PROGRESS IN

MOLECULAR BIOLOGY AND TRANSLATIONAL SCIENCE

VOLUME 98

THE BRAIN AS A DRUG TARGET

EDITED BY

SHAFIQUR RAHMAN

PROGRESS IN

Molecular Biology and Translational Science

Volume 98

This page intentionally left blank

PROGRESS IN

Molecular Biology and Translational Science

The Brain as a Drug Target

edited by

Shafiqur Rahman

Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, South Dakota, USA

Volume 98

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON
NEW YORK • OXFORD • PARIS • SAN DIEGO
SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
32 Jamestown Road, London, NW1 7BY, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
30 Corporate Drive, Suite 400, Burlington, MA 01803, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

This book is printed on acid-free paper. ♾

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-385506-0
ISSN: 1877-1173

Printed and Bound in the USA
11 12 13 14 10 9 8 7 6 5 4 3 2 1

Contents

Contributors ix
Preface xi

Monoamine Transporters: Vulnerable and Vital Doorkeepers 1

Zhicheng Lin, Juan J. Canales, Thröstur Björgvinsson, Morgane Thomsen, Hong Qu, Qing-Rong Liu, Gonzalo E. Torres, and S. Barak Caine

I. Introduction 2
II. Clinical Benefits: Demonstration of the Medical Roles of Monoamine Transporters 3
III. Preclinical Indications—Behavioral Pharmacology 6
IV. Molecular Study: cDNA Cloning and Structure Activity Relationship ... 11
V. Protein Regulations 14
VI. Animal Genetics 17
VII. The Transporter Genes as Risk Factors 20
VIII. Perspectives for Medication Development 24
References 27

Therapeutic Targets in Alzheimer's Disease and Related Tauopathies 47

Christopher P. Corbo and Alejandra del C. Alonso

I. Introduction to the Disease 48
II. Attempts at Abeta Therapy 53
III. Tau Hypothesis 55
IV. Tau Theraputic Targets 66
V. Concluding Remarks 71
References 72

Therapeutic Targets for Neuroprotection and/or Enhancement of Functional Recovery Following Traumatic Brain Injury 85

Matthew L. Kelso and James R. Pauly

I. Introduction 86
II. Primary Injury Models 87
III. Receptor-Based Targets for Neuroprotection 89
IV. Targeting Enzymatic Activity for Neuroprotection 104
V. Other Promising Strategies 107
VI. Future Directions for Optimization of Treatment Regimens 114
References 116

Insulin, Synaptic Function, and Opportunities for Neuroprotection 133

John G. Mielke and Yu-Tian Wang

I. Introduction 134
II. Insulin Within the Central Nervous System 135
III. Insulin Receptors Within the Brain 140
IV. Insulin Signaling and Synaptic Function 148
V. Therapeutic Opportunities: Insulin and Neuroprotection 158
VI. General Summary 167
References 168

Molecular Alterations in Glioblastoma: Potential Targets for Immunotherapy 187

Azizul Haque, Naren L. Banik, and Swapan K. Ray

I. Introduction 188
II. Genetic Alterations in Glioblastoma 190
III. Ags and Biomarkers in Glioblastoma 195
IV. Ag-Specific Immunotherapy for Glioblastoma 202
V. Biomarker-Specific Chemotherapy for Glioblastoma 206
VI. Cytokine-Based Immunotherapy for Glioblastoma 212
VII. Chemoimmunotherapy for Glioblastoma 216
VIII. Tumor-Associated Molecules Influencing Glioblastoma Immunotherapy 218
IX. Conclusions 224
References 226

Molecular Signaling and Translational Significance of the Corticotropin Releasing Factor System 235

Patrick J. Ronan and Cliff H. Summers

I. Introduction to the Corticotropin Releasing Factor Peptide Family 236
II. Neural CRF Distribution..... 237
III. CRF Binding and Signal Transduction 241
IV. Genes and Gene Systems Activated by CRF 244
V. Interactions with Other Neural Circuits 247
VI. Translational Significance of CRF..... 253
VII. Integrated CRF Function 264
References..... 267

Molecular Targets of Alcohol Action: Translational Research for Pharmacotherapy Development and Screening 293

Giorgio Gorini, Richard L. Bell, and R. Dayne Mayfield

I. Introduction 294
II. Alcohol Abuse and Dependence, Significance, and Treatment Needs 296
III. Preclinical Strategies for Identification of Novel Targets of Alcohol Action..... 297
IV. Translational Strategies and Need for Continued Biomarkers Development..... 327
References 331

Brain Nicotinic Receptors as Emerging Targets for Drug Addiction: Neurobiology to Translational Research 349

Shafiqur Rahman

I. Introduction 350
II. Brain nAChRs in Nicotine Addiction..... 351
III. Brain nAChRs in Alcohol Dependence 353
IV. Brain nAChRs in Other Addictive Disorders..... 355
V. Limitations in nAChR-Based Translational Research 357
VI. Conclusions and Future Perspectives..... 358
References 359

Glutamatergic Neuroplasticity in Cocaine Addiction . . . 367

Joachim D. Uys and Kathryn J. Reissner

I. Introduction 368
II. Neurocircuitry of Addiction 369
III. Adaptations in Synaptic Plasticity 373
IV. Glutamate Homeostasis Hypothesis of Cocaine Addiction 378
V. Redox Regulation of Neurons 381
VI. Promise for Glutamate-Mediated Pharmacotherapies for Addiction 384
VII. Conclusion 389
References 389

Role of the Serotonergic System in Alcohol Dependence: From Animal Models to Clinics 401

Youssef Sari, Verity R. Johnson, and Jason M. Weedman

I. Introduction 402
II. The Role of 5-HTT in Alcohol-Directed Neuroadaptation, Intoxication Response, and Potential for Abuse and Dependence 405
III. Serotonergic Receptors: Molecular, Pharmacological, and Physiological Aspects and Their Role in Alcohol Dependence 412
IV. Interactions Between the Serotonergic System and Other Neurotransmitter Systems in the Modulation of Alcohol Consumption ... 415
V. Serotonergic System as a Potential Therapeutic Target in Alcohol Dependence/Addiction 419
VI. Conclusions 426
References 427

Index 445

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Alejandra del C. Alonso, College of Staten Island, Program in Developmental Neuroscience, The Graduate Center, City University of New York (CUNY), Staten Island, New York, USA (47)

Naren L. Banik, Department of Neurosciences, Medical University of South Carolina, Charleston, South Carolina, USA (187)

Richard L. Bell, Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine; and Department of Psychology, Purdue School of Science, Indiana University Purdue University at Indianapolis, Indianapolis, Indiana, USA (293)

Thröstur Björgvinsson, Behavioral Health Partial Hospital and Psychology Internship Programs, McLean Hospital/Harvard Medical School, Belmont, Massachusetts, USA (1)

S. Barak Caine, Department of Psychiatry, Harvard Medical School and Division of Alcohol and Drug Abuse, McLean Hospital, Belmont, Massachusetts, USA (1)

Juan J. Canales, Department of Psychology, Behavioural Neuroscience, University of Canterbury, Christchurch, New Zealand (1)

Christopher P. Corbo, College of Staten Island, Program in Developmental Neuroscience, The Graduate Center, City University of New York (CUNY), Staten Island, New York, USA (47)

Giorgio Gorini, Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, Texas, USA (293)

Azizul Haque, Department of Microbiology and Immunology; Hollings Cancer Center; and Children's Research Institute, Medical University of South Carolina, Charleston, South Carolina, USA (187)

Verity R. Johnson, Department of Psychological and Brain Sciences, Indiana University Bloomington, Bloomington, Indiana, USA (401)

Matthew L. Kelso, Department of Pharmacy Practice, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska, USA (85)

Zhicheng Lin, Department of Psychiatry, Harvard Medical School and Division of Alcohol and Drug Abuse, McLean Hospital, Belmont, Massachusetts, USA (1)

Qing-Rong Liu, Behavioral Neuroscience Branch, Intramural Research Program, National Institute on Drug Abuse, NIH/DHHS, Baltimore, Maryland, USA (1)

R. Dayne Mayfield, Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, Texas, USA (293)

John G. Mielke, Faculty of Applied Health Sciences, Department of Health Studies and Gerontology, University of Waterloo, Waterloo, Ontario, Canada (133)

James R. Pauly, Department of Pharmaceutical Sciences, College of Pharmacy; and Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, Kentucky, USA (85)

Hong Qu, Center for Bioinformatics, National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing, China (1)

Shafiqur Rahman, Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, South Dakota, USA (349)

Swapan K. Ray, Department of Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, South Carolina, USA (187)

Kathryn J. Reissner, Department of Neurosciences, Medical University of South Carolina, Charleston, South Carolina, USA (367)

Patrick J. Ronan, Avera Research Institute, Sioux Falls; Research Service, Sioux Falls VA Medical Center, Sioux Falls; Neuroscience Group, Division of Basic Biomedical Sciences; and Department of Psychiatry, University of South Dakota School of Medicine, Vermillion, South Dakota, USA (235)

Youssef Sari, Department of Pharmacology, Health Science Campus, College of Pharmacy and Pharmaceutical Sciences, University of Toledo, Toledo, Ohio, USA (401)

Cliff H. Summers, Neuroscience Group, Division of Basic Biomedical Sciences; and Department of Biology, University of South Dakota School of Medicine, Vermillion, South Dakota, USA (235)

Morgane Thomsen, Department of Psychiatry, Harvard Medical School and Division of Alcohol and Drug Abuse, McLean Hospital, Belmont, Massachusetts, USA (1)

Gonzalo E. Torres, Department of Neurobiology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA (1)

Joachim D. Uys, Department of Cell and Molecular Pharmacology, Medical University of South Carolina, Charleston, South Carolina, USA (367)

Yu-Tian Wang, Brain Research Centre, University of British Columbia, Vancouver, British Columbia, Canada (133)

Jason M. Weedman, Department of Psychological and Brain Sciences, Indiana University Bloomington, Bloomington, Indiana, USA (401)

Preface

Translational neuroscience research refers to knowledge through fundamental understanding on brain structure and function to develop novel prevention and treatment strategies for neurological and psychiatric disorders.[1] Among many human diseases, brain disorders provide the biggest challenges to translational research in the era of novel pathways to drug development.[2] These challenges involve many demanding research efforts from target identification, the predictability in animal models, and disease model validation, to understanding complex pharmacodynamic and pharmacokinetic markers.[3] Therefore, translational research offers an opportunity to bridge the gap between preclinical research and drug development in humans. This volume, *Brain as a Drug Target*, consists of ten chapters written by eminent experts in the field. The volume covers important aspects of preclinical and clinical research on many important neurological, psychiatric, and drug-addictive disorders associated with multiple brain targets. The chapters in this volume cover current information, discuss some of the latest concepts in preclinical research, analyze breakthrough findings, define novel approaches, and target multiple brain substrates including monoamine transporters, tau protein, brain insulin receptors, glioblastoma, corticotropin releasing factor, molecular biomarkers for alcohol effects, neuronal nicotinic receptors, glutamatergic system, and serotonergic system for medication development, and clinical management of these neuronal disorders.

Lin *et al.* focus on monoamine transporters as important brain targets implicated in multiple neuropsychiatric disorders. They review molecular studies of these transporters, which reveal a wealth of information on the transporters' structure–activity relationship, neuropharmacology, cell biology, biochemistry, and pharmacogenetics related to the human genes encoding these transporters. Implications of this scientific insight will provide new opportunity to develop transporter-specific medications for Parkinson's disease, schizophrenia, depression, and other brain disorders.

Corbo and Alonso present therapeutic targets in Alzheimer's disease (AD) and related tauopathies. The authors describe important research efforts dedicated to attack the plaques and, in more detail, the process of neurofibrillary degeneration, linked to the presence of the hyperphosphorylated microtubule-associated protein tau. Thus, in addition to discussing current data on these

important molecular mechanisms of AD, this chapter also provides evidence-based understanding of the use of these mechanisms in defining strategies for future therapeutics with identified putative targets.

Kelso and Pauly provide preclinical evidence for pharmacotherapy of traumatic brain injury. They provide important information on various animal models of the primary mechanical trauma, as well as current knowledge of the complex biochemical mechanisms. They also highlight some of the promising molecular and cellular targets that have been identified for future drug development strategies. The translational value of the extensive preclinical work reviewed in this chapter provides great importance in understanding this complex disorder for prevention and treatment.

Mielke and Wang provide an emerging relationship between insulin and brain, and its relevance to synaptic function and neuroprotection. The authors describe important preclinical studies that support the critical role of insulin on brain targets. They also discuss the novel role of insulin on the modulation of ligand-gated ion channel trafficking, including tone of synaptic transmission by regulating cell surface expression of inhibitory and excitatory receptors. Insulin-mediated neuroprotection in the absence of hypoglycemia is also reviewed. Overall, the research highlighted in this chapter has profound implications for future translational research and therapeutic opportunities in the area of neuroprotection.

Haque, Banik, and Ray focus on glioblastoma, the most common and deadly brain tumor. The authors provide information on molecular interactions among glioblastoma tumors, host immune cells, and the tumor microenvironment. Knowledge from this research may lead to novel integrated approaches for the simultaneous control of tumor escape pathways and the activation of antitumor immune responses. The progress and latest lines of research in the field are reviewed in this chapter and shed new light for future translational research and new chemoimmunotherapeutics against glioblastoma.

Ronan and Summers present a detailed review on the corticotropin releasing factor (CRF) system implicated in various neuropsychiatric disorders, including stress. The authors describe the detailed molecular signaling of the CRF system, and its interaction with other key brain transmitter systems. They provide preclinical studies highlighting the importance of the CRF signaling system in psychiatric disorders and discuss the limitations given the complexity of its signaling in the brain. Overall, this comprehensive chapter provides important knowledge and enhances the understanding for future translational research and therapeutics for several psychiatric disorders.

Gorini, Bell, and Mayfield describe the reliable brain biomarkers and targets for alcohol effects relevant to alcohol use disorders. They highlight latest research identifying neurobiological systems associated with these targets and possible pharmacotherapies. The authors summarize evidence from

animal and human studies, and sketch the challenges facing the fields of proteomics and genomics. Finally, better understanding and ideas on profiling these molecular technologies are discussed for future drug development and clinical utility for alcohol use disorders.

Rahman presents an overview on the brain nicotinic receptor system with compelling evidence that nicotinic receptors could serve as potential targets for pharmacotherapy of several addictive disorders, including nicotine addiction and alcohol dependence. The author discusses the preclinical research literature involving nicotinic receptors in the brain reward system implicated in drug addiction. The review highlights the preclinical and clinical research on a number of important compounds that target brain nicotinic receptors. The progress and latest lines of research in the nicotinic receptor field reviewed in this chapter shed new light for future translational research for improved and new therapeutics in the clinical management of addictive disorders, including nicotine addiction and alcohol dependence.

Uys and Reissner focus on neuroplasticity in cocaine addiction by targeting brain glutamatergic system. The authors describe preclinical studies involving glutamatergic transmission in cocaine-related behavior. They also discuss evidence for adaptations in glutamatergic neuroplasticity as a mechanism for cocaine addiction. Finally, the authors discuss progress in the development of glutamate-based pharmacotherapies for the treatment of cocaine addiction in humans.

Sari, Johnson, and, Weedman cover the role of brain serotonergic system in alcohol dependence. The authors provide the current understanding of the brain serotonin system and its relevance to alcohol-mediated dependence and craving. They also discuss preclinical studies related to the interactions of serotonin system and other neurotransmitter systems. The chapter highlights the serotonin transporter and its possible role in alcoholism. The authors emphasize the contribution of several serotonergic receptors and discuss their relationship with alcohol dependence. Finally, they assess the serotonin system as an important brain target for future translational research and pharmacotherapy of alcohol dependence.

Overall, these ten comprehensive chapters provide an extensive overview, new insights into our current knowledge on different targets, and essential directions for future research related to many neurological, neuropsychiatric disorders, and drug addiction. I hope that the information provided on multiple topics and relevant research summarized in this volume will attract new ideas and stimulate additional investigations for effective prevention and treatment strategies for these devastating brain disorders.

Acknowledgments

I would like to thank all the authors for their outstanding contributions to this volume. I am very thankful to Dr. P. Michael Conn, the Editor-in-Chief, the Book Series, and for the opportunity and his guidance. Finally, I also thank Editors Ms. Lisa Tickner and Ms. Delsy Retchagar of Elsevier for their support in bringing this volume together. Special thanks to my wife and daughters for their understanding and love.

Shafiqur Rahman
College of Pharmacy, South Dakota State
University, Brookings, South Dakota, USA

References

1. Finkelstein R, Miller T, Baughman R. The challenge of translational research—a perspective from NINDS. *Nat Neurosci* 2002;**5**:1029–30.
2. Hurko O, Ryan JL. Translational research in central nervous system drug discovery. *NeuroRx* 2005;**2**:671–82.
3. Fox GB, Chin CL, Luo F, Day M, Cox BF. Translational neuroimaging of the CNS: novel pathways to drug development. *Mol Interv* 2009;**9**:302–13.

Monoamine Transporters: Vulnerable and Vital Doorkeepers

Zhicheng Lin,[*] Juan J. Canales,[†] Thröstur Björgvinsson,[‡] Morgane Thomsen,[*] Hong Qu,[§] Qing-Rong Liu,[¶] Gonzalo E. Torres,[‖] and S. Barak Caine[*]

[*]*Department of Psychiatry, Harvard Medical School and Division of Alcohol and Drug Abuse, McLean Hospital, Belmont, Massachusetts, USA*

[†]*Department of Psychology, Behavioural Neuroscience, University of Canterbury, Christchurch, New Zealand*

[‡]*Behavioral Health Partial Hospital and Psychology Internship Programs, McLean Hospital/Harvard Medical School, Belmont, Massachusetts, USA*

[§]*Center for Bioinformatics, National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing, China*

[¶]*Behavioral Neuroscience Branch, Intramural Research Program, National Institute on Drug Abuse, NIH/DHHS, Baltimore, Maryland, USA*

[‖]*Department of Neurobiology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA*

I. Introduction 2
II. Clinical Benefits: Demonstration of the Medical Roles of Monoamine Transporters 3
A. Depression 4
B. Obsessive Compulsive Disorder (OCD) and Other Anxiety Disorders 4
C. Chronic Pain Syndrome 5
D. Attention Deficit Hyperactivity Disorder (ADHD) 5
E. Cigarette Smoking 5
III. Preclinical Indications—Behavioral Pharmacology 6
A. DAT 6
B. SERT 7

Progress in Molecular Biology and Translational Science, Vol. 98
DOI: 10.1016/B978-0-12-385506-0.00001-6

1877-1173/11 $35.00

C. NET 9
D. VMAT2 10
IV. Molecular Study: cDNA Cloning and Structure Activity Relationship 11
A. cDNA Cloning and Characterization 12
B. SAR of the Plasma Membrane Transporters 12
V. Protein Regulations 14
A. Subcellular Localization 14
B. Drug Regulation 15
C. DAT Regulators 15
D. SERT and NET Regulators 17
E. VMAT2 Regulators 17
VI. Animal Genetics 17
A. DAT 18
B. SERT 18
C. NET 19
D. VMAT2 19
VII. The Transporter Genes as Risk Factors 20
A. Gene Structures 21
B. hDAT 21
C. hSERT 23
D. hNET 23
E. hVMATs 23
VIII. Perspectives for Medication Development 24
A. Medication Issues 24
B. New Targets for Medications 26
References 27

Transporters of dopamine, serotonin, and norepinephrine have been empirically used as medication targets for several mental illnesses in the last decades. These protein-*targeted* medications are effective only for subpopulations of patients with transporter-related brain disorders. Since the cDNA clonings in early 1990s, molecular studies of these transporters have revealed a wealth of information about the transporters' structure–activity relationship (SAR), neuropharmacology, cell biology, biochemistry, pharmacogenetics, and the diseases related to the human genes encoding these transporters among related regulators. Such new information creates a unique opportunity to develop transporter-*specific* medications based on SAR, mRNA, DNA, and perhaps transporter trafficking regulation for a number of highly relevant diseases including substance abuse, depression, schizophrenia, and Parkinson's disease.

I. Introduction

Monoamine transporters are transmembrane proteins located in plasma membranes of monoaminergic neurons, including the dopamine transporter (DAT), serotonin transporter (SERT, also expressed in platelets), and norepinephrine transporter (NET).[1,2] These proteins use ion (Na^+, Cl^-) gradients as

energy sources to move monoamines into or out of neurons. The major function of these transporters is to terminate monoamine transmission by inward transport of substrates away from the synaptic cleft. In the membrane of intracellular synaptic vesicles is the vesicular monoamine transporters 1 and 2 (VMAT1 and VMAT2), which use a proton gradient as the energy source to sequester cytosolic monoamines into the vesicles and then release the monoamines into the synaptic cleft by exocytosis. Therefore, the overall function of these four transporters is to regulate spatio-temporal components of monoamine transmission. Loss of a transporter could cause severe disease or lethality. For instance, two loss-of-function DAT mutants, L368Q and P395L, cause infantile parkinsonism-dystonia in humans.[3] Complete deletion of the VMAT2 gene causes developmental defect and embryonic lethality in mice.[4–6]

Because of the exclusive expression of each transporter in the corresponding neurons, these transporters are often used as markers of specific neurons. DAT is expressed in dopaminergic neurons that project mainly from the ventral tegmental area (VTA) and substantia nigra to prefrontal cortex, nucleus accumbens, and striatum; SERT plays its role in the pons and upper brain stem; NET is localized in the locus coeruleus and the lateral tegmental group that project into many other brain regions. VMAT1 is expressed transiently during brain development, and VMAT2 is the main vesicular transporter in these monoaminergic neurons.[7] Importantly, these monoaminergic neurons intervene with each other and with many other types of neurons and innervate various brain regions including cortex, hippocampus, amygdale, and hypothalamus.

The extensive distribution of these transporters determines their central roles in neurotransmission and are ideal medication targets for a spectrum of monoamine-related neuropsychiatric disorders, including attention deficit hyperactivity disorder (ADHD), depression, anxiety, addiction, narcolepsy, fatigue, obesity, eating disorder, other mood disorders, schizophrenia (SCZ), bipolar disorder, and Parkinson's disease. On the other hand, the central roles have also presented these plasma membrane proteins as functional targets for drugs of abuse such as alcohol, cocaine, methamphetamine, and MDMA (3,4-methylenedioxymethamphetamine or Ecstasy). In this chapter, we summarize the recent progress in our understanding of the contribution of these monoamine transporters to brain function and diseases.

II. Clinical Benefits: Demonstration of the Medical Roles of Monoamine Transporters

Due to amino acid sequence and proposed structural similarity among the three plasma membrane transporters (DAT, SERT, and NET), many monoamine transporter inhibitors have affinity for all three transporters. Unlike the

other sections below that discuss individual transporters, this section categorizes the main diseases and their treatments with monoamine transporter inhibitors.

A. Depression

Depression is the most common disease that is treated by directly targeting the NET,[8] SETR,[9] DAT (e.g., Refs. 10,11), and/or some combinations of the three (e.g., Ref. 12).

First developed in the 1950s in an attempt to improve the effectiveness of chlorpromazine, monoamine oxidase inhibitors (MAOI) and tricyclic antidepressants (TCAs) function by inhibiting the reuptake of serotonin, norepinephrine, and dopamine through blocking each respective neurotransmitter transporter (SERT, NET, DAT).[13] Each class of drugs acts on all three of these monoamine systems, with most TCAs primarily inhibiting NET and SERT.[14] These medications were then superseded by the selective serotonin reuptake inhibitors (SSRIs) as antidepressants. As the most commonly prescribed antidepressant medication, SSRIs are posited to work more effectively within the complex central nervous–neural circuit–gene system in the epidemiology of depression,[15] and thus have far less adverse side effects in comparison to TCAs and MAOIs.[9] Hundreds of placebo-controlled trials have demonstrated benefits in moderate to severe depression, particularly in those with symptoms of more acute major depressive episodes and dysthymia[9,16,17] and melancholic depression.[18,19] SSRIs also possess strong therapeutic activity for various DSM-IV-TR disorders (e.g., panic) as described below.

The most recently utilized class of antidepressants falls under selective norepinephrine/dopamine reuptake inhibitors (SNDRIs), with bupropion (Welbutrin) as the most commonly prescribed one. Bupropion is an effective and generally well-tolerated option in the treatment of moderate to severe major depressive disorder (e.g., Ref. 20). In addition, bupropion has been shown to be as effective as many common psychopharmacological medications in managing symptoms of depression.[21] Trials have demonstrated that SSRIs appear to be more effective in the treatment of moderate/acute depression, whereas SNDRIs may be advantageous in the treatment of chronic depression.[14]

B. Obsessive Compulsive Disorder (OCD) and Other Anxiety Disorders

Clomipramine, an inhibitor of SERT, NET, DAT and some receptors, was discovered by the Spanish psychiatrist Lopez-Ibor in the 1960s for treating OCD symptoms and the efficacy was subsequently confirmed by many other trials (see Refs. 22–24). SSRIs have been used for OCD but the clomipramine

effect size appeared to be larger than that of the SSRIs (e.g., Ref. 25). Regardless, clomipramine and SSRIs remain an integral part of "best practice" management of OCD.

In fact, drugs acting on the monoamine transporters, specifically SERT, have efficacy in other anxiety disorders too. Panic disorder w/agoraphobia was originally named in DSM III in 1980 following research in the USA described as "pharmacodissection" using the NET inhibitor imipramine.[26] Recent studies show the benefits of norepinephrine and serotonin reuptake inhibitors in the treatment of panic agoraphobia,[27,28] social anxiety disorder,[29] generalized anxiety disorder,[30] and posttraumatic stress disorder.[31]

C. Chronic Pain Syndrome

In addition to new interventions (e.g., the use of sodium oxybate in the treatment of fibromyalgia pain, and insomnia), TCAs, SNDRIs, and SSRIs are promising medications for fibromyalgia pain.[32,33] TCAs have been shown to improve the symptoms of pain, poor sleep, and fatigue associated with fibromyalgia but still show greater side effects (e.g., Ref. 34). The results of SNRIs are more promising,[35] although current effect sizes are smaller than trials using TCAs. The efficacy of pain treatments seems to be better achieved by balanced inhibition of multiple monoamine transporters.[33]

D. Attention Deficit Hyperactivity Disorder (ADHD)

Atomoxetine, a NET inhibitor, has been shown in randomized clinical trials to significantly reduce ADHD symptoms in both comorbid and noncomorbid children,[36] adolescents, and adults with ADHD.[37] While stimulants including the DAT inhibitors methylphenidate and amphetamine remain the most frequently prescribed medication in treating ADHD, SNDRIs are currently the leading second-line alternatives.[38,39]

E. Cigarette Smoking

Reviews on the effect of cigarette smoking postulate that chronic exposure to nicotine elicits depressogenic changes in serotonin formation and release in the hippocampus.[40] These changes may contribute to the symptoms of depression experienced by many smokers when they first quit. The research examining this relationship has resulted in clinically significant findings. For instance, medications such as bupropion and nortriptyline have been shown to be efficacious in the treatment of cigarette smoking.[41] Moreover, it has been shown that a treatment modality that includes nicotine replacement/cessation therapy is recommended for individuals who are highly nicotine dependent and who have a current or past history of major depressive disorder.[42]

In summary, DAT serves as a medication target for ADHD, depression, OCD, smoking cessation, as well as narocolepsy and Parkinson's disease which are not mentioned here; SERT for depression, anxiety, OCD, and pain; and NET for depression, OCD, anxiety, and ADHD, which are diseases that affect approximately 20% of the population.

III. Preclinical Indications—Behavioral Pharmacology

During the last 40 years of preclinical and human psychopharmacology research, the monoamine transporters have been recognized to play a central role in modulating a wide variety of physiological and behavioral functions, including locomotion, autonomic function, and hormone regulation, and to make a fundamental contribution to emotional and cognitive function, neurotoxicity, and mental disease. Such a central role is paralleled by the breadth of monoaminergic projections into the neocortex, basal ganglia, and limbic forebrain. These pharmacologic and anatomical findings have also been instrumental for the identification and development of medications that are currently used as pharmacotherapies to treat a variety of behavioral disorders, such as major depression, OCD, anxiety, ADHD, and addiction.

A. DAT

The pioneering investigations by Arvid Carlsson and Kjell Fuxe established dopamine (DA) as a neurotransmitter in the late 1950s,[43] and the topography of the dopaminergic innervation of the central nervous system was soon delineated. The mesolimbic DA system originating in the VTA innervates the nucleus accumbens of the ventral striatum, where DA is postulated to participate in the control of exploratory activity, reward-related processes, and reinforcement, both natural and drug-induced.[44,45] In turn, the nigrostriatal contingent of DA fibers projecting into the dorsal striatum (i.e., caudate and putamen nuclei in humans) is linked to the control of movement, as revealed by the clinical phenomenology associated with basal ganglia disorders,[46] and for "chunking" action repertoires and habits.[47] Further, the innervation of the prefrontal cortex by the mesocortical DA pathway has been proposed to modulate various aspects of executive function, including working memory function, planning, and attention.[48] Historically, the emerging view of DA as an important transmitter has been reinforced by two additional findings: First, the clinical efficacy of antipsychotic medications was observed to correlate with the binding affinity for DA D_2 receptors. Second, observations showed that most abused drugs, especially psychomotor stimulants such as cocaine and amphetamine (AMPH), exert psychoactive effects through interactions with the DA system, some of which involved mainly the DAT.

Preclinical experiments and human data have demonstrated that the DAT is involved in the behavioral reinforcing and euphorogenic effects of stimulant drugs. The ability of cocaine-like drugs to maintain self-administration in rodents is correlated with their potency in inhibiting the DAT.[49] The idea is that cocaine binding to DAT may increase extracellular DA concentration by blocking the reuptake activity and inducing the release of reserve pool of DA,[50] activating DA receptors. Moreover, the self-reported "high" induced by stimulants in humans appears to be a function of both the rate of DAT occupancy by the stimulant and the speed of stimulant delivery into the brain.[51] This evidence suggested that both the binding affinity for the DAT and the pharmacokinetic/pharmacodynamic properties are important characteristics that predict the psychopharmacological effects of drugs acting at the DAT. In addition, preclinical behavioral assays including tests of locomotor activity, conditioned place preference, drug discrimination, and self-administration indicated that various DAT inhibitors differ from prototypical stimulants such as cocaine and AMPH. This evidence fueled speculation that it might be possible to design molecules that bind to the DAT and prevent the actions of AMPH and cocaine at the DAT but lack psychomotor stimulant-like effects. However, AMPH or cocaine antagonism without blocking DA reuptake activity has not been successful due to overlapping DAT sites for stimulant binding and DA recognition.

An alternative to antagonism approach is a substitute approach: a slow-onset, long-acting competing agonist could be used to treat stimulant addiction. As indicated, the specific pharmacokinetic/dynamic features and rate of DAT occupancy are important factors that influence the cocaine-like properties of DAT inhibitors.[51] Different DA uptake inhibitors display specific modes of interaction with the DAT, leading to specific conformational changes of the protein,[52,53] and the different DAT conformations are related to the ability of the inhibitors to induce locomotor activity and substitute for cocaine in discrimination assays.[54] It is currently believed that the rational design and development of high-affinity, long-acting DAT ligands with specific pharmacokinetic/dynamic properties might lead to the discovery of optimal medications for stimulant addiction.[55,56] A variety of molecules based on 3-aryltropanes (WIN compounds),[57] 1,4-dialkylpiperazines (e.g., GBR 12909 and its analogues),[55] and analogs of benztropine (BZT)[56] have all been synthesized, tested *in vitro* for binding at different transporters and receptors, and evaluated in preclinical models. Of these, BZT analogs exhibit ideal characteristics in preclinical assays, blocking the behavioral effects of cocaine and AMPH and exhibiting weak abuse liability in place preference and self-administration assays.[58–60]

B. SERT

5-HT-containing neurons of the raphe regions located in the pons and upper brain stem extensively innervate the diencephalon and telencephalon, providing input to the hypothalamus, habenula, thalamus, amygdala, striatum,

and cortical mantle.[61] The 5-HT system is believed to regulate mood, emotion, learning, memory, sleep, and appetite. Of the chemical neurotransmitters, 5-HT is perhaps the most widely implicated in the treatment of mental illnesses.[62] The 5-HT transporter (SERT) is responsible for taking up 5-HT from the synapse and is indeed the target of a wide range of molecules that inhibit the uptake process. Two aspects have been decisive to confirm the key functions of 5-HT and the importance of inhibiting 5-HT transport as therapeutic principle: First is the discovery that 5-HT is structurally related to many psychotropic agents, including lysergic acid diethylamide (LSD) and psilocin[63]. Second is the finding that many drugs with psychoactive properties, including cocaine, AMPH, Ecstasy, TCAs, and SSRIs, effectively interact with SERT to block 5-HT uptake.[64] These findings, particularly the latter, confirmed a critical role for 5-HT in the regulation of mood and affect and marked a milestone in neuropsychopharmacology and psychiatry research.

Over the last decades, tremendous strides have been made in the treatment of major depressive illness, anxiety, and eating disorders. The introduction of the first generation of monoamine transporter inhibitors, that is, the TCAs, which target both NET and SERT with variable affinity, revolutionized the management of affective disorders. However, the widespread activity of TCAs at multiple biological sites, including not only monoamine transporters but also noradrenergic, histaminergic, and muscarinic receptors, is associated with unwanted side effects.[65] The development of SSRIs, such as fluoxetine, citalopram, and fluvoxamine, led to fewer side effects while retaining full clinical efficacy. In animal models of depression, such as the learned helplessness and forced swimming tests, SSRIs have systematically displayed strong activity suggestive of clinical efficacy.[66,67] These models provide opportunities to assay new compounds and investigate the mechanisms underlying preclinical efficacy. Indeed, in spite of adequate treatment with current antidepressant medication, a large proportion of patients do not receive full symptom remission when treated with SSRIs, and new approaches are presently pursued. A promising lead for improved therapeutic effects is the development of triple uptake inhibitors targeting SERT, NET, and DAT (SNDRIs). Several of these compounds are in advanced stages of development, though none has yet been approved for human use. In animal models, the SNDRI, DOV 21,947, inhibits reuptake of 5-HT, NE, and DA (EC_{50} of 12, 23, and 96 nM at human recombinant transporters, respectively) and exhibits antidepressant effects in the forced swim and tail suspension test.[68] DOV 102,677, another SNDRI (EC_{50} of 129, 103, and 133 nM at human recombinant transporters, respectively), was as effective as methylphenidate in reducing the amplitude of the startle response in juvenile mice, in addition to showing an antidepressant profile.[69] Preclinical indications suggest that "triple inhibitors" may produce a more rapid onset of action and greater efficacy than traditional antidepressants.[70] In addition, an emerging

literature indicates that nonclassical transporters such as the plasma membrane monoamine transporter (PMAT) and organic cation transporters (OCTs), which subserve promiscuous uptake of biogenic amines, may constitute new targets for improved antidepressant action, alone or in combination with SSRIs.[71]

It is noticed that NET and SERT are also binding sites for psychomotor stimulants including cocaine, AMPH, methamphetamine, or Ecstasy. Although DA seems to underlie the reinforcing effects more closely, several studies have suggested that these binding activities contribute to at least some of the reinforcing properties,[72–76] warranting medication targets for drug addiction.

C. NET

There are two major noradrenergic neuronal clusterings in the brain: the locus coeruleus and the lateral tegmental group, which provide extensive innervation to the striatum, amygdala, hypothalamus, thalamus, cerebellum, and neocortex.[77] Such widespread ascending projections have been implicated in the modulation of arousal, sleep, and cognitive processes.[78,79] An important additional function of the NE system is to control the endocrine and the autonomic nervous system, which play a fundamental role in anxiety and the stress response.[80]

The NE system is an important target for a wide range of drugs used for the treatment of mood, anxiety, and behavioral disorders, including major depression, generalized anxiety disorder, and ADHD. The neurobiological links between stress, anxiety, and depression have long been postulated, but the identification of such relationship has only begun to emerge. The locus coeruleus is uniquely placed to integrate both exteroceptive cues and internal visceral/endocrine information, and to influence stress- and fear-related anatomical structures, including amygdala, periaqueductal gray, and neocortex.[81] A great deal of evidence suggests that the NE system and the corticotrophin-releasing factor (CRF) pathways co-regulate their activation in response to fear and stress.[82] Pharmacological inhibition of NE transport with NET blockers that display little or no activity at other monoamine transporters exerts activity in several animal models of stress and depression. Atomoxetine and reboxetine, which exhibit 50- to100-fold preference at human NET versus other monoamine transporters,[83] show strong activity in animal models of depression that is predictive of therapeutic effects in humans. For example, the therapeutic-like effects of reboxetine have been assayed in the olfactory bulbectomized (OB) rat model of depression, reducing immobility time in the forced swim test and hyperactivity in the open field.[84] Reboxetine also attenuates the physiological responses associated with swim stress, including 5-HT elevations in amygdala and prefrontal cortex and activation of hypothalamic–pituitary–adrenal axis.[85] The antidepressant-like effects of reboxetine in the forced swim test are blocked by 6-hydroxydopamine lesions of the ventral noradrenergic bundle, suggesting that enhanced noradrenergic activity mediates the effects of