration (De Vry *et al.*, 1993; Curzon *et al.*, 1992). Accordingly, the rapid reversal of brain stimulation deficits by δ receptor activation, as well as the prevention of long term pathology, may be consistent with an enhancement of 5-HT within the A10 region. In this respect, anhedonia associated with δ A variations may not represent hypofunction of the mesocorticolimbic system *per se*, but rather, reflect a dysregulation of the "coding" of at least some types of reward owing to a disruption in the complex interactions between DA and other neurotransmitters and neuropeptides which interact within the A10 area. Such a proposal may account for the relative paucity of data supporting a consistent role for DA in depression despite its well defined role in motivation and reward processing.

Owing to the rapid reversal of brain stimulation performance deficits by μ or δ receptor stimulation and the prevention of long term pathology by δ receptor ligands, it is

tempting to speculate that opioid receptor agonists may be of value in treating depression. However, it must be considered that while μ and δ receptor agonists antagonized immediate and/or long term brain stimulation performance deficits from the dorsal A10 region, μ receptor agonists provoked self-stimulation deficits from the ventral A10 region which is ordinarily resistant to pathology, while δ receptor activation interfered with the recovery of self-stimulation responding, and presumably neurochemical adaptation, from the interpeduncular region. Accordingly, activation of opioid receptors does not have a uniform protective or therapeutic effect with respect to neurochemical and behavioral adaptation to stressors. In fact, excessive opioid activity within some brain regions appears to produce or prolong behavioral pathology. In this respect, it is not surprising that non-selective opioid compounds which stimulate opioid receptors throughout the brain are not effective treatments for clinical depression. For example, the administration of β -endorphin, methadone, or morphine, have been noted to be ineffective in alleviating depressive symptomatology or to provoke only transient mood elevations in depressed patients (Schmauss *et al.*, 1988; Berger *et al.*, 1987; Catlin *et al.*, 1982; Pickar *et al.*, 1982). Nevertheless, as more selective opioid receptor agonists are developed and the distribution of opioid receptor subtypes in the brain is more precisely defined, it may be possible to selectively activate opioid receptors in discreet brain regions which convey therapeutic antidepressant effects.

It should also be emphasized that the current investigation revealed that the efficacy of opioid receptor agonists in ameliorating long term motivational variations varied according to the time at which the opioid agonists were administered relative to stressor imposition. In this respect, the efficacy of selective μ and δ receptor agonists appears to be dependent upon the neurochemical variations onto which they are superimposed. Accordingly, selective opioid receptor ligands may be effective therapeutic agents in only subtypes of depression, comparable to the varied response rates of depressed populations to all classes of antidepressant agents (Joffe *et al.*, 1995). There is evidence from post-

mortem analyses and PET scans that binding for μ and δ receptors is increased in the frontal and temporal cortices of at least some depressed individuals, potentially representing compensatory changes attributable to reduced levels of endogenous agonists (Frost, 1993; Caldecott-Hazard *et al.*, 1991b; Biegon *et al.*, 1990; Holiday *et al.*, 1986). However, considering peripheral indicators of endogenous levels of opioid peptides, or any central neurotransmitter or neuropeptide for that matter are inadequate, identifying depressed individuals who may be responsive to opioid ligands is clearly not feasible at the present time.

It should be further noted that many classes of antidepressant agents influence endogenous opioid systems. For example, chronic treatment with numerous antidepressants (e.g., imipramine, amitriptyline, citalopram, rolipram) has been noted to increase central met-enkephalin, leu-enkephalin, and β -endorphin levels and to enhance central opioid receptor density in mice and rats (Crawley *et al.*, 1986; Przewlock *et al.*, 1985; DeFelipe *et al.*, 1985; Baraldi *et al.*, 1983; Antkiewicz-Michaluk *et al.*, 1984). Electroconvulsive shock in infrahuman subjects has also been observed to enhance central met-enkephalin, β -endorphin and dynorphin levels, and to enhance the biosynthesis of the opioid precursors, pro-enkephalin and pro-dynorphin (reviewed in Yamada *et al.*, 1995). Accordingly, the therapeutic efficacy of current antidepressant treatments may be attributable, at least in part, to an enhancement of endogenous opioid activity. Owing to the relatively rapid effects of enkephalinase inhibitors in reversing behavioral pathology in animal models of depression (e.g., Tajador-Real *et al.*, 1993; Baamonde *et al.*, 1992; de Felipe *et al.*, 1989), the further development of agents which augment endogenous opioid levels may provide effective means of treating depression with a shorter lag to obtain therapeutic efficacy than classical antidepressants. In this respect, enkephalinase inhibitors have recently been developed (e.g., RB 101) which appear to result in selective activation of δ receptors and do not promote tolerance nor physiological dependence in mice with chronic administration (Noble *et al.*, 1992a, 1992b; Fournie-Zabinski *et al.*, 1992).

3 OF/DE 3

PM-1 3 1/2" x 4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

Pharmacological agents which selectively enhance central opioid activity may prove to have antidepressant properties owing to their modulatory role on central DA activity and/or additional neurotransmitters and neuropeptides within the diverse central systems involved in reward processing.

In conclusion, insight into the neurochemical variations associated with the expression of depressive symptomatology has progressed rapidly over the last four decades, owing in part, to the data derived from animal models of depression. In the 1950s and early 1960s, depression was attributed simply to a hypofunction within noradrenergic systems. Over the next three decades, various hypotheses were put forth which linked depression to alterations of cholinergic, dopaminergic and serotonergic activity. It is now apparent that depression involves a dysregulation among a variety of neurotransmitter and neuropeptide systems, potentially reflecting a failure of these systems to initiate adaptive changes in response to stressful life circumstances. As the understanding of the mechanisms of action of antidepressant medications has progressed from the enhancement of synaptic amine levels, to variations in the density or sensitivity of central receptors, and ultimately, to variations in intracellular signaling pathways and gene transcription, the complexity of the neural variations contributing to depressive illnesses becomes more apparent. Continued research into the environmental conditions which provoke behavioral pathology, the neurochemical variations associated with the emergence or prevention of depression, and the pharmacological agents which reverse behavioral pathology may provide a further understanding of the etiology and effective treatment of depression.

References

- Abercrombie ED, Keefe KA, DiFrischia DF, Zigmond MJ. Differential effects of stress on in vivo dopamine release in the striatum, nucleus accumbens, and the medial frontal cortex. Journal of Neurochemistry 52: 1655-1658, 1989.
- Adell A, Garcia-Marquez C, Armario A. Chronic stress increases serotonin and noreadrenaline in rat brain and sensitizes their responses to a further acute stress. Journal of Neurochemistry 50: 1678-1681, 1988.
- Adler B, Goodman RR, Pasternak GW. Opioid peptide receptors. In A Blorklund, T Hokfelt, MJ Kuhar (eds.), Handbook of Chemical Neuroanatomy: Neuropeptides in the CNS. Elsevier, New York, 1990, 359-394.
- Agneter E, Hoffmann IS, Singer EA, Cubedda LX. Behavior of mesocortical dopamine terminals during single and repetitive stimulation: comparison with nigrostriatal neurons. The Journal of Pharmacology and Experimental Therapeutics 269: 470-476, 1994.
- Akil H. Molecular and integrative mechanism of stress responsiveness: implications for studying affective disorders. In Neurobiology of Affective Disorders, Raven Press, New York, 1993, 51- 58.
- Akil H, Bronstein D, Mansour A. Overview of the endogenous opioid systems: anatomical, biochemical and functional issues. In RJ Rodgers, SJ Cooper (eds.), Endorphins, Opiates and Behavioral Processes. John Wiley and Sons, New York, 1988, 1-23.
- Albanese A, Minciacchi D. Organization of the ascending projections from the ventral tegmental area: a multiple fluorescent retrograde tracer study in the rat. The Journal of Comparative Neurology 216: 406-420, 1983.
- Allen D, Johnson AE, Tempel A, Zukin RS, Luine VN, McEwan BS. Serotonergic lesions decrease μ and δ opiate receptor binding in discrete areas of the hypothalamus and in the central gray. Brain Research 625: 269-275, 1993.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders IV. American Psychiatric Association, Washington, 1994.

Ames D. Depressive disorders among elderly people in long term institutional care. Australian and New Zealand Journal of Psychiatry 27: 379-391, 1993.

Ango A, Federman I, Navarro V, Padua M, Velazquez G. Reward and reinforcement produced by drinking water: Role of opioids and dopamine receptor subtypes. Pharmacology, Biochemistry and Behavior 46: 183-194, 1993.

Angst J. Current issues in the classification and epidemiology of mood disorders. In J Mendlewicz, N Brunello, SZ Langer, G Racagni (eds.), New Pharmacological Approaches to the Therapy of Mood Disorders. Basel, Karger, 1993, 1-7.

Anisman H, Zacharko RM. Depression as a consequence of inadequate neurochemical adaptation in response to stressors. British Journal of Psychiatry 160: 36-43, 1992a.

Anisman H, Zacharko RM. Resistance to stress: multiple neurochemical, behavioral and genetic factors. Journal of Psychopharmacology 6: 8-10, 1992b.

Anisman H, Zalcman S, Shanks N, Zacharko RM. Multisystem regulation of performance deficits induced by stressors. In A Boulton, G Baker & M Iverson (eds.), Animal Models in Psychiatry. Human Press, New York, 1991, 1-57.

Anisman H, Zacharko RM. Multiple neurochemical and behavioral consequences of stressors: implications for depression. Pharmacological Therapeutics 46: 119-136, 1990.

Anseau M, von Frenckell R, Cerfontaine JL, Papart P, Franck G, Timsit-Berthier M, Grenen V, Legros JJ. Blunted response of growth hormone to clonidine and apomorphine in endogenous depression. British Journal of Psychiatry 153: 65-71, 1988.

Antelman M, Knopf S, Caggiula AR, Kocan D, Lysle DT, Edwards DJ. Stress and enhanced dopamine utilization in the frontal cortex: Myth and reality. Annals of the New York Academy of Sciences, 537: 262-272, 1988.

Antkiewicz-Michaluk L, Rokosz-Pelc A, Vetulani J. Increase in cortical ^3H Naloxone binding site density after chronic administration of antidepressant agents. European Journal of Pharmacology, 102, 179-181, 1984.

Arborelius L, Nomikos GG, Hacksell U, Svensson TH. (R)-8-OH-DPAT preferentially increases dopamine release in rat medial prefrontal cortex. Acta Psychiatrica Scandinavica 148: 465-466, 1993.

Areso P, Frazer A. Effect of repeated administration of novel stressors on central β -adrenoceptors. Journal of Neural Transmission 86: 229-235, 1991.

Artymyshyn R, Murray M. Substance P in the interpeduncular nucleus of the rat: Normal distribution and the effects of deafferentation. The Journal of Comparative Neurology 231: 78-90, 1985.

Arvidsson U, Dado RJ, Riedl M, Lee JH, Law PV, Loh HH, Elde R, Wessendorf MW. δ opioid receptor immunoreactivity: Distribution in brain stem and spinal cord and relationship to biogenic amines and enkephalin. The Journal of Neuroscience 15: 1215-1235, 1995.

Asberg M, Bertilsson L, Mortenson B, Scalia CP, Thoren D. CSF monoamine metabolites in depression. Acta Psychiatrica Scandinavica 69: 201-219, 1984.

Aston-Jones G, Chiang C, Alexinsky T. Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. In CD Barnes & O Pompeiano (eds.), Neurobiology of the Locus Coeruleus, Progress in Brain Research 88: 501-520, 1991.

Austin MC, Kalivas PW. Blockade of enkephalinergic and GABAergic mediated locomotion in the nucleus accumbens by muscimol in the ventral pallidum. Japanese Journal of Pharmacology 115: 487-489, 1991.

Baamonde A, Dauge V, Ruiz-Gayo M. Antidepressant effects of endogenous enkephalins protected by systemic RB 101 are mediated by opioid δ and D_1 receptor stimulation. European Journal of Pharmacology 216: 157-166, 1992.

Badiani A, Leone P, Noel MB, Stewart J. Ventral tegmental opioid mechanisms and modulation of ingestive behavior. Brain Research 670: 264-276, 1995.

Balleine B. The acquisition of self-stimulation of the medial prefrontal cortex following exposure to escapable or inescapable footshock. Behavioral Brain Research 43: 167-174, 1991.

Bals-Kubik R, Ableitner A, Herz A, Shippenberg TS. Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. Journal of Pharmacology and Experimental Therapeutics 264: 489-495, 1993.

Bals-Kubik R, Shippenberg TS, Herz A: Involvement of central μ and δ opioid receptors in mediating the reinforcing effects of β -endorphin in the rat. European Journal of Pharmacology 175: 63-69, 1990a.

Bals-Kubik R, Shippenberg TS, Herz A: Neuroanatomical substrates mediating the motivational effects of opioids. in JM van Ree, AH Mulder, VM Wiegart, TB van Wimersma-Geidarus (eds.), New Leads in Opioid Research, Excerpta Medica, Amsterdam, 1990b, pp 11-12,.

Bannon MJ, Roth RH. Pharmacology of mesocortical dopamine neurons. Pharmacological Reviews 35: 53-68, 1983.

Baraldi M, Poggioli R, Vergoni AV, Bertolini A. Antidepressants and opiate interactions: Pharmacological and biochemical evidence. Pharmacological Research Communications 15: 843-857, 1983.

Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: A meta analysis. Neuroscience and Biobehavioral Reviews 19: 39-51, 1995.

Barr GA. Behavioral effects of opiates during development. In M Miller (ed.), Development of the Central Nervous System: Effects of alcohol and opiates. New York, Wiley Press, 1992, 221-254.

Barr GA, Rossi G. Conditioned place preference from ventral tegmental injection of morphine in neonatal rats. Developmental Brain Research 66: 133-136, 1992a.

Barr GA, Hoffer MA, Wang S. Role of the nucleus accumbens in the effects of morphine on separation induced crying in infant rats. Society for Neuroscience Abstracts 18: 660, 1992b.

Bauco P, Wang Y, Wise RA. Lack of sensitization or tolerance to the facilitating effect of ventral tegmental area morphine on lateral hypothalamic brain stimulation reward. Brain Research 617: 303-308, 1993.

Baumeister AA, Hurry H, Curtis W, Charney TM, Wolf E, Leoni RR. The antinociceptive and motivational effects of intranigral injection of opioid agonists. Neuropharmacology 32: 1229-1303, 1993.

Bayer VE, Pickel VM. GABA-labeled terminals form proportionally more synapses with dopaminergic neurons having low densities of tyrosine hydroxylase activity in rat ventral tegmental area. Brain Research, 559: 44-55, 1991.

Bechara A, vand der Kooy. Lesions of the tegmental pedunculopontine nucleus: Effects on the locomotor activity induced by morphine and amphetamine. Pharmacology, Biochemistry and Behavior 42: 9-18, 1992.

Beck CHM. Acute treatment with antidepressant drugs selectively increases the expression of *c-fos* in the rat brain. Journal of Psychiatric Neuroscience 20: 25-32, 1995.

Beck CHM, Fibiger HC. Conditioned fear induced changes in behavior in the expression of the immediate early gene *c-fos*: With and without diazepam pretreatment. The Journal of Neurochemistry 15: 709-720, 1995a.

Beck CHM, Fibiger HC. Chronic desipramine alters stress-induced behaviors and regional expression of the immediate early gene, *c-fos*. Pharmacology, Biochemistry and Behavior. 51: 331-338, 1995b.

Berger PA, Nemeroff CB. Opioid peptides in affective disorders. In HY Meltzer (ed.), Psychopharmacology: The third generation of progress. New York, Raven Press, 1987, 637-646.

Bertolucci-D'Angio M, Serrano A, Driscoll P, Scatton B. Involvement of mesocorticolimbic dopaminergic systems in emotional states. Progress in Brain Research 85: 405-417, 1990.

Bilsky EJ, Calderon SN, Wang T, Bernstein RN, Davis P, Hruby VJ, McNutt RW, Rothman RB, Rice KC, Porreca F. SNC 80, a selective nonpeptidic and systemically active opioid delta agonist. The Journal of Pharmacology and Experimental Therapeutics 273: 359-366, 1995.

Blake MJ, Stein EA, Czech DA. Drinking induced alterations in reward pathways: an in vivo autoradiographic analysis. Brain Research 413: 111-119, 1987.

Blanc G, Herve D, Simon H, Lisoprawski A, Glowinski J, Tassin JP. Response to stress of mesocortico-frontal dopaminergic neurones in rats after long term isolation. Nature 284: 265-267, 1980.

Blustein JE, Whitehouse WG, Calcagnetti DJ, Troisi JR, Margules DL, Bersch PJ. Reduction of learned helplessness by central administration of quaternary naloxone. Physiology and Behavior 51: 1075-1078, 1992.

Boadle-Bider MC, Corley KC, Graves L. Increasing activity of tryptophan hydroxylase from cortex and midbrain of male Fisher 344 rats in response to acute and repeated sound stress. Brain Research 482: 306-316, 1989.

Boix F, Sandor P, Nogueira PJC, Huston JP, Schwarting RKW. Relationship between dopamine release in the nucleus accumbens and place preference induced by substance P injected into the nucleus basalis magnocellularis region. Neuroscience 64: 1045-1055, 1995.

Borowski TB, Kokkinidis L. Long term influence of *d*-amphetamine on mesolimbic brain stimulation reward: Comparison to chronic haloperidol and naloxone effects. Pharmacology, Biochemistry and Behavior 43: 1-15, 1992.

Borsisni F, Melia A. The forced swimming test: Its contribution to the understanding of the mechanisms of action of antidepressant drugs. In GL Gessa, G Serra (eds.), Dopamine and Mental Depression. Oxford, Pergamon Press, 1990, 63-76.

Bowers J, Zacharko RM, Anisman H. Evaluation of stressor effects on intracranial self-stimulation from the nucleus accumbens and the substantia nigra in a current intensity paradigm. Behavioral Brain Research 23: 85-93, 1987.

Bozarth. Ventral tegmental reward system. In J Engel, L Oreland (eds.), Brain Reward Systems and Drugs of Abuse. Raven Press, New York, 1987, 1-17.

Bozarth MA. Neuroanatomical boundaries of the reward-relevant opiate-receptor field in the ventral tegmental area as mapped by the conditioned place preference method in rats. Brain Research 414: 77-84, 1987.

Bozarth MA, Wise RA. Intracranial self-administration of morphine into the ventral tegmental area in rats. Life Sciences 28: 551-555, 1980.

Bradberry CW, Lory JD, Roth RH. The anxiogenic β -carboline FG 7142 selectively increases DA release in rat prefrontal cortex as measured by microdialysis. Journal of Neurochemistry 56: 748-752, 1991.

Braff DL, Geyer MA (1990). Sensorimotor gating and schizophrenia. Archives of General Psychiatry 47: 181-188.

Britt MD, Wise RA. Ventral tegmental site of opiate reward: Antagonism by a hydrophilic opiate receptor blocker. Brain Research 258: 105-108, 1983.

Broekkamp CL, Phillips AG, Cools AR. Facilitation of self-stimulation behavior following intracerebral microinjection of opioids into the ventral tegmental area. Pharmacology, Biochemistry and Behavior 11: 289-295, 1979.

Broekkamp CL, Van den Boggard JH, Heijnen HJ, Rops RH, Cools AR, Van Rossum JM. Separation of inhibiting and stimulating effects of morphine on self-stimulation behavior by intracerebral microinjections. European Journal of Pharmacology 4: 443-446, 1976.

Brown AS, Gershon S. Dopamine and Depression. Journal of Neural Transmission 91: 75-109, 1993.

Brown GW. Life events and affective disorder: Implications and limitations. Psychosomatic Medicine 55: 248-259, 1993.

Brown GW, Adler Z, Bifulco A. Life events, difficulties and recovery from chronic depression. British Journal of Psychiatry, 156, 169-179, 1988.

Bunney BS, Chiodo LA, Grace AA: Midbrain dopamine system electrophysiological functioning: A review and new hypothesis. Synapse 9: 79-94, 1991.

Buzas B, Izenwasser S, Portoghese PS, Cox BM. Evidence for delta opioid receptor subtypes regulating adenylyl cyclase activity in rat brain. Life Sciences 54: 101-106, 1994.

Cabib S, Puglisi-Allegra S. Opposite responses of mesolimbic dopamine system to controllable and uncontrollable aversive experiences. The Journal of Neuroscience 14: 333-3340, 1994.

Cabib S, Puglisi-Allegra S. Genotype-dependent effect of chronic stress on apomorphine-induced alterations of striatal and mesolimbic dopamine metabolism in response to apomorphine. Brain Research 542: 91-96, 1991.

Cabib, S, Oliverio A, Puglisi-Allegra S. Stress induced decrease in 3-methoxytyramine in the nucleus accumbens of the mouse is prevented by naltrexone pretreatment. Life Sciences 45: 1031-1037, 1989.

Cabib S, Kempf E, Schlee C, Oliverio A, Puglisi-Allegra S. Effects of immobilization stress on dopamine and its metabolites in different brain areas of the mouse: role of genotype and stress duration. Brain Research 441: 153-160, 1988.

Cador, M, Rivet JM, Kelley AE, Le Moal M, Stinus L: Substance P, neurotensin and enkephalin injections into the ventral tegmental area: comparative study on dopamine turnover in several forebrain structures. Brain Research 486: 357-363, 1989.

Cador, M, Kelley AE, Le Moal M, Stinus L: D-Ala-Met-enkephalin injection into the ventral tegmental area: Effect on investigatory and spontaneous motor behavior in the rat. Psychopharmacology 96: 332-342, 1988.

Cador, M, Kelley AE, Le Moal M, Stinus L: Ventral tegmental area infusion of substance P, neurotensin and enkephalin: Differential effects on feeding behavior. Neuroscience 18: 659-669, 1986.

Calcagnetti D, Keck RJ, Quatella LA, Scheter MD. Blockade of cocaine induced conditioned place preference: Relevance to cocaine abuse therapeutics. Life Sciences 56: 475-483, 1995.

Caldecott-Hazard S, Morgan DG, DeLeon-Jones F, Overstreet DH, Janowsky D: Clinical and biochemical aspects of depressive disorders II. Transmitter/receptor theories. Synapse 9: 251-301, 1991a.

Caldecott-Hazard S, Guze BH, Kling MA, Baxter LR: Clinical and biochemical aspects of depressive disorders II. Introduction, classification and research techniques. Synapse 9: 185-211, 1991b.

Calenco-Choukroun G, Dauge V, Gacel G, Feger J, Roques BP: Opioid δ and endogenous enkephalins induce differential emotional reactivity than μ agonists after injection in the rat ventral tegmental area. Psychopharmacology 103: 493-502, 1991.

Calenco-Choukroun G, Dauge V, Gacel G, Roques BP: Lesion of DA mesolimbic neurons blocks behavioral effects induced by the endogenous enkephalins but not a μ agonist. European Journal of Pharmacology 209: 267-271, 1991.

Cameron DL, Williams JT. Cocaine inhibits GABA release in the VTA through endogenous 5-HT. Journal of Neuroscience 14: 6763-6767, 1994.

Cameron DL, Williams JT. Dopamine D1 receptors facilitate transmitter release. Nature 366: 344-347, 1993.

Carden SE, Bortot AT, Hofer MA. U50,488 and pentylentetrazol, but not naltrexone, elicit ultrasonic vocalization from rat pups in the home cage. Society for Neuroscience Abstracts 18: 659, 1992.

Carelli RM, Deadwyler SA. A comparison of nucleus accumbens neuronal firing patterns during cocaine self administration and water reinforcement in rats. The Journal of Neuroscience 14: 7735-7746, 1994.

Catlin DH, Gorelick, DA, Gerner RH: Clinical pharmacology of β -endorphin in depression and schizophrenia. In K Vereby (ed.), Opioids in mental illness. Annals of the New York Academy of Sciences 398, 1982, 434-447.

Cervo L, Grignaschio G, Samanin R. The role of the mesolimbic dopaminergic system in the desipramine effect in the forced swimming test. European Journal of Pharmacology 13: 178: 129-133, 1990.

Cha XY, Xu H, Rice KC, Porreca F, Lai J, Ananthans S, Rothman RB. Opioid peptide receptor studies: Identification of a novel δ opioid receptor binding site in rat brain membranes. Peptides 16: 191-198, 1995.

Chang JY, Sawyer SE, Lee RS, Woodward DJ. Electrophysiological and pharmacological evidence for the role of the nucleus accumbens in cocaine self administration in freely moving rats. Journal of Neuroscience 14: 1224-1244, 1994.

Charney DS, Deutch AY, Krystal JH, Southwick SM, Davis MD (1993). Psychobiological mechanisms of posttraumatic stress disorder. Archives of General Psychiatry 50: 294-305.

Chen NH, Reith MEA. Autoregulation and monoamine interactions in the ventral tegmental area in the absence and presence of cocaine: a microdialysis study in freely moving rats. Journal of Pharmacology and Experimental Therapeutics 271: 1597-1610, 1994.

Chen Y, Mestek A, Liu J, Hurley JA, Yu L: Molecular cloning and functional expression of the μ -opioid receptor from rat brain. Molecular Pharmacology 44: 8-12, 1993.

Chrousos GP, Gold PW (1992). The concepts of stress and stress system disorders: Overview of physical and behavioral homeostasis. Journal of the American Medical Association 267: 1244-1252.

Churchill L, Kalivas PW. A topographically organized gamma-aminobutyric projection from the ventral pallidum to the nucleus accumbens in the rat. The Journal of Comparative Neurology 345: 579-595, 1994.

Churchill L, Dilts RP, Kalivas PW. Autoradiographic localization of γ -aminobutyric acid receptors within the ventral tegmental area. Neurochemistry Research 101-106, 1992a.

Churchill L, Kalivas PW. Dopamine depletion produces augmented behavioral responses to a μ , but not a δ -opioid receptor agonist in the nucleus accumbens: Lack of a role for receptor upregulation. Synapse 11: 47-57, 1992b.

Claustre Y, Rivy JP, Dennis T, Scatton B. Pharmacological studies on stress induced increase in frontal cortical dopamine metabolism in the rat. Journal of Pharmacology and Experimental Therapeutics 283: 693-699, 1986.

Contestabile A, Flumerfelt BA. Afferent connections of the interpeduncular nucleus and topographic organization of the habenulo-interpeduncular pathway: An HRP study in the rat. Journal of Comprehensive Neurology 196: 253-270, 1981.

Crawley JN, Corwin RL. Biological actions of cholecystokinin. Peptides 15: 731-755, 1994.

Crow TJ. Specific monoamine systems as reward pathways: Evidence for the hypothesis that activation of the ventral mesencephalon dopaminergic neurones and noradrenergic neurones of the locus coeruleus complex will support self-stimulation responding. In A Wauquier & ET Rolls (eds.), Brain Stimulation Reward. Elsevier Publishing, New York, 1976, 211-237.

Cubeddu LX, Hoffman IS, Talmaciu R. Is the release of dopamine from medial prefrontal cortex modulated by presynaptic receptors? Comparisons with nigrostriatal and mesolimbic terminals. Annals of the New York Academy of Sciences 604: 452-461, 1990.

Cummings JL. Depression and Parkinson's disease: A review. American Journal of Psychiatry 149: 443-454, 1992.

Cunningham ST, Kelley AE. Opiate infusion into nucleus accumbens: contrasting effects on motor activity and responding for conditioned reward. Brain Research 588: 104-114, 1992.

Curzon G, kennett GA, Sarna GS, Whitton PS. The effects of tianeptine and other antidepressants on a rat model of depression. British Journal of Psychiatry 160: 51-55, 1992.

Dalgard OS, Bjork S, Tamis K. Social support, negative life events and mental health. British Journal of Psychiatry 166: 29-34, 1995.

D'Angio M, Scatton B. Feeding or exposure to food odours increases extracellular DOPAC levels (as measured by in vitro voltammetry) in the prefrontal cortex of food-deprived rats. Neuroscience Letters 96: 223-228, 1989.

D'Angio M, Serrano A, Driscoll P, Scatton B. Stressful environmental stimuli increase extracellular DOPAC levels in the prefrontal cortex of hypoemotional (Roman high-avoidance) but not hyperemotional (Roman low-avoidance) rats. An in vivo voltammetric study. Brain Research 451: 237-247, 1988.

D'Angio M, Serrano A, Rivy JP, Scatton B. Tail-pinch stress increases extracellular DOPAC levels (as measured by in vivo voltammetry) in the rat nucleus accumbens but not frontal cortex: Antagonism by diazepam and zolpidem. Brain Research 409: 169-174, 1987.

D'Aquila PS, Collu M, Pani L, Cessa GL, Serra G. Antidepressant-like effect of selective dopamine D1 receptor agonists in the behavioral despair animal model of depression. European Journal of Pharmacology 262: 107-111, 1994.

Darko DF, Risch SC, Gillin JC, Golshan S. Association of β -endorphin with specific clinical symptoms of depression. American Journal of Psychiatry 149: 1162-1167, 1992.

Dauge V, Kalivas PW, Duffy T, Roques BP: Effect of inhibiting enkephalin catabolism in the VTA on motor activity and extracellular dopamine. Brain Research 599: 209-214, 1992.

David V, Cazala P. A comparative study of self-administration of morphine into the amygdala and the ventral tegmental area in mice. Behavioral Brain Research 65: 205-211, 1995.

Debonnel G, Gaudreau P, Querion R, de Motigny C. Effects of long term haloperidol treatment on the responsiveness of accumbens neurons to cholecystokinin and dopamine: electrophysiological and radioligand binding studies in the rat. Journal of Neuroscience 10: 469-478, 1990.

de Felipe MD, Jimenez I, Castro A, Fuentes JA. Antidepressant action of imipramine and iprindole in mice is enhanced by inhibitors of enkephalin degrading peptidases. European Journal of Pharmacology 159: 175-180, 1989.

Delagrangé P, Cenu MH, Rougeau A, Burer P, Bouyer JJ. Effects of locus coeruleus lesions on vigilance and attentive behavior in the cat. Behavioral Brain Research 53: 155-165, 1993.

Delay-Goyet P, Zajac JM, Roques BP: Improved quantitative autoradiography of rat brain δ opioid binding sites using ^3H DSTBULET, a new highly potent and selective linear enkephalin analogue. Neurochemistry International 16: 341-368, 1990.

Del Zompo M, Bocchetta A, Bernardi F, Burrai C, Corsini GU: Clinical evidence for a role of dopaminergic system in depressive syndromes. In GL Gessa, G Serra (eds.), Dopamine and Mental Depression. Pergamon Press, Oxford, 1990, 177-184.

DeMontis GM, Devoto P, Gessa GL, Meloni D, Porcella A, Saba P, Serra G, Tagliamonte A. Central dopaminergic transmission is selectively increased in the limbic system of rats chronically exposed to antidepressants. European Journal of Pharmacology 180: 31-35, 1990.

DeMontis GM, Devoto P, Gessa GL, Meloni D, Porcella A, Saba P, Serra G, Tagliamonte A. Chronic imipramine reduces [3H]SCH 23390 binding and DA sensitive adenylate cyclase in limbic system. European Journal of Pharmacology 167: 299-304, 1989.

DePaoli AM, Hurley KM, Yasada K, Reisine T, Bell G. Distribution of kappa opioid receptors mRNA in adult mouse brain: an in situ hybridization histochemistry study. Molecular and Cellular Neurosciences 5: 327-335, 1994.

Deutch AY, Cameron DS. Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. Neuroscience 46: 49-56, 1992.

Deutch AY, Lee MC, Gillham MH, Cameron DA, Goldstein M, Iadarola MJ. Stress selectively increases Fos protein in dopamine neurons innervating the prefrontal cortex. Cerebral Cortex 1: 273-292, 1991.

Deutch AY, Roth RH. The determinants of stress-induced activation of the prefrontal cortical dopamine system. Progress in Brain Research 85: 367-403, 1990.

Deutch AY, Tam SY, Roth RH. Footshock and conditioned stress increase 3, 4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. Brain Research, 333: 143-146, 1985a.

Deutch AY, Maggio JE, Bannon MJ, Kalivas PW, Tam SY, Goldstein M, Roth RH. Substance K and substance P differentially modulate mesolimbic and mesocortical systems. Peptides 6: 113-122, 1985b.

Devine DP, Wise RA. Self-administration of morphine, (D-Ala², N-Me-Phe⁴-Gly⁵-ol)-enkephalin (DAGO), and (D-Pen², D-Pen⁵)-enkephalin (DPDPE) into the ventral tegmentum of the rat. Journal of Neuroscience 14: 1978-1984, 1994.

Devine DP, Leaoe P, Pocock D, Wise RA: Differential involvement of ventral tegmental mu, delta, and kappa opioid receptors in modulation of basal mesolimbic dopamine release: in vivo microdialysis studies. Journal of Pharmacology and Experimental Therapeutics 266: 1236-1246, 1993a.

Devine DP, Leone P, Wise RA: Mesolimbic dopamine neurotransmission is increased by administration of μ -opioid receptor antagonists. European Journal of Pharmacology 243: 55-64, 1993b.

Devine DP, Leone P, Carlezon WA, Wise RA: Ventral mesencephalic delta opioid receptors are involved in modulation of basal mesolimbic dopamine neurotransmission: an anatomical localization study. Brain Research 622: 348-352, 1993c.

De Vry J, Schreiber R. Comparison of acute and repeated treatment with the 5-HT_{1A} receptor ligands 8-OH-DPAT and ipsapirone in animal models of anxiety and depression. Drug Development Research 30: 91-103, 1993.

De Witte PH, Heidbreder CH, Roques BP. Kelatorphan, a potent enkephalinase inhibitor, and opioid receptor agonists DAGO and DTLET, differentially modulate self-stimulation behavior depending on the site of administration. Neuropharmacology 28: 667-676, 1989.

Di Chiara G, Imperato A. Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. Journal of Pharmacology and Experimental Therapeutics 244: 1067-1080, 1988.

Dickson R. Stressor provoked alterations in locomotor activity, exploration, and self-stimulation from the ventral tegmental area following intraventricular administration of U50, 488 in the mouse. Unpublished master's thesis, Carleton University, Ottawa, Ontario, Canada, 1993.

Diehl DJ, Gershon S. The role of dopamine in mood disorders. Comprehensive Psychiatry 33: 115-120, 1992.

Dilts RP, Kalivas PW: Autoradiographic localization of delta opioid receptors within the mesocorticolimbic dopamine system using radioiodinated DPDPE. Synapse 6: 121-132, 1990.

Dilts RP, Kalivas PW: Autoradiographic localization of μ opioid and neurotensin receptors within the mesolimbic dopamine system. Brain Research 488: 311-327, 1989.

Drugan RC, Basile AS, Ha JH, Ferland RJ. The protective effects of stress control may be mediated by increased brain levels of benzodiazapine receptor agonists. Brain Research 661: 127-136, 1994.

Druhan JP, Deschamps SE, Stewart J. D-amphetamine-like stimulus properties are produced by morphine injections into the ventral tegmental area but not into the nucleus accumbens. Behavioral Brain Research 59: 41-51, 1993.

Dunn AJ. Changes in plasma and brain tryptophan and 5-Hydroxyindoleacetic acid after foot shock stress. Life Sciences 42: 1847-1853, 1988.

Dunn AJ, File SE. Cold restraint alters dopamine metabolism in frontal cortex, nucleus accumbens and neostriatum. Physiology and Behavior 31: 511-513, 1983.

Edwards E, Kornich W, Van Houtten P, Henn FA. In vitro neurotransmitter release in an animal model of depression. Neurochemistry International 21: 29-35, 1992.

Elliot PJ, Mason GS, Graham EA, Turpin MP, Hagan RM. Modulation of the rat mesolimbic pathway by neurokinins. Behavioural Brain Research 51: 77-82, 1992.

Emmerson PJ, Liu MR, Woods JH, Medjhradsky F. Binding affinity and selectivity of opioids at mu, delta, and kappa receptors in monkey brain membrane. Journal of Pharmacology and Experimental Therapeutics 271: 1630-1637, 1994.

Etienne PE, Zubensko GS. Does captopril elevate mood? Trends in Pharmacology 8: 329-331, 1987.

Fawcett J, Clark DC, Scheftner WA, Hedeker D. Differences between anhedonic and normally hedonic depressive states. American Journal of Psychiatry 140: 1027-1030, 1983.

Fadda F, Argioloas A, Melis MR, Tissari AH, Onali PL, Gessa GL. Stress induced increase in 3, 4-Dihydroxyphenylacetic acid (DOPAC) levels in the cerebral cortex and in nucleus accumbens: Reversal by diazepam. Life Sciences 23: 2219-2224, 1978.

Fallon JH, Ciofi P. Dynorphin containing neurons. In A Blorklund, T Hokfelt, MJ Kuhar (eds.), Handbook of Chemical Neuroanatomy: Neuropeptides in the CNS. Elsevier, New York, 1990, 1-130.

Fallon JH, Leslie FM. Distribution of dynorphin and enkephalin peptides in the rat brain. Journal of Comprehensive Neurology 249: 293-336. 1986.

Fallon HF. Topographic organization of ascending dopaminergic projections. Annals of the New York Academy of Sciences 537: 1-9, 1988.

Ferris RM, Cooper BR. Mechanism of antidepressant activity of bupropion. Journal of Clinical Psychology Monographs 11: 2-14, 1993.

Fibiger HC, LePiane FG, Jakubovik A, Phillips AG. The role of dopamine in intracranial self-stimulation of the ventral tegmental area. The Journal of Neuroscience 7: 3888-3896, 1987.

Finlay JM, Zigmond MJ, Abercrombie ED. Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: Effects of diazepam. Neuroscience 64: 619-628, 1995.

Fiorino DF, Courty A, Fibiger HC, Phillips AG. Electrical stimulation of reward sites in the ventral tegmental area increases dopamine transmission in the nucleus accumbens of the rat. Behavioral Brain Research 55: 131-141, 1993.

Foote SL, Berridge CW, Adams LM, Pineda JA. Electrophysiological evidence for the involvement of the locus coeruleus in alerting, orienting and attending. In CD Barnes & O Pompeiano (Eds.), Neurobiology of the Locus Coeruleus, Progress in Brain Research 88: 521-533, 1991.

Fouriez G, Franc S. Apomorphine and electrical self-stimulation of rat brain. Behavioral Brain Research 52: 73-80, 1992.

Frost JJ. Receptor imaging by PET and SPECT: Focus on the opioid receptor. Journal of Receptor Research 13: 39-53, 1993.

Fuxe K, Li XM, Bjelke B, Hedlund PB, Biagini G, Agnati LF. Possible mechanisms for the powerful actions of neuropeptides. Annals of the New York Academy of Sciences 718: 42-59, 1995.

Fuxe K, Agnati LF. Two principal modes of electrochemical communication in the brain: Volume versus wiring transmission. In K Fuxe, L Agnati (eds.), Volume Transmission in the Brain: Novel Mechanisms for Neural Transmission, New York, Raven Press, 1991, 1-9.

Fuxe K, Agnati LF, Kalia M, Goldstein M, Andersson K, Harfstrand A. In E Fluckiger, EE Muller, MO Thoner (eds.), The Dopaminergic System. Springer-Verlag, New York, 1985, 11-26.

Gaetano DC, Imperato A: Opposite effects of mu and kappa agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. The Journal of Pharmacology and Experimental Therapeutics 244: 1067-1080, 1987.

Gambarana C, Ghiglieria O, Tagliamonte A, D'Alessandra N, de Montis MG. Crucial role of D₁ dopamine receptors in mediating the antidepressant effects of imipramine. Pharmacology, Biochemistry and Behavior 50: 147-151, 1995.

Ganon FG. Nonlinear relationship between impulse flow and dopamine released by midbrain dopaminergic neurons as studies by in vivo electrochemistry. Neuroscience 24: 19-28, 1988.

Gariono RF, Graves PM. Burst firing induced in midbrain dopamine neurons by stimulation of the medial prefrontal cortex and anterior cingulate cortices. Brain Research 462: 194-198, 1988.

Gasbari A, Verney C, Innacenzi R, Campana E, Pacitti C. Mesolimbic dopaminergic neurons innervating the hippocampal formation in the rat: A combined retrograde tracing and immunohistochemical study. Brain Research 668: 71-79, 1994.

Geoffroy M, Christensen AV. Psychomotor stimulants versus antidepressants in the learned helplessness model of depression. Drug Development Research 29: 48-55, 1993.

German DC, Manaye KF. Midbrain dopaminergic neurons (nuclei A8, A9, and A10): three dimensional reconstruction in the rat. The Journal of Comparative Neurology 331: 297-309, 1993.

German DC, Speciale SG, Manaye KF, Sadeq M: Opioid receptors in midbrain dopaminergic regions of the rat I. Mu receptor autoradiography. Journal of Neural Transmission 91: 39-52, 1993.

Giles DE, Roffwarg HP, Schlessler MA, Rush AJ. Which endogenous depressive symptoms relate to REM latency reduction? Biological Psychiatry 21: 473-482, 1986.

Giorgio O, Pibri MG, Ongini E, Trampus M, Biggio G: The neutral endopeptidase-24.11 (enkephalinase) inhibitor, SCH 32615, increases dopamine metabolism in the nucleus accumbens of the rat. European Journal of Pharmacology 196: 137-142, 1991.

Golden RN, Rudorfer MV, Sherer MA, Linnoila M, Potter WZ. Bupropion in depression. I. Biochemical effects and clinical response. Archives of General Psychiatry 130: 139-143, 1988.

Goldstein LE, Rasmussen AM, Bunney BS, Roth RH. The NMDA glycine site antagonist (+)-HA-966 selectively regulates conditioned stress-induced metabolic activation of the mesoprefrontal cortical but not serotonin systems: A behavioral, neuroendocrine, and neurochemical study in the rat. Journal of Neuroscience 14: 4937-4950, 1994.

Gosnell BA, Patel CK. Centrally administered μ and δ opioid agonists increase operant responding for saccharin. Pharmacology, Biochemistry and Behavior 45: 979-982, 1993.

Grace AA, Bunney BS. Induction of depolarization block in midbrain dopamine neurons by repeated administration of haloperidol: Analysis using in vivo intracellular recording. The Journal of Pharmacology and Experimental Therapeutics 238: 1092-1099, 1986.

Grant SJ, Sonti G. Buprenorphine and morphine produce equivalent increases in extracellular single unit activity of dopamine neurons in the ventral tegmental area in vivo. Synapse 16: 181-187, 1994.

Griffiths J, Shanks N, Anisman H. Strain-specific alterations in consumption of a palatable diet following repeated stressor exposure. Pharmacology, Biochemistry and Behavior 42: 219-227, 1992.

Grimcher PW, Giovino AA, Margolin DH, Hoebel BG. Endogenous opioid reward induced by an enkephalinase inhibitor, thiorphin, injected into the ventral midbrain. Behavioral Neuroscience 98: 262-268, 1984.

Gross-Iseroff R, Cohen E, Shavit Y. Comparisons of μ opioid receptors in brains of rats bred for high or low rate of self stimulation. Physiology and Behavior 51: 1093-1096, 1992.

Guan XM, McBride WJ. Serotonin microinfusion into the ventral tegmental area increases accumbens dopamine release. Brain Research Bulletin 23: 541-547, 1989.

Halliday GM, Tork I. Serotonin-like immunoreactive cells and fibres in the rat mesencephalic tegmentum. Brain Research Bulletin 22: 725-735, 1989.

Halliday GM, Tork I. Substance P-like immunoreactive fibres in the ventromedial mesencephalic tegmentum of the rat. Brain Research Bulletin 21: 659-670, 1988.

Hamill GS, Lenn NJ. The subnuclear organization of the rat interpeduncular nucleus: A light and electron microscopic study. The Journal of Comparative Neurology 222: 396-408, 1984a.

Hamill GS, Olschowka JA, Lenn NJ, Jacobowitz DM. The subnuclear distribution of substance P, cholecystokinin, vasoactive intestinal peptides, somatostatin, leu-enkephalin, dopamine- β -hydroxylase, and serotonin in the rat interpeduncular nucleus. The Journal of Comparative Neurology 226: 580-596, 1984b.

Hamill GS, Jacobowitz DM. A study of afferent projections to the rat interpeduncular nucleus. Brain Research Bulletin 13: 527-539, 1984c.

Hand TH, Franklin KB. 6-OHDA lesions of the ventral tegmental area block morphine-induced but not amphetamine-induced facilitation of self-stimulation. Brain Research 328: 233-241, 1985.

Handley SL, McBane JW. An assessment of the elevated X-maze for studying anxiety and anxiety modulating drugs. Journal of Pharmacological and Toxicological Methods 29: 129-138, 1993.

Heidbreder CH, Gewiss M, Lallemand S, Roques BP, De Witte PH. Inhibition of enkephalin catabolism and activation of mu or delta opioid receptors elicit opposite effects on reward and motility in the ventral mesencephalon. Neuropharmacology 31: 293-298, 1992.

Heinsbroek RP, van Haaren F, Feenstra MG, Boon P, van de Poll NE. Controllable and uncontrollable footshock and monoaminergic activity in the frontal cortex of male and female rats. Brain Research 551: 247-255, 1991.

Heninger GR. The biological basis of affective disorders: An overview. Neurobiology of Affective Disorders. Raven Press: New York, 1993, 2-6.

Henry DJ, Wise RA, Rompre PP, White FJ. Acute depolarization block of A10 dopamine neurons: interactions of morphine with dopamine antagonists. Brain Research 596: 231-237, 1992.

Herman JP, Stinus L, LeMoal M. Repeated stress increases locomotor response to amphetamine. Psychopharmacology 84: 431-435, 1984.

Herve D, Pickel VM, Joh TH, Beaudet A. Serotonin axon terminals in the ventral tegmental area of the rat: Fine structure and synaptic input to dopaminergic neurons. Brain Research 435: 71-83, 1987.

Herve D, Simon H, Blanc G, LeMoal M, Glowinski J, Tassin JP. Opposite changes in dopamine utilization in the nucleus accumbens and frontal cortex after electrolytic lesions of the median raphe in the rat. Brain Research 1216: 422-428, 1981.

Higgins GA, Nguyen P, Sellers EM. Morphine place conditioning is differentially affected by CCKA and CCKB receptor antagonists. Brain Research 572: 208-215, 1992.

Hirsch SR, Gainer P, Bowen J. The triggering hypothesis of the role of life events in schizophrenia. British Journal of Psychiatry 161: 84-87, 1992.

Hoffmann IS, Talmaciu RK, Ferro CP, Cubeddu LX. Sustained high release at rapid stimulation rates and reduced functional autoreceptors characterize prefrontal cortex dopamine terminals. Journal of Pharmacology and Experimental Therapeutics 245: 761-772, 1988.

Hokfelt T. Neuropeptides in perspective: The last ten years. Neuron 7: 867-879, 1991.

Hooks MS, Kalivas PW. The role of mesoaccumbens-pallidal circuitry in novelty induced behavioral activation. Neuroscience 64: 587-597, 1995.

Hooks MS, Colvin AC, Juncos JL, Justice JB. Individual differences in basal and cocaine stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. Brain Research 587: 306-312, 1992.

Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse 9: 121-128, 1991.

Hunt GE, Atrens DM, Jackson DM. Reward summation and the effects of dopamine D₁ and D₂ agonists and antagonists on fixed interval responding for brain stimulation. Pharmacology, Biochemistry and Behavior 48: 853-862, 1994.

Hunt N, Bruce-Jones W, Silverstone T. Life events and relapse in bipolar affective disorder. Journal of Affective Disorders 25: 13-20, 1991.

Hunziker MHL. Opioid nature of learned helplessness and stress induced analgesia observed with reexposure to shock. Behavioral Pharmacology 3: 117-121, 1992.

Hurd YL, Pristupa ZB, Herman MM, Niznik HB, Klunman JE. The dopamine transporter and dopamine D₂ receptor messenger RNAs are differentially expressed in limbic and motor related subpopulations of human mesencephalic neurons. Neuroscience 63: 357-362, 1994.

Ikai Y, Takada M, Shinonaga Y, Mizuno N. Dopaminergic and non-dopaminergic neurons in the ventral tegmental area of the rat project, respectively, to the cerebellar cortex and deep cerebellar nuclei. Neuroscience 51: 719-728, 1992.

Imperato A, Obinu MC, Cabib S, Cestari V, Puglisi-Allegra S. Effects of subchronic minaprine on dopamine release in the ventral striatum and on immobility in the forced swimming test. Neuroscience Letters 166: 69-72, 1994.

Imperato A, Puglisi-Allegra S, Casolini P, Angelucci L. Changes in dopamine and acetylcholine release during and following stress are independent of the pituitary-adrenocortical axis. Brain Research 538: 111-117, 1991.

Imperato A, Puglisi-Allegra S, Zocchi A, Scrocco MG, Casolini P, Angelucci L. Stress activation of limbic and cortical dopamine release is prevented by ICS 205-930 but not diazepam European Journal of Pharmacology 178: 211-214, 1990a.

Imperato A, Honore T, Jensen L. Dopamine release in the nucleus caudate and nucleus accumbens is under glutaminergic control through non-NMDA receptor: a study in freely moving rats. Brain Research 530: 223-228, 1990b.

Inoue T, Tsuchiya K, Koyama T. Regional changes in dopamine and serotonin activation with various intensities of physical and psychological stress in the rat brain. Pharmacology, Biochemistry and Behavior 49: 911-920, 1995.

Inverizzi R, Pozzi L, Vallebuona F, Bonini I, Sacchetti G, Samanin R. Effects of amineptine on regional extracellular concentrations of dopamine and noradrenaline in the rat brain. Journal of Pharmacology and Experimental Therapeutics 262: 769-774, 1992a.

Inverizzi R, Pozzi L, Garattini S, Samanin R. Tianeptine increases the extracellular concentrations of dopamine in the nucleus accumbens by a serotonin-independent mechanism. Neuropharmacology 31: 221-227, 1992b.

Iuvone PM, Dunn AJ. Tyrosine hydroxylase activity in mesocortical 3,4-dihydroxyphenylethylamine neurons following footshock. Journal of Neurochemistry 47: 837-844, 1986.

Ivengar S, Kim HS, Marien MR, McHugh D, Wood PL: Modulation of mesolimbic dopaminergic projections by beta-endorphin in the rat. Neuropharmacology 28: 123-128, 1989.

Iwahori N, Koaori N, Kameda S. Terminal patterns of the fasciculus retroflex in the interpeduncular nucleus of the mouse: a Golgi study. Anatomy and Embryology 187: 523-528, 1993a.

Iwahori N, Koaori N, Kameda S, Yahara H. Terminal patterns of the tegmental afferents in the interpeduncular nucleus: a Golgi study in the mouse. Anatomy and Embryology 188: 593-599, 1993b.

Jackson RL, Alexander JH, Maier SF. Learned helplessness, inactivity and associative deficits: Effects of inescapable shock on response choice escape learning. Journal of Experimental Psychology 6: 1-20, 1980.

Jayaraman A, Nishimori T, Dobner P, Uhl GR. Cholecystokinin and neurotensin mRNAs are differentially expressed in subnuclei of the ventral tegmental area. Journal of Comprehensive Neurology 296: 291-302, 1990.

Jenck F, Moreau JL, Mutel V, Martin JR. Brain 5-HT_{1C} receptors and antidepressants. Progress in Neuro-Psychopharmacology and Biological Psychiatry 18: 563-574, 1994.

Jenck F, Gratton A, Wise RA. Opioid receptor subtypes associated with ventral tegmental facilitation and periaqueductal gray inhibition of feeding. Brain Research 423: 39-44, 1987.

Jenck F, Gratton A, Wise RA. Opioid receptor subtypes associated with ventral tegmental facilitation of lateral hypothalamic brain stimulation reward. Brain Research 423: 34-38, 1987.

Joffe RT, Levitt AJ. Antidepressant failure: Augmentation or substitution? Journal of Psychiatric Neuroscience 20: 7-9, 1995.

Johnson PI, Stellar JR. Effects of accumbens DALA microinjections on brain stimulation reward and behavioral activation in intact and 6-OHDA treated rats. Psychopharmacology 114: 665-671, 1994.

Johnson PI, Stellar JR. Comparison of delta opiate receptor agonist induced reward and motor effects between the ventral pallidum and dorsal striatum. Neuropharmacology 33: 1171-1182, 1994.

Johnson SW, North RA. Two types of neurons in the rat ventral tegmental area and their synaptic inputs. Journal of Physiology 450: 455-468, 1992a.

Johnson SW, North RA. Opicids excite dopamine neurons by hyperpolarization of local interneurons. Journal of Neuroscience 12: 438-489, 1992b.

Johnson SW, Mercuri NB, North RA. 5-hydroxytryptamine_{1B} receptors block the GABA_B synaptic potential in rat dopamine neurons. Journal of Neuroscience 208: 435-346, 1992c.

Kalivas PW, Duffy P. Time course of extracellular dopamine and behavioral sensitization to cocaine. Dopamine axon terminals. The Journal of Neuroscience 13: 266-275, 1993.

Kalivas PW. Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Research Reviews 18: 75-113, 1993.

Kalivas PW, Churchill L, Klitenick MA. GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. Neuroscience 57: 1047-1060, 1993.

Kalivas PW, Duffy P. A comparison of axonal and somatodendritic dopamine release using in vivo dialysis. Journal of Neurochemistry 56: 961-967, 1991.

Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug and stress induced sensitization of motor activity. Brain Research Reviews 16: 223-244, 1991.

Kalivas PW, Duffy P, Eberhart H. Modulation of A10 dopamine neurons by γ -aminobutyric acid agonists. Journal of Pharmacology and Experimental Therapeutics 253: 858-866, 1990.

Kalivas PW, Duffy P, Barrow J. Regulation of the mesocortical dopamine system by glutamic acid receptor subtypes. Journal of Pharmacology and Experimental Therapeutics 251: 378-387, 1989.

Kalivas PW, Duffy P, Dilts R, Abhold R. Enkephalin modulation of A10 dopamine neurons: A role in dopamine sensitization. Annals of the New York Academy of Sciences 537: 405-414, 1988.

Kalivas PW, Abhold R. Enkephalin release into the ventral tegmental area in response to stress: Modulation of mesocorticolimbic dopamine. Brain Research 414: 339-348, 1987a.

Kalivas PW, Duffy P. Sensitization to repeated morphine injection in the rat: Possible involvement of A10 dopamine neurons. Journal of Pharmacology and Experimental Therapeutics 241: 204-212, 1987b.

Kalivas PW, Richardson-Carlson R. Endogenous enkephalin modulation of dopamine neurons in the ventral tegmental area. American Journal of Physiology 251: R243-R249, 1986.

Kalivas PW. Interactions between neuropeptides and dopamine neurons in the ventromedial mesencephalon. Neuroscience and Biobehavioral Reviews 9: 573-587, 1985.

Kamata K, Yoshida S, Kameyama T. Antagonism of footshock stress-induced inhibition of intracranial self-stimulation by naloxone or methamphetamine. Brain Research, 371: 197-200, 1986.

Kapur S, Mann JJ. Role of the dopaminergic system in depression. Biological Psychiatry 32: 1-17, 1992.

Katoh A, Nabeshima T, Kameyama T. Interaction between enkephalinergic and dopaminergic systems in stressful situations. European Journal of Pharmacology 193: 95-99, 1991.

Katoh A, Nabeshima T, Kameyama T. Behavioral changes induced by stressful situations: Effects of enkephalins, dynorphin and their interactions. The Journal of Pharmacology and Experimental Therapeutics 253: 600-607, 1990.

Katz RJ. Endorphins, exploration and activity. In RJ Rodgers, SJ Cooper (eds.), Endorphins, Opiates and Behavioural Processes. John Wiley and Sons, Chichester, 1988, 249-267.

Katz RJ, Gormezano G. A rapid inexpensive technique for assessing the reinforcing effects of opiate drugs. Pharmacology, Biochemistry and Behavior 11: 231-233, 1979.

Kelland MD, Zhang J, Chiodo LA, Freeman AS. Receptor selectivity of cholecystokinin effects on mesoaccumbens dopamine neurons. Synapse 8: 137-143.

Keller RW, Zigmond MJ, Stricker EM. Striatal dopamine release in freely moving rats during cold stress. Society for Neuroscience Abstracts 7: 155, 1983.

Kelley AE, Delf JM. Dopamine and conditioned reinforcement II. Contrasting effects of amphetamine microinjection into the nucleus accumbens with peptide microinjection into the ventral tegmental area. Psychopharmacology 103: 197-203, 1991.

Kelley AE, Cadot M, Stinus L, LeMoal M. Neurectensin, substance P, neurokinin and enkephalin: Injection into ventral tegmental area in the rat produces differential effects on operant responding. Psychopharmacology 97: 243-252, 1989.

Kelley, AE, Cadot M. Behavioral evidence for differential neuropeptide modulation of the mesolimbic dopamine system. Annals of the New York Academy of Sciences 537: 415-434, 1988.

Kemali D. A multicenter Italian study of amineptine. Clinical Neuropharmacology 12: 41-50, 1989.

Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ. The clinical characteristics of major depression as indices of the familial risk to illness. British Journal of Psychiatry 165: 66-72, 1994.

Kendler KS, Kessler RC, Neale MC. The prediction of major depression in women: Toward an integrated etiological model. American Journal of Psychiatry 150: 1139-1148, 1993.

Kendler KS, Neale MC, Kessler RC. A population based twin study of major depression in women. Archives of General Psychiatry 49: 252-266, 1992.

Kennet GA. 5-HT_{1C} receptor antagonists have anxiolytic-like actions in the rat social interaction model. Psychopharmacology 107: 379-384, 1992.

Kessler RC, McGongle KA, Swartz M, Blazer DG, Nelson CB. Sex and depression in the national comorbidity survey I: Lifetime prevalence, chronicity and recurrence. Journal of Affective Disorders 29: 85-96, 1993.

Kim HS, Iyenger S, Wood PL. Reversal of the actions of morphine on mesocortical dopamine metabolism in the rat by the kappa agonist MR-2034: Tentative μ^2 control of mesocortical dopaminergic projections. Life Sciences 41: 1711-1715, 1987.

Kim HS, Iyengar S, Wood PL. Opiate actions on mesocortical dopamine metabolism in the rat. Life Sciences 39: 2033-2036, 1986.

Kiyatkin EA, Wise RA, Gratton A. Drug and behavior-associated changes in dopamine related electrochemical signals during intravenous heroin self-administration in rats. Synapse 14: 60-72, 1993.

Kiyatkin EA. Morphine-induced modification of the functional properties of ventral tegmental neurons in conscious rat. International Journal of Neuroscience 41: 57-70, 1988.

Klimek V, Zak-Knapik J, Mackowiak M. Effects of repeated treatment with fluoxetine and citalopram, 5-HT uptake inhibitors, on 5-HT_{1a} and 5-HT₂ receptors in the rat brain. Journal of Psychiatric Neuroscience 19: 63-67.

Klimek V, Maj J. Repeated administration of antidepressants enhances agonist affinity for mesolimbic D₂ receptors. Journal of Pharmacology 41: 555-558, 1989.

Klimek V, Nielsen M. Chronic treatment with antidepressants decreases the number of 3H-SCH 23390 binding sites in the rat striatum and limbic system. European Journal of Pharmacology 139: 163-169, 1987.

Klitenick MA, Kalivas PW. Behavioral and neurochemical studies of opioid effects in the pedunculo-pontine nucleus and mediodorsal thalamus. Journal of Pharmacology and Experimental Therapeutics 269: 427-438, 1994.

Klitenick MA, Deutch AY, Churchill L, Kalivas PW. Topography and functional role of dopaminergic projections from the ventral mesencephalic tegmentum to the ventral pallidum. Neuroscience 50: 371-386, 1992a.

Klitenick MA, DeWitte P, Kalivas PW. Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study. The Journal of Neuroscience 12: 2623-2632, 1992b.

Knapp CM, Kornetsky C. Bromocriptine, a D₂ receptor agonist, lowers the threshold for rewarding brain stimulation. Pharmacology, Biochemistry and Behavior 49: 901-904, 1995.

Knauper B, Wittchen HU. Diagnosing major depression in the elderly: Evidence for response bias in standardized diagnostic interviews. Journal of Psychiatric Research 28: 147-164, 1994.

Kornetsky C, Bain B. Effects of opiates on rewarding brain stimulation. In JE Smith, JD Lane (eds.), The neurobiology of opiate reward processes. Amsterdam, Elsevier Biomedical Press, 1983, 237-256.

Kosobud AEK, Harris GC, Chapin JK. Behavioral associations of neuronal activity in the ventral tegmental area of the rat. The Journal of Neuroscience 14: 7117-7129, 1994.

Kostrzewa RM. Dopamine receptor supersensitivity. Neuroscience and Biobehavioral Reviews 19: 1-7, 1995.

Krappman P, Hasenohrl RU, Frisch C, Huston JP. Self-administration of neurokinin substance P into the ventromedial caudate putamen. Neuroscience 62: 1093-1101, 1994.

Krishnan KR, Rayasam K, Reed D, Smith M, Chapell P, Saunders WB, Ritchie JC, Carroll BJ, Nemeroff CB. The corticotropin releasing factor stimulation test in patients with major depression: relationship to dexamethasone suppression test results. Depression 1: 133-136, 1993.

Latimer LG, Duffy P, Kalivas PW. Mu opioid receptor involvement in enkephalin activation of dopamine neurons in the ventral tegmental area. Journal of Pharmacology and Experimental Therapeutics 241: 328-336, 1987.

Lavielle S, Tassin JP, Thierry AM, Blanc G, Herve D, Barthelemy C, Glowinski J. Blockade by benzodiazapines of the selective high increase in dopamine turnover induced by stress in mesocortical dopaminergic neurons of the rat. Brain Research 168: 585-594, 1978.

Le Moal M, Simon H. Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiology Reviews 71: 155-234, 1991.

Le Moal M. Simon H. Self-stimulation of some fiber systems traced posteriorly from the ventral tegmental area. in A Wauquier & ET Rolls (eds.), Brain Stimulation Reward. Elsevier Publishing, New York. 1976, 55-60.

Leone P, Pocock D, Wise RA: Morphine-dopamine interactions: Ventral tegmental morphine increases nucleus accumbens dopamine release. Pharmacology, Biochemistry and Behavior 39: 469-472, 1991.

Lever JR, Scheffel U, Kinter CM, Ravert HT, Dannals RF, Wagner HN, Frost JJ. In vivo binding of N1 ([11C]methyl)natrindole to δ receptors in mouse brain. European Journal of Pharmacology 216: 459-460, 1992.

Liang CL, Kozlowski GP, German DC. Leucine⁵-enkephalin afferents to midbrain dopaminergic neurons: light and electron microscopic examination. Journal of Comparative Neurology 332: 269-281, 1993.

Lisansky J, Hauger R, Strassman R. β -endorphin response to low dosage of human corticotropin releasing hormone during metyrapone administration in depression. Endocrinology Research 18: 241-260, 1992.

Ljungberg T, Apicella P, Schultz W. Responses of monkey dopamine neurons during learning of behavioral reactions. Journal of Neurophysiology 67: 145-163, 1992.

Loas G, Salinas JD, Pierson A, Guelfi JD, Samuel-Lajeunesse B. Anhedonia and blunted affect in major depressive disorder. Comprehensive Psychology 35: 366-372, 1994.

Loas G, Salinas JD, Guelfi JD, Samuel-Lajeunesse B. Physical anhedonia in major depressive disorder. Journal of Affective Disorders 25: 139-146, 1992.

Lora A, Fava E. Provoking agents, vulnerability factors and depression in an Italian setting: A replication of Brown and Harris's model. Journal of Affective Disorders 24: 227-236, 1992.

Lorens SA, Hata N, Handa RJ, Van der Kar LD, Guschwan M, Goral J, Lee JM, Hamilton ME, Bethea CL, Clancy J. Neurochemical, endocrine and immunological responses to stress in young and old fischer 344 male rats. Neurobiology of Aging 11: 39-50, 1990.

Ltief G, Mavirsakalion MR. Life events and panic disorder/agoraphobia. Comprehensive Psychiatry 36: 118-122, 1995.

Lutz RA, Pfister HP. Opioid receptors and their pharmacological profiles. Journal of Receptor Research 12: 261-286, 1992.

Lykouras L, Markianos M, Hatzimanolis J, Malbioris D, Stefanis C. Biogenic amine metabolites in delusional (psychotic) depression and melancholic subtypes of major depression. Progress in Neuropsychopharmacology and Biological Psychiatry 18: 1261-1271, 1994.

Maes M, Claes M, Vanderwoude M, Schotte C, Martin M, Block P, Cosyns P. Adrenocorticotropin hormone, β -endorphin and cortisol responses to oCRF in melancholic patients. Psychological Medicine 22: 317-329, 1992.

Maddeaux C, Zacharko RM. Intraventricular administration of D-Ala²-Met⁵-Enkephalinamide induces rapid recovery of responding for electrical brain stimulation from the ventral tegmental area following uncontrollable footshock. Brain Research Bulletin 28: 337-341, 1992.

Maier SF, Sherman JE, Lewis JW, Tewrman GW, Liebeskind JC. The opioid/non-opioid nature of stress induced analgesia and learned helplessness. Animal Behaviour Proceedings 9: 80-90, 1983.

Mallek-Akmadi P. Substance P and neuropsychiatric disorders: An overview. Neuroscience and Biobehavioral Reviews 16: 365-369, 1992.

Malyszko J, Urano T, Yakada Y, Takada A. Amino acid, serotonin and 5-Hydroxyindoleacetic acid following footshock in rats. Brain Research Bulletin 36: 137-140, 1995.

Mann JJ, Kapur S. A dopaminergic hypothesis of major depression. Clinical Neuropsychopharmacology 18: S57-S65, 1995.

Mann JJ, Aarsons SF, Wilner PJ. A controlled study of the antidepressant efficacy and side effects of deprenyl. Archives of General Psychiatry 46: 45-50, 1989.

Mansour A, Fox CA, Thompson RC, Akil H, Watson SJ. Mu opioid receptor mRNA expression in the rat CNS: comparison to mu receptor binding. Brain Research 643: 245-265, 1994.

Mansour A, Thompson RC, Akil H, Watson SJ. Delta opioid receptor mRNA distribution in the brain: comparison to delta receptor binding and proenkephalin mRNA. Journal of Chemistry and Neuroanatomy 6: 351-362, 1993.

Mansour A, Khachaturian H, Lewis MR, Akil H, Watson SJ. Anatomy of CNS opioid receptors. Trends in Neurosciences 11: 308-314, 1988.

Mansour A, Khachaturian H, Lewis MR, Akil H, Watson SJ. Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain and midbrain. Journal of Neuroscience 7: 2445-2467, 1987.

Mantz J, Thierry AM, Glowinski J. Effect of noxious tail pinch on the discharge rate of mesocortical and mesolimbic dopamine neurons. Selective activation of the mesocortical system. Brain Research 476: 377-381, 1989.

Manzanares J, Durham RA, Lookingland KJ, Moore KE. δ -opioid receptor mediated regulation of central dopaminergic neurons in the rat. European Journal of Pharmacology 249: 107-112, 1993.

Manzanares J, Lookingland KJ, Moore KE. Kappa opioid receptor mediated regulation of dopaminergic neurons in the rat brain. Journal of Pharmacology and Experimental Therapeutics 256: 500-505, 1991.

Masuko S, Nakajami S, Nakajami Y. Dissociated high purity dopaminergic neuron cultures from the substantia nigra and the ventral tegmental area of the postnatal rat. Neuroscience 49: 347-364, 1992.

Matthews K, Forbes N, Reid I. Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. Physiology and Behavior 57: 241-248, 1995.

Matthews RT, German DC. Electrophysiological evidence for the excitation of rat ventral tegmental area dopamine neurons by morphine. Neuroscience, 11, 617-625, 1984.

Mattia A, Vanderah T, Mosberg HI, Porreca F. Lack of antinociceptive cross tolerance between (D-pen², D-pen²-enkephalin and (d-Ala²)deltorphin II in mice: evidence for delta receptor subtypes. Journal of Pharmacology and Experimental Therapeutics 258: 583-598, 1991.

Mattioli R, Aguilar C, Vasconcelos L. Reinforcing properties of the neuropeptide substance P in *Cassius auratus*: evidence of dopaminergic involvement. Pharmacology, Biochemistry and Behavior 50: 77-81, 1995.

Mayeux R. Parkinson's disease. Journal of Clinical Psychiatry 51: 20-23, 1990.

McCullough LD, Salamone JD. Anxiogenic drugs β -CCE and FG 7142 increase extracellular dopamine levels in the nucleus accumbens. Psychopharmacology 109: 379-382, 1992.

McGregor A, Roberts DCS. Effects of medial prefrontal cortex injections of SCH 23390 on intravenous cocaine self administration under both a fixed and progressive ratio schedule of reinforcement. Behavioral Brain Research 67: 75-80, 1995.

McGregor A, Roberts DCS. Dopaminergic antagonism within the nucleus accumbens or amygdala produces differential effects on intravenous cocaine self administration under fixed and progressive ratio schedules. Brain Research 624: 245-252, 1993.

McGuffin P, Katz R. The genetics of depression and manic depressive disorder. British Journal of Psychiatry 155: 294-304, 1989.

Melia KR, Nestler EJ, Duman RS. Chronic imipramine treatment normalizes levels of tyrosine hydroxylase in the locus coeruleus of chronically stressed rats. Psychopharmacology 108: 23-26, 1992.

Melis MR, Angiolas A. Dopamine and sexual behavior. Neuroscience and Biobehavioral Reviews 19: 19-38, 1995.

Mendella PD. Intraventricular administration of DPDPE in the CD-1 mouse: Variations of activity, exploration and intracranial self-stimulation from the dorsal A10 region following uncontrollable footshock. Unpublished Master's thesis, Carleton University, Ottawa, Ontario, Canada, 1994.

Mereu G, Lilliu V, Vargiu D, Muntonu AL, Diana M, Gessa GL. Depolarization inactivation of dopamine neurons: An artifact? The Journal of Neurochemistry 15: 1144-1149, 1995.

Meyer ME, McLaurin BI, Meyer ME. Intra-accumbens δ_1 -opioid agonist, pCL-DPDPE, differentially affects patterns of locomotor activity. Pharmacology, Biochemistry and Behavior 51: 359-362, 1995.

Meyer ME, Meyer ME. Behavioral effects of opioid peptide agonists DAMGO, DPDPE, and DAKLI on locomotor activities. Pharmacology, Biochemistry and Behavior. 45: 315-320, 1993.

Michael-Titus A, Dourmap N, Costentin J. Mu and delta opioid receptors control differently the horizontal and vertical components of locomotor activity in mice. Neuropeptides 13: 235-242, 1989.

Mickley GA, Mulvihill MA, Postler MA. Brain mu and delta opioid receptors mediate different locomotor hyperactivity responses of the C57BL/6J mouse. Psychopharmacology 101: 332-337, 1990.

Miller JD, Speciale SG, McMillen BA, German DC. Naloxone antagonism of stress-induced augmentation of frontal cortex dopamine metabolism. European Journal of Pharmacology 98: 437-439, 1984.

Miller R, Wickens JR, Beninger RJ. Dopamine D1 and D2 receptors in relation to reward and performance: A case for the D1 receptor as a primary site of therapeutic action of neuroleptic drugs. Progress in Neurobiology 34: 143-183, 1990.

Mogenson GJ, Jones DL, Yim CH. From motivation to action: Functional interface between the limbic system and the motor system. Progress in Neurobiology 14: 69-97, 1980.

Mokhtari M, Tafani JA, Zajac JM. Binding in vivo of selective μ and δ opioid antagonists: Localization by autoradiography. Neuropeptides 25: 183-191, 1993.

Molina VA, Volosin M, Cancela L, Keller E, Murua VA, Basso AM. Effect of chronic variable stress on monoamine receptors: Influence of imipramine administration. Pharmacology, Biochemistry and Behavior 35: 335-340, 1990.

Monleon S, D'Aquila P, Passa A, Simon VM, Brain PF, Willner P. Attenuation of sucrose consumption in mice by chronic mild stress and its restoration by imipramine. Psychopharmacology 117: 453-457, 1995.

Moore KE. Hypothalamic dopaminergic neuronal systems. In HY Meltzer (ed.), Psychopharmacology: The Third Generation of Progress. New York, Raven Press, 1987, 127-140.

Moreau JL, Bourson A, Jenck F, Martin JR, Mortas P. Curative effects of the atypical antidepressant mianserin in the chronic mild stress-induced anhedonia model of depression. Journal of Psychiatry and Neuroscience 19: 51056, 1994.

Moreau JL, Jenck F, Martin JR, Mortas P, Haefely WE. Antidepressant treatment prevents chronic mild unpredictable stress induced anhedonia as assessed by ventral tegmental area self-stimulation behavior in rats. European Journal of Neuropharmacology 2: 43-49, 1992.

Moskowitz AS, Goodman RR. Light microscopic autoradiographic localization of opioid binding sites in the mouse central nervous system. Journal of Neuroscience 4: 331-1342, 1984.

Mouret J, Lemoine P, Minuit MP, Robelin N. Immediate and long lasting treatment of dopamine dependent depressions by L-tyrosine. A clinical and polygraphic study. Comptes Rendus De L'Academie Des Sciences, Paris 306: 93-98, 1988.

Mouret J, Lemoine P, Minuit MP. Sleep polygraphic, clinical and therapeutic markers of dopamine dependent depression. Comptes Rendus De L'Academie Des Sciences, Paris 305: 301-306, 1987.

Mucha RF, Herz A. Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. Psychopharmacology 86: 274-280, 1985.

Muscat R, Papp M, Wilner P. Antidepressant like effect of dopamine agonists in an animal model of depression. Biological Psychiatry 31: 937-946, 1992.

Muscat R, Sampson D, Willner P. Dopaminergic mechanism of imipramine action in an animal model of depression. Biological Psychiatry 28: 223-230, 1990.

Nabeshima T, Katoh A, Wada M, Kameyama T. Stress induced changes in met-enkephalin, leu-enkephalin and dynorphin concentrations. Life Sciences 51: 211-217, 1992.

Nabeshima T, Kamei H, Kameyama T. Opioid κ receptors correlate with the development of conditioned suppression of motility in mice. European Journal of Pharmacology 152: 129-133, 1988.

Nakahara D, Fuchikami K, Ozaki O, Iwasaki T, Nagatsu T. Differential effects of self-stimulation on dopamine release and metabolism in the rat medial frontal cortex, nucleus accumbens and striatum studied by in vivo microdialysis. Brain Research 574: 164-170, 1992.

Nakata Y, Chang KJ, Mitchell CL, Hong JS. Repeated electroconvulsive shock downregulates the opioid receptors in rat brain. Brain Research 346: 160-163, 1985.

Negri L, Potenza RL, Corsi R, Melchiorri P. Evidence for two subtypes of delta opioid receptors in rat brain. European Journal of Pharmacology 196: 335-336, 1991.

Neilsen EB, Anderson PH. GRB 12909: A new potent and selective dopamine uptake inhibitor. In GL Gessa, G Sera (eds.), Dopamine and Mental Depression. Oxford, Pergamon Press, 1990, 77-90.

Nisenbaum LK, Zigmond MJ, Sved AF, Abercrombie ED. Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. The Journal of Neuroscience 11: 1478-1484, 1991.

Noel MB, Wise RA. Ventral tegmental area injection of selective μ or δ opioids enhance feeding in food deprived rats. Brain Research 673: 304-312, 1995.

Nowak G, Skolnick P, Paul IA. Down-regulation of dopamine₁ (D₁) receptors by chronic imipramine is species specific. Pharmacology, Biochemistry and Behavior 39: 769-771, 1991.

Oades RD, Halliday GM. Ventral tegmental (A10) system: neurobiology I. Anatomy and connectivity. Brain Research Review 12: 117-165, 1987.

Oakley NR, Hayes AG, Sheehan MJ. Effects of typical and atypical neuroleptics on the behavioral consequences of activation by muscimol of mesolimbic and nigro-striatal dopaminergic pathways in the rat. Psychopharmacology 105: 204-208, 1991.

Olds ME. Enhanced dopamine metabolism in accumbens leads to motor activity and concurrently, to increased output from non-dopamine neurons in the ventral tegmental area and substantia nigra. Physiology and Behavior 51: 39-50, 1991.

Olson GA, Olson RD, Kastin AJ. Endogenous Opioids. 1993. Peptides 15: 1513-1556, 1994.

Olson GA, Olson RD, Kastin AJ. Endogenous Opiates: 1992. Peptides 14: 1339-1378, 1993.

Okada F, Harikawa T, Tokimitsu Y, Nomura Y. Chronic desipramine treatment influences D1 stimulation and D2 inhibition (dual control) of adenylate cyclase by desipramine in rat striatum. Neurochemistry International 24: 23-27, 1994.

Overstreet DH. The Flinders sensitive line rats: A genetic model of depression. Neuroscience and Biobehavioral Reviews 17: 51-68, 1993.

Paes de Souza M, Tropa J. Evaluation of the efficacy of amineptine in a population of 1229 depressed patients: Results of a multicenter study carried out by 135 general practitioners. Clinical Neuropharmacology 12: 77-86, 1989.

Papp M, Klínek V, Willner P. Parallel changes in dopamine D2 receptor binding in limbic forebrain associated with chronic mild stress induced anhedonia and its reversal by imipramine. Psychopharmacology 115: 441-446, 1994.

Papp M, Muscat R, Willner P. Subsensitivity to rewarding and locomotor stimulant effects of dopamine agonists following chronic mild stress. Psychopharmacology 110: 152-158, 1993.

Papp M, Muscat R, Willner P. Additive effects of chronic treatment with antidepressant drugs and intermittent treatment with a dopamine agonist. European Neuropsychopharmacology 2: 121-125, 1992.

Parent A, Lavoie B. The heterogeneity of the mesostriatal dopaminergic system as revealed in normal and parkinsonian monkeys. Advances in Neurology 60: 25-33, 1993.

Paris JM, Cunningham KA. Habenular lesions decrease the responsiveness of the dorsal raphe serotonin neurons to cocaine. Pharmacology, Biochemistry and Behavior 49: 555-560, 1994.

Parker G, Pavlovic H. Prediction of response to antidepressant medication by a sign-based index of melancholia. Australian and New Zealand Journal of Psychiatry 27: 56-61, 1993.

Parker G, Ertchic H. Psychomotor change as a feature of depressive disorders: An historical overview. Australian and New Zealand Journal of Psychiatry 26: 146-155, 1992.

Pasternak GW. Pharmacological mechanisms of opioid analgesics. Clinical Neuropharmacology 16: 1-18, 1993.

Perego C, Vetrugno GC, De Simoni MG, Algeri S. Aging prolongs the stress induced release of noreadrenaline in rat hypothalamus. Neuroscience Letters 157: 127-130, 1993.

Pert A, Hulsebus R. Effect of morphine on intracranial self-stimulation behavior following brain amine depletion. Life Sciences 17: 19-20, 1975.

Phillips AG, Atkinson LJ, Blackburn JR, Blaha CD. Increased extracellular dopamine in the nucleus accumbens of the rat elicited by a conditioned stimulus for food: an electrochemical study. Canadian Journal of Physiology and Pharmacology 71: 387-393, 1993.

Phillips AG, Pfaus JG, Blaha CD. Dopamine and motivated behavior; insights provided by in vivo dialysis. In P Willner, J Scheel-Kruger (eds.), The Mesolimbic Dopamine System: From Motivation to Action. John Wiley and Sons, Chichester, 1991, 199-224.

Phillips AG, Fibiger HC. Neuroanatomical bases of intracranial self stimulation: Untangling the Gordian knot. In JM Liebmann, SJ Cooper (eds.), The Neuropharmacological Basis of Reward. Oxford, University Press, 1989, 66-105.

Phillips AG. Brain reward circuitry: a case for separate systems. Brain Research Bulletin 12: 195-201, 1984.

Phillips AG, LePiane FG, Fibiger HC. Dopaminergic mediation of reward produced by direct injection of enkephalin into the ventral tegmental area of the rat. Life Sciences 33: 2505-2511, 1983.

Phillips AG, LePiane FG. Reward produced by microinjection of D-Ala²-Met⁵-enkephalinamide into the ventral tegmental area. Behavioral Brain Research 5: 225-229, 1982.

Phillips AG, LePiane FG. Reinforcing effects of morphine microinjections into the ventral tegmental area. Pharmacology, Biochemistry and Behavior 12: 965-968, 1980.

Phillips GD, Robbins TW, Everitt BJ. Mesoaccumbens dopamine-opiate interactions in the control over behavior by a conditioned reinforcer. Psychopharmacology 114: 345-359, 1994.

Phillipson OT. A golgi study of the ventral tegmental area of tsai and interfascicular nucleus in the rat. Journal of Comprehensive Neurology 187: 99-116, 1979a.

Phillipson OT. The cytoarchitecture of the interfascicular nucleus and ventral tegmental area of tsai in the rat. Journal of Comprehensive Neurology 187: 85-98, 1979b.

Pickar D, Extein I, Gold PW, Naber D, Summers RS, Goodwin FK. Endorphins in affective disorders. In NS Shah, AG Donald (eds.), Endorphins and opiate agonists in psychiatric illness. New York: Plenum Press, 1982, 375-397.

Pitchot W, Anseau M, Moreno AG, Hansenne M, von Freckell R. Dopaminergic function in panic disorder: Comparison with major and minor depression. Biological Psychiatry 32: 1004-1011, 1992.

Pitchot W, Hansenne M, Moreno AG, von Frencckell R, Ansseau M. Psychopathological correlates of dopaminergic disturbances in major depression. Neuropsychobiology 24: 169-172, 1991.

Plaznik A, Kostowski W. The effects of antidepressants and electroconvulsive shocks on the functioning of the mesolimbic dopaminergic system: a behavioral study. European Journal of Pharmacology 135: 389-396, 1987.

Pollard H, Bouthenet ML, Moreau J, Souil E, Verroust P, Ronco P, Schwartz JC. Detailed immunohistochemical mapping of enkephalinase (EC3.4.24.11) in rat central nervous system: Comparison with enkephalins and substance P. Neuroscience 30: 339-376, 1989.

Post RM. Episode and stress sensitization in the longitudinal course of affective disorders: role of proto-oncogenes and other transcription factors. Neurobiology of Affective Disorders, Raven Press, New York, 1993, 65-68.

Post RM. Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. American Journal of Psychiatry 149: 999-1010, 1992.

Prince CR, Anisman H. Acute and chronic stress effects on performance in a forced swim task. Behavioral Neural Biology 84: 99-119, 1984.

Prisco S, Pagannone S, Esposito E. Serotonin-dopamine interaction in the rat ventral tegmental area: an electrophysiological study in vivo. The Journal of Pharmacology and Experimental Therapeutics 271: 83-90, 1994.

Przewlocki R, Lason W, Majeed NH, Przewlocka B. Antidepressants and endogenous opioid peptide systems. Neuropeptides 5: 575-578, 1985.

Puglisi-Allegra S, Imperato A, Angelucci L, Cabib S. Acute stress induces time-dependent responses in dopamine mesolimbic system. Brain Research 554: 217-222, 1991.

Rampello L, Nicoletti G, Raffaele R. Dopaminergic hypothesis for retarded depression: a symptom profile for predicting therapeutic responses. Acta Psychiatrica Scandinavica 84: 552-554, 1991.

Rasmussen K. CCK, schizophrenia and anxiety. Annals of the New York Academy of Sciences 713: 300-311, 1994.

Rasmusson AN, Goldstein LE, Deutch AY, Bunney BS, Roth RH. 5-HT_{1a} agonist $\pm$ 8-OH-DPAT modulates basal and stress-induced changes in medial prefrontal cortical dopamine. Synapse 18: 218-224, 1994.

Raynor K, Kong H, Mestek A, Bye LS, Tian M, Liu J, Yu L, Reisine T. Characterization of the cloned human mu opioid receptor. Journal of Pharmacology and Experimental Therapeutics 272: 423-428, 1995.

Retaux S, Trovero F, Besson MJ. Role of dopamine in the plasticity of glutamic acid decarboxylase messenger RNA in the rat frontal cortex and the nucleus accumbens. European Journal of Neuroscience 6: 1782-1791, 1994.

Rice ME, Richards CD, Nedergaard S, Hounsgaard J, Nicholson C, Greenfield C. Direct monitoring of dopamine and 5-HT release in substantia nigra and ventral tegmental area in vitro. Experimental Brain Research 100: 395-406, 1994.

Ring HA, Bench CJ, Trimble MR, Brooks DJ, Frackowiak RSJ, Dolan RJ. Depression in Parkinson's disease: A positron emission study. British Journal of Psychiatry 165: 333-339, 1994.

Risch SC. β -endorphin hypersecretion in depression: Possible cholinergic mechanisms. Biological Psychiatry 17: 1071-1079, 1982.

Robertson GS, Juan N. D₁ and D₂ dopamine receptors differentially increase Fos-like immunoreactivity in accumbal projections to the ventral pallidum and midbrain. Neuroscience 64: 1019-1034, 1995.

Robinson TE, Becker JB, Young EA, Akil H, Castenada E. The effects of footshock stress on regional brain dopamine metabolism and pituitary β -endorphin release in rats previously sensitized to amphetamine. Neuropharmacology 26: 679-691, 1987.

Romppe PP, Wise RA. Behavioral evidence for midbrain dopamine depolarization inactivation. Brain Research, 477, 152-156, 1989a.

Romppe PP, Wise RA. Opioid-neuroleptic interaction in brain stem self-stimulation. Brain Research 477: 144-151, 1989b.

Romppe PP, Shizgal P. Electrophysiological characteristics of neurons in forebrain regions implicated in self-stimulation of the medial forebrain bundle in the rat. Brain Research 364: 338-349, 1986.

Ronken E, Van Muiswinkel FL, Mulder AH, Schoffelmeer AN: Opioid receptor-mediated inhibition of evoked catecholamine release from cultured neurons of rat mesencephalon and locus coeruleus. European Journal of Pharmacology 230: 349-355, 1993.

Rosetti ZL, Lai M, Hmaidan Y, Gessa GL. Depletion of mesolimbic dopamine during behavioral despair: partial reversal by chronic imipramine. European Journal of Pharmacology 242: 313-315, 1993.

Rouge-Pont F, Piazza PV, Kharouby M, Le Moal M, Simon H. Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals predisposed to amphetamine self-administration. A microdialysis study. Brain Research 602: 169-174, 1993.

Roy A. Recent biological studies on suicide. Suicide and Life-Threatening Behavior 24: 10-14, 1994.

Roy A, Pickar D, Douillet P, Karoum F, Linnoila M. Urinary monoamines and monoamine metabolites in subtypes of unipolar depressive disorder and normal controls. Psychological Medicine 16: 541-546, 1986.

Rudski JM, Grace MK, Billington CJ, Levine AS. Increased food intake following morphine or NPT administration into the ventral tegmental area of rats. Society for Neuroscience Abstracts 18: 1427, 1992.

Sacchetti G, Bonini I, Waterloos GC, Samanin R. Tianeptine raises dopamine and blocks stress-induced noreadrenaline release in the rat frontal cortex. European Journal of Pharmacology 236: 171-175, 1993.

Salamone JD. The involvement of nucleus accumbens dopamine in appetitive and aversive motivation. Behavioral Brain Research 61: 117-133, 1994.

Sandyk R. Pineal melatonin functions and the depression of Parkinson's disease: a hypothesis. International Journal of Neuroscience 51: 73-77, 1990.

Sandyk R. The "therapeutic lag" of antidepressants and the opioid system. International Journal of Neuroscience 34: 115-116, 1987.

Schmauss C, Emrich HM. Narcotic antagonist and opioid treatment in psychiatry. In RJ Rodgers, SJ Cooper (eds.), Endorphins, Opiates and Behavioral Processes. John Wiley and Sons, New York, 1988, 327-351.

Schultz W, Apicella P, Ljungberg T. Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. The Journal of Neuroscience 13: 900-913, 1993.

Schwartz JC, Levesque D, Martres MP, Sojloff P. Dopamine D₃ receptor: Basic and clinical aspects. Clinical Neuropharmacology 16: 295-314, 1993.

Self DW, Nestler EJ. Molecular mechanisms of drug reinforcement and addiction. Annual Review of Neuroscience 18: 463-495, 1995.

Self DW, Stein L. Receptor subtypes in opioid and stimulant reward. Pharmacology and Toxicology 70: 87-94, 1992.

Seroogy KB, Danagaran K, Lim S, Haycock JW, Fallon JH. Ventral mesencephalon neurons containing both cholecystikinin and tyrosine hydroxylase-like immunoreactives project to forebrain regions. Journal of Comprehensive Neurology 279: 397-414, 1989.

Seroogy K, Ceccatelli S, Schalling M, Hokfelt T, Frey P, Walsh J, Dockray G, Brown J, Buchan A, Goldstein M. A subpopulation of dopaminergic neurons in rat ventral mesencephalon contains both neurotensin and cholecystokinin. Brain Research 455: 88-98, 1988.

Serra G, Collu M, D'aquila PS, Gessa GL. Role of the mesolimbic dopamine system in the mechanism of action of antidepressants. Pharmacology and Toxicology 71: 72-78, 1992.

Serra G, Culla M, D'Aquila PS, DeMontis MG, Gessa GL. On the mechanisms involved in the behavioral supersensitivity to DA agonists induced by chronic antidepressants. In GL Gessa, G Serra (eds.), Dopamine in Mental Depression. Pergamon Press, Oxford, 1990, 121-138.

Sesack SR, Pickel VM: Dual ultrastructural localization of enkephalin and tyrosine hydroxylase immunoreactivity in the rat ventral tegmental area: multiple substrates for opiate-dopamine interaction. The Journal of Neuroscience 12: 1335-1350, 1992.

Settle EC. Bupropion: a novel antidepressant-Update 1989. International Drug Therapy Newsletter 24: 29-36, 1989.

Shanks N, Griffiths J, Anisman H. Central catecholamine alterations induced by stressor exposure: analyses in recombinant inbred strains of mice. Behavioral Brain Research 63: 25-33, 1994.

Shanks N, Francis D, Zalcman S, Meaney MJ, Anisman H. Alterations in central catecholamines associated with immune responding in adult and aged mice. Brain Research 666: 77-87, 1994.

Shanks N, Zalcman S, Zacharko RM, Anisman H. Alterations in central norepinephrine, dopamine and serotonin in several strains of mice following acute stressor exposure. Pharmacology, Biochemistry and Behavior 38: 69-75, 1991.

Shanks N, Anisman H. Strain specific effects of antidepressants on escape deficits induced by inescapable shock. Psychopharmacology 99: 122-128, 1989.

Shanks N, Anisman A. Stressor-provoked behavioral changes in six strains of mice. Behavioral Neuroscience 102: 894-905, 1988.

Shaud-Chagny MF, Chergui K, Chouvet G, Ganon F. Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. Neuroscience 49: 63-72, 1992.

Shepard PD, German DC. A subpopulation of mesocortical dopamine neurons possesses autoreceptors. European Journal of Pharmacology 98: 455-456, 1984.

Shibata H, Suzuki T, Matsushita M. Afferent projections to the interpeduncular nucleus in the rat, as studied by retrograde and anterograde transport of wheat germ agglutinin conjugated to horseradish peroxidase. The Journal of Comparative Neurology 248: 272-284, 1986.

Shinoda K, Michigami T, Awano K, Shiotani Y. Analysis of the rat interpeduncular subnuclei by immunocytochemical double-staining for enkephalin and substance P, with some reference to the coexistence of both peptides. The Journal of Comparative Neurology 271: 243-256, 1988.

Sieghart W. Pharmacology of benzodiazapine receptors: an update. Journal of Psychiatry and Neuroscience 19: 24-29, 1994.

Simon EJ. Opioid receptors and endogenous opioid peptides. Medicinal Research Reviews 11: 357-374, 1991.

Singh J, Desiraju T, Nagaraja TN, Raju TR. Facilitation of self-stimulation of ventral tegmentum by microinjections of opioid receptor subtype agonists. Physiology and Behavior 55: 627-632, 1994.

Slater p, Gross AJ. Receptors for opioid peptides in brain. Methods in Neuroscience 5: 459-478, 1991.

Smith JA, Loughlin SE, Leslie FM. Kappa opioid inhibition of [3H] dopamine release from ventral mesencephalic dissociated cell cultures. Molecular Pharmacology 42: 575-583, 1992.

Smith AP, Lee NM. Pharmacology of dynorphin. Annual Review of Pharmacology and Toxicology 28: 120-140, 1988.

Soares-de-Silva P, Garret MC. A kinetic study of the rate of formation of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the rat brain: implication for the origin of DOPAC. Neuropharmacology 29: 869-874, 1990.

Sorg BA, Kalivas PW. Effects of cocaine and footshock stress on extracellular dopamine levels in the medial prefrontal cortex. Neuroscience 53: 695-703, 1993.

Spanagel R, Herz A, Shippenberg TS: Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proceedings of the National Academy of Science 89: 2046-2050, 1992.

Spanagel R, Herz A, Shippenberg TS: The effects of opioid peptides on dopamine release in the nucleus accumbens: An in vivo microdialysis study. Journal of Neurochemistry 55: 1734-1740, 1990a.

Spanagel R, Herz A, Shippenberg TS: Identification of the opioid receptor types mediating β -endorphin induced alterations in dopamine release in the nucleus accumbens. European Journal of Pharmacology 190: 177-184, 1990b.

Speciale SG, Manaye KF, Sadeq M, German DC. Opioid receptors in midbrain dopaminergic regions of the rat II. Kappa and delta receptor autoradiography. Journal of Neural Transmission 91: 53-66, 1993.

Speciale SG, Fuchs I. Footshock stress induced elevations in regional brain endogenous opioids. Society for Neuroscience Abstracts 12: 411, 1986.

Stein EA, Hiller JM, Simons EJ. Effects of stress on opioid receptor binding in the rat central nervous system. Neuroscience 51: 683-690, 1992.

Stinus L, Cador M, LeMoal M. Interactions between endogenous opioids and dopamine within the nucleus accumbens. Annals of the New York Academy of Science 654: 254-273, 1992.

Stokes PE. Current issues in the treatment of major depression. Journal of Clinical Psychopharmacology 13: 2S-9S, 1993.

Stone EA. Noradrenergic receptor mechanisms in stress adaptation. In S Puglisi-Allegra, A Oliverio (Eds.), Psychobiology of Stress. Kluwer Academic Publishers: Netherlands, 1990, pp 151-160.

Stone EA. Central cyclic-AMP-linked noradrenergic receptors: new findings on properties as related to the actions of stress. Neuroscience and Biobehavioral Reviews 11: 391-398, 1987.

Stoner L, Linkowski P, Mendlewicz J. Biological markers as classifiers for depression: A multivariate study. Progress in Neuropsychopharmacology and Biological Psychiatry 18: 899-914, 1994.

Sugita S, Johnson SW, North RA. Synaptic inputs to GABAA and GABAB receptors originate from discrete afferent neurons. Neuroscience Letters 134: 63-72, 1992.

Swanson LW. The ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Research Bulletin 9: 321-353, 1982.

Tanda G, Carboni E, Frau R, Di Chiara G. Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? Psychopharmacology 115: 285-288, 1994.

Tanaka M, Yoshishige I, Tsuda A, Tsujimaru S, Shirao A, Oguchi M. Met-enkephalin, injected during the early phase of stress, attenuates stress-induced increases in noradrenaline release in rat brain regions. Pharmacology, Biochemistry and Behavior 32: 791-795, 1989.

Tejedor-Real P, Mico JA, Maldonado R, Roques BP, Gibert-Rahola J. Effect of mixed (RB 38A) and selective (RB 38B) inhibitors of enkephalin degrading enzymes on a model of depression in the rat. Biological Psychiatry 34: 100-107, 1993.

Thapar A, McGuffin P. A twin study of depressive symptoms in childhood. British Journal of Psychiatry 165, 259-265, 1994.

Thornton EW, Murray M, Connors-Eckenrode T, Haun F. Dissociation of behavioral changes in rats resulting from lesions of the habenula versus fasciculus retroflexus and their possible anatomical substrates. Behavioral Neuroscience 108: 1150-1162, 1994.

Tiu SC, Li WY, Luo CB, Yew DT. Habenulo-interpeduncular descending pathways and their relationship to enkephalin and somatostatin-immunoreactive neurons in the interpeduncular nucleus of human fetuses. Neuroscience 53: 489-493, 1993.

Tork I, Halliday G, Schneiber T, Turner S. The organization of the mesencephalic ventromedial area in the cat. In R Bandler (ed.), Modulation of Sensorimotor Activity During Alterations in Behavioral States. Alan Liss, New York, 1984, 39-73.

Trulson ME, Arasteh K. Morphine increases the activity of midbrain dopamine neurons in vivo. European Journal of Pharmacology 114: 105-109, 1985.

Tsuji S, Nakai Y, Koh T, Takahashi U, Usui T, Imura H. Effects of food deprivation and high fat diet on opioid receptor binding in rat brain. Neuroscience Letters 72: 169-173, 1986.

Vaccarino FJ. Nucleus accumbens dopamine-CCK interactions in psychostimulant reward and related behavior. Neuroscience and Biobehavioral Reviews 18: 207-214, 1994.

Vaccarino FJ, Bloom FE, Koob GF. Blockade of nucleus accumbens opiate receptors attenuates intravenous heroin reward in the rat. Psychopharmacology 85: 37-42, 1985.

Vadasz C, Laszlovszky I, De Simone PA, Fleischer A. Genetic aspects of dopamine receptor binding in the mouse and rat brain: An overview. Journal of Neurochemistry 59: 793-808, 1992.

Vancova M, Arluison M, Boyer PA, Bourgoin S, Quignon M. Enkephalin containing nerve cell bodies in the substantia nigra of the rat: demonstration by immunohistochemical and in situ hybridization. Brain Research Bulletin 27: 19-27, 1991.

Wang GJ, Volkow ND, Logan J, Fowler JS, Schyler D, MacGregor RR, Hitzemann RJ, Gur RC, Wolf AP. Evaluation of age related changes in serotonin 5-HT₂ and dopamine D₂ receptor availability in healthy human subjects. Life Sciences 56: 249-253, 1995.

Wang T, French ED. NMDA, kainate and AMPA depolarize nondopamine neurons in the rat ventral tegmentum. Brain Research Bulletin 36: 39-43, 1995.

Wang T, O'Conner WT, Ungerstedt U, French ED. N-methyl-D aspartic acid biphasically regulates the biochemical and electrophysiological response of A10 dopamine neurons in the ventral tegmental area: in vivo microdialysis and in vitro electrophysiological studies. Brain Research 666: 255-262, 1994.

Wang T, French ED. L-glutamate excitation of A10 dopamine neurons is preferentially mediated by activation of NMDA receptors: extra- and intracellular electrophysiological studies in brain slices. Brain Research 627: 299-306, 1993.

Weber E, Roth KA, Barchas JD. Immunohistochemical distribution of alpha-neoendorphin/dynorphin neuronal systems in the rat brain: Evidence for colocalization. Proceeding of the National Academy of Sciences 79: 3062-3066, 1982, 1982.

Weisler RH, Johnson JA, Lineberry CG, Samara B, Branconnier RJ, Billow AA. Comparison of bupropion and trazadone for the treatment of major depression. Journal of Clinical Psychopharmacology 14: 170-179, 1994.

Weiss JM. Stress induced depression: critical neurochemical and electrophysiological changes. In J Madden (Ed.), Neurobiology of Learning, Emotion and Affect. Raven Press: New York, 1991, pp 123-154.

Weiss JM, Simson PE. Electrophysiology of the locus coeruleus: Implications for stress induced depression. In GF Koob, CL Ehlers & DJ Kupfer (Eds.), Animal Models of Depression. Birkhauser: Boston, 1989, pp. 111-134.

Wells BG, Marken PA. Bromocriptine in the treatment of depression. Discoveries in Clinical Psychology 23: 601-602, 1989.

West TEG, Wise RA. Nucleus accumbens injections of mu and delta but not kappa opioids facilitate hypothalamic brain stimulation reward. Society for Neuroscience Abstracts 21: 34, 1989.

White NM. Reward or reinforcement: what's the difference? Neuroscience and Biobehavioral Reviews 13: 181-186, 1989.

Willner P, Lappas S, Cheeta S, Muscat R. Reversal of stress induced anhedonia by the DA receptor agonist promipexole. Psychopharmacology 117: ?, 1995.

Willner P, Muscat R, Papp M. Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neuroscience and Biobehavioral Reviews 16: 525-534, 1992.

Willner P. Animal models as simulations of depression. Trends in Pharmacological Sciences 12: 131-136, 1991.

Willner P, Klimek V, Golembiowska K, Muscat R. Changes in mesolimbic dopamine may explain stress-induced anhedonia. Psychobiology 19: 79-84, 1991.

Wise RA, Keeb K. Psychomotor-stimulant sensitization: a unitary phenomenon? Behavioral Pharmacology 4: 339-349, 1993.

Wise RA, Hoffman DC. Localization of drug reward mechanisms by intracranial injections. Synapse 10: 247-263, 1992.

Wise RA, Rompre PP. Brain dopamine and reward. Annual Review of Psychology, 40: 191-225, 1989.

Wise RA. The role of reward pathways in the development of drug dependence. Pharmacological Therapeutics 35: 227-263, 1987.

Wise RA, Bozarth MA. Action of drugs of abuse on brain reward systems: an update with specific attention to opiates. Pharmacology, Biochemistry and Behavior. 17: 239-243, 1982.

Wittchen HU, Knauper B, Kessler RC. Lifetime risk of depression. British Journal of Psychiatry 165: 16-22, 1994.

Wolfe C, Zacharko RM. Desmethylinipramine promotes recovery of self-stimulation from the prefrontal cortex following footshock. Brain Research Bulletin 27: 601-604, 1991a.

Wolfe C, Zacharko RM. Intraventricular administration of D-Ala²-Met⁵-Enkephalinamide exacerbates stressor induced disruptions in ICSS from the ventral VTA. The IBRO World Congress of Neuroscience 3: 388, 1991b.

Wolfe C, Zacharko RM. D-Ala²-Met⁵-Enkephalinamide attenuates stressor induced alterations in intracranial self-stimulation from the dorsal VTA. Society for Neuroscience Abstracts 16: 44, 1990.

Wollemann MS, Benyhe S, Simon J. The kappa-opioid receptor: evidence for the different subtypes. Life Sciences 52: 599-611, 1993.

Wong LS, Eshel G, Dreher J, Ong J, Jackson DM. Role of dopamine and GABA in the control of motor activity elicited from the rat nucleus accumbens. Pharmacology, Biochemistry and Behavior 38: 829-835, 1991.

Wood PL, Altar CA. Dopamine release in vivo from nigrostriatal, mesolimbic and mesocortical neurons: utility of 3-methoxytyramine measurements. Pharmacology Reviews 40: 163-187, 1988.

Yamada K, Nabeshima T. Stress induced behavioral responses and multiple opioid system in the brain. Behavioral Brain Research 67: 133-145, 1995.

Yellowlees PM, Kaushik AV. An examination of the association between life problems and psychiatric disorders in a rural patient population. Australian and New Zealand Journal of Psychiatry 28: 50-57, 1994.

Yoburn BC, Lutfy K, Candido J: Species differences in μ and δ opioid receptors. European Journal of Pharmacology 193: 105-108, 1991.

Young EA, Haskett RF, Murphy-Weinberg V, Watson SJ, Akil H. Loss of glucocorticoid fast feedback in depression. Archives of General Psychiatry 48: 693-699, 1991.

Zacharko RM, Anisman H. Stressor induced anhedonia in the mesocorticolimbic system. Neuroscience and Biobehavioral Reviews 15: 391-405, 1991.

Zacharko RM, Gilmore W, MacNeil G, Kasian M, Anisman H. Stressor induced variations of intracranial self-stimulation from the mesocortex in several strains of mice. Brain Research, 533: 353-357, 1990a.

Zacharko RM, Kasian M, MacNeil G, Anisman H. Stressor induced behavioral alterations in intracranial self-stimulation from the ventral tegmental area: Evidence for regional variations Behavioral Brain Research 25: 617-621, 1990b.

Zacharko RM, Kasian M, Irwin J, Zalzman S, LaLonde G, MacNeil G, Anisman H. Behavioral characterization of intracranial self-stimulation from mesolimbic, mesocortical, nigrostriatal, hypothalamic and extrahypothalamic sites in the non-inbred CD-1 mouse strain. Behavioral Brain Research 36: 251-281, 1990c.

Zacharko RM, Lalonde GT, Kasian M, Anisman H. Strain-specific effects of inescapable shock on intracranial self-stimulation from the nucleus accumbens. Brain Research 426: 164-168, 1987.

Zacharko RM, Bowers WJ, Kelley MS, Anisman H. Prevention of stressor induced disturbances of self-stimulation by desmethylinipramine. Brain Research 321: 175-179, 1984.

Zacharko RM, Bowers WJ, Kokkinidis L, Anisman H. Region-specific reductions of intracranial self-stimulation after uncontrollable stress: Possible effects on reward processes. Behavioral Brain Research 9: 129-141, 1983.

Zamir N, Palkovitz M, Brownstein M. Distribution of immunoreactive met-enkephalin-arg6-gly7-leu8 and leu-enkephalin in discrete regions of the rat-brain. Brain Research 326: 1-8, 1985.

Zarrindast MR, Zarghi A. Morphine stimulates locomotor activity by an indirect dopaminergic mechanism: Possible D₁ and D₂ receptor involvement. General Pharmacology 23: 1221-1225, 1992.

Zastawny RL, George SR, Nguyen T, Cheng R, Tsatsos J, Briones-Urbina R, O'Dowd B. Cloning, characterization, and distribution of mu opioid receptor in rat brain. Journal of Neurochemistry 62: 2099-2105, 1994.

Zbigniew S, Obuchowicz E, Kawolski J, Plech A. Leu-enkephalin content in rat brain during prolonged treatment with imipramine or amitriptyline. Neuropeptides 7: 113-124, 1986.

Zetterstrom T, Sharp T, Collin AK, Ungerstedt U. In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs: implications for the origin of extracellular DOPAC. European Journal of Pharmacology 148: 327-334, 1988.

Zhang H, Kiyatkin EA, Stein EA. Behavioral and pharmacological modulation of ventral tegmental dendritic dopamine release. Brain Research 656: 59-70, 1994.

Zis AP, Garland JE. Opioid peptides and depression: the neuroendocrine approach. Bailliere's Clinical Endocrinology and Metabolism 5: 97-117, 1991.

Zubenko GS, Nixon RA. Mood elevating effects of captopril in depressed patients. American Journal of Psychiatry, 141: 110-111, 1984.

APPENDIX A
ANOVA SUMMARY TABLE FOR EXPERIMENT 1

Baseline Activity

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	1464	1	1464	0.03	.8720
D	38574	4	9643	0.17	.9514
S x D	4506	4	1126	0.02	.9992
Error	2793848	50	55876		
T	876678	14	62619	77.02	.0000*
T x S	6725	14	480	0.59	.8735
T x D	52563	56	938	1.15	.2114
T x S x D	43988	56	785	0.97	.5471
Error	569112	700	813		

Post-Shock and Drug Activity

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	294533	1	294533	1.57	.2157
D	663909	4	165977	0.89	.4791
S x D	649860	4	162465	0.87	.4900
Error	9364674	50	187293		
A	597656	2	298828	6.88	.0016*
A x S	889953	2	444976	10.25	.0001*
A x D	669899	8	83737	1.93	.0638
A x S x D	800544	8	100068	2.30	.0261*
Error	4342714	100	43427		
T	2553054	14	182361	68.71	.0000*
T x S	27179	14	1941	0.73	.7434
T x D	182826	56	3264	1.23	.1270
T x S x D	171112	56	3055	1.15	.2157
Error	1857807	700	2654		
A x T	98830	28	3529	1.84	.0051*
A x T x S	81190	28	2899	1.51	.0434*
A x T x D	2390456	112	2134	1.11	.2133
A x T x S x D	262359	112	2342	1.22	.0667
Error	2691431	1400	1922		

S = SHOCK

D = DRUG

A = DAY

T = TIME

APPENDIX B
ANOVA SUMMARY TABLE FOR EXPERIMENT 1

Baseline Rearing

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	13440	1	13440	1.49	.2286
D	5942	4	1485	0.17	.9548
S x D	13269	4	3317	0.37	.8293
Error	359694	50	8992		
T	12966	14	926	3.22	.0001*
T x S	2618	14	187	0.65	.8225
T x D	13190	56	235	0.82	.8219
T x S x D	15054	56	268	0.94	.6104
Error	160981	560	287		

Post-Shock and Drug Rearing

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	136188	1	136188	4.08	.0501*
D	62127	4	15531	0.47	.7607
S x D	145317	4	36329	1.09	.3752
Error	1335002	50	33375		
A	141809	2	70904	11.77	.0000*
A x S	100643	2	50321	8.35	.0005*
A x D	54305	8	6788	1.13	.3546
A x S x D	41766	8	5220	0.87	.5482
Error	481976	80	6024		
T	24046	14	1717	3.24	.0001*
T x S	5393	14	385	0.73	.7486
T x D	36698	56	655	1.24	.1249
T x S x D	28819	56	514	0.97	.5388
Error	297027	560	530		
A x T	14206	28	507	1.16	.2542
A x T x S	14379	28	513	1.18	.2394
A x T x D	47177	112	421	0.97	.5803
A x T x S x D	43917	112	392	0.90	.7592
Error	487988	1120	435		

S = SHOCK
D = DRUG
A = DAY
T = TIME

APPENDIX C
ANOVA SUMMARY TABLE FOR EXPERIMENT 2a

Baseline Brain Stimulation: Dorsal VTA-Propylactic

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	26888	1	26888	0.00	.9971
D	689259	3	229753	1.11	.3563
S x D	111497	3	37165	0.18	.9096
Error	8277119	40	206927		
T	1119374	14	79955	14.83	.0000*
T x S	36057	14	2575	0.48	.9451
T x D	266903	56	6354	1.18	.2100
T x S x D	1277843	56	3043	0.56	.9882
Error	3020065	560	5392		

Post-Shock and Drug Brain Stimulation

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	2072599	1	2072599	3.41	.0722
D	6634183	3	2211394	3.64	.0206*
S x D	2945059	3	981686	1.62	.2010
Error	24313926	40	607848		
A	17030588	2	851529	9.64	.0002*
A x S	209977	2	104988	1.19	.3098
A x D	161656	6	269427	3.05	.0097*
A x S x D	787319	6	131219	1.49	.1935
Error	7063857	80	88298		
T	3350039	14	239288	30.17	.0000*
T x S	79490	14	5677	0.72	.7592
T x D	207673	42	4944	0.62	.9703
T x S x D	356561	42	8489	1.07	.3563
Error	4441045	560	7930		
A x T	198815	28	7100	1.31	.1293
A x T x S	169851	28	6066	1.12	.3042
A x T x D	568877	84	6772	1.25	.0685
A x T x S x D	457952	84	5451	1.01	.4655
Error	60647774	1120	5414		

S = SHOCK

D = DRUG

A = DAY

T = TIME

APPENDIX D
ANOVA SUMMARY TABLE FOR EXPERIMENT 2b

Baseline Brain Stimulation: Dorsal VTA-Therapeutic

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	289166	1	289166	2.19	.1498
D	104137	2	52068	0.39	.6775
S x D	9162	2	4581	0.03	.9659
Error	3692203	28	131864		
T	1742536	14	124466	16.68	.0000*
T x S	101575	14	7255	0.97	.4810
T x D	312389	28	11156	1.50	.0528
T x S x D	116629	28	4165	0.56	.9683
Error	2924926	392	7461		

Immediate Pre-Drug Brain Stimulation

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	1476903	1	1476903	27.08	.0000*
D	76096	2	38048	0.70	.5062
S x D	4492	2	2246	0.04	.9597
Error	1526996	28	54535		
T	808297	4	202074	20.20	.0000*
T x S	378729	4	94682	9.46	.0000*
T x D	56880	8	7110	0.71	.6816
T x S x D	181580	8	22697	2.27	.0275*
Error	1120431	112	10003		

S = SHOCK
D = DRUG
A = DAY
T = TIME

APPENDIX D
ANOVA SUMMARY TABLE FOR EXPERIMENT 2b

Dorsal VTA-Therapeutic: Immediate Post-Drug Brain Stimulation

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	1497950	1	1497950	16.11	.0004*
D	153193	2	76596	0.82	.4491
S x D	444527	2	222263	2.39	.1101
Error	2603672	28	92988		
T	67971	9	7552	1.02	.4225
T x S	138863	9	15429	2.09	.0310*
T x D	175463	18	9747	1.32	.1753
T x S x D	145124	18	8062	1.09	.3610
Error	1861542	252	7387		

Long Term Post-Drug Brain Stimulation

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	1925735	1	1925735	6.01	.0207*
D	3025245	2	1512622	4.72	.0171*
S x D	1745892	2	872946	2.73	.0829
Error	8967678	28	320274		
A	387214	1	387214	3.02	.0934
A x S	1000	1	1000	0.01	.9303
A x D	52201	2	26100	0.20	.8172
A x S x D	310516	2	155258	1.21	.3135
Error	3594781	28	128385		
T	1684598	14	120328	11.23	.0000*
T x S	106999	14	7642	0.71	.7611
T x D	498611	28	17807	1.66	.0200*
T x S x D	334491	28	11946	1.12	.3158
Error	4198916	392	10711		
A x T	175016	14	12501	1.77	.0410*
A x T x S	95853	14	6846	0.97	.4841
A x T x D	2738322	28	9779	1.38	.0950
A x T x S x D	168475	28	6016	0.85	.6859
Error	2768519	392	7062		

S = SHOCK

D = DRUG

A = DAY

T = TIME

APPENDIX E
ANOVA SUMMARY TABLE FOR EXPERIMENT 3

Baseline Brain Stimulation: Ventral VTA

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	160921	1	160921	0.68	.4149
D	1009297	3	336432	1.42	.2513
S x D	25190	3	8396	0.04	.9909
Error	9481572	40	237039		
T	1570246	14	112160	13.38	.0000*
T x S	252289	14	18020	2.15	.0086*
T x D	463440	42	11034	1.32	.0925
T x S x D	173617	42	4133	0.49	.9971
Error	4695634	560	83385		

Post-Shock and Drug Brain Stimulation

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	194636	1	194636	0.44	.5096
D	8266063	3	27555354	6.27	.0014*
S x D	2283196	3	761065	1.73	.1760
Error	1758546	40	439636		
A	4122816	2	2061408	8.98	.0003*
A x S	181053	2	90526	0.39	.6755
A x D	4393362	6	732227	3.19	.0074*
A x S x D	344867	6	57477	0.25	.9578
Error	18373273	80	229665		
T	2492602	14	178043	16.86	.0000*
T x S	119829	14	8559	0.81	.6582
T x D	605014	42	14405	1.36	.0672
T x S x D	788609	42	18776	1.78	.0024*
Error	5914510	560	10561		
A x T	167648	28	5987	0.75	.8284
A x T x S	182733	28	6526	0.81	.7435
A x T x D	676616	84	8054	1.00	.4745
A x T x S x D	613882	84	7308	0.91	.7040
Error	8993268	1120	8029		

S = SHOCK

D = DRUG

A = DAY

T = TIME

APPENDIX F
ANOVA SUMMARY TABLE FOR EXPERIMENT 4

Baseline Brain Stimulation: Interpeduncular Nucleus

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	189353	1	189353	1.95	.1701
D	367600	4	91900	0.95	.4469
S x D	53196	4	13299	0.14	.9676
Error	9481572	50	237039		
T	312142	14	22295	3.25	.0001*
T x S	86718	14	6194	0.90	.5554
T x D	496688	56	8869	1.29	.0815
T x S x D	415756	56	7424	1.08	.3237
Error	3841065	560	6859		

Post-Shock and Drug Brain Stimulation

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	Probability
S	4029795	1	4029795	9.87	.0032*
D	6852234	4	17130058	4.19	.0063*
S x D	1532788	4	383197	0.94	.4518
Error	1633739	40	408434		
A	8005003	2	4002518	18.52	.0000*
A x S	3954058	2	1977029	9.15	.0003*
A x D	3429745	8	428718	1.98	.0590
A x S x D	3524626	8	440578	2.04	.0521*
Error	17288582	80	216107		
T	1104228	14	78873	8.02	.0000*
T x S	46425	14	3316	0.34	.9890
T x D	639792	56	11424	1.16	.2041
T x S x D	482729	56	8620	0.88	.7244
Error	5504928	560	9830		
A x T	389007	28	13893	1.75	.0096*
A x T x S	194772	28	6956	0.88	.6539
A x T x D	729703	112	6515	0.82	.9104
A x T x S x D	948017	112	8464	1.06	.3125
Error	8903199	1120	7949		

S = SHOCK

D = DRUG

A = DAY

T = TIME

END

0 1 - 0 5 - 9 6

FIN